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

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CHRONOLOGICAL LIST OF SESSIONS

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WEDNESDAY

THEMATIC LIST OF SESSIONS

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Session Number	Session Title	Type	Day and Time Mon.	Tue.	Wed.	Thu.	Fri.
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626.	Chemical senses: central pathways III	Poster					Fri AM
49.	Chemical senses: peripheral mechanisms I	Poster	Mon AM				
251.	Chemical senses: peripheral mechanisms II	Poster		Tue PM			
438.	Chemical senses: peripheral mechanisms III	Slide				Thu AM	

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

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

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

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

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

Theme J: Disorders of the Nervous System

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

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

395.5

DEXFENFLURAMINE INHIBITS BASAL AND CRF-INDUCED ACTH AND β-ENDORPHIN RELEASE DURING IN VITRO PERIFUSION FROM PITUITARIES OF ADULT MALE RAT. B. Kerdelhue, V. Lenoir*, F. Lacour*, S. Melik*, O. Arnaud*, C. Nathan* and J. Duhault*. Lab. for Reproductive Neurobiology, CNRS, 78350 jouy-en-josas ; and IRIS, 92415 Courbevoie, FRANCE.

It has been demonstrated by in vivo experiments that dexfenfluramine (DF) markedly decreases the stimulatory action of environmental stress on corticosterone release. The present study examined in vitro the action of various concentrations of DF on the basal as well on the CRF-induced release of ACTH and β-Endorphin (β-EP). Anterior pituitaries (AP) were removed from 30 days old male rats sacrificed and placed in a perifusion system (1 AP/chamber ;chambers volume : 500 µl ;fraction collection : 10 min ;perifusion rate :500 µl/min ;perifusion medium: medium 199 containing I.B.M.X. : 0.2 mM ; Hepes : 20 mM ; Bacitracin : 0.1 mM ; BSA : 1 mg/ml ; pH : 7.4). After a 90 min equilibration period, DF (10^{-10}M to 10^{-6}M) was added for 60 min during which an application of a 10 min CRF 10^{-9}M pulse was performed at the end. Additional fractions were collected for 90 min after the end of the CRF pulse. ACTH and β-EP concentrations were determined by specific RIA's. The results show a significant reduction of basal ACTH (but not β-EP) release after exposure to DF. The inhibitory effect started at 10^{-10}M of DF and was concentration-dependent. From 10^{-10}M of DF there was a significant progressive reduction (20 % to 50 %) of the amplitude of the CRF-induced ACTH or β-EP surge. These results show that DF inhibits the secretion of ACTH and β-EP in basal and CRF-stimulated conditions. Together with recent observations from our laboratory showing an inhibitory effect of DF on CRF release rate from hypothalami,these results imply that the inhibitory effect of DF on corticosterone secretion is due to an action at the hypothalamo-pituitary corticotropic complex.

395.6

THE ANTIDEPRESSANTS FLUOXETINE, IDAZOXAN, AND PHENELZINE ALTER CORTICOTROPIN-RELEASING HORMONE AND TYROSINE HYDROXYLASE mRNA LEVELS IN RAT BRAIN: THERAPEUTIC IMPLICATIONS. L. S. Brady, P. W. Gold, M. Herkenham, A. B. Lynn, and Harvey J. Whitfield, Jr. Section on Functional Neuroanatomy, Clinical Neuroendocrinology. Branch, NIMH, Bethesda, MD 20892.

Antidepressant agents exert their therapeutic efficacy only after prolonged administration. We used *in situ* hybridization histochemistry to examine the effects of short-term (2 weeks) and long-term (8 weeks) administration of activating antidepressants that are preferentially effective in treating atypical depression–a subtype of depression associated with lethargy and hypersomnia–on the expression of genes encoding stress-responsive, arousal-producing neurotransmitters. Daily administration (5 mg/kg, ip) of the 5-HT reuptake inhibitor fluoxetine, the α_2-adrenergic antagonist idazoxan, and the nonspecific MAO A and B inhibitor phenelzine to rats increased tyrosine hydroxylase (TH) mRNA levels by 70-150% in the locus coeruleus (LC) after 2 weeks of drug and by 71-115% after 8 weeks. The drugs decreased corticotropin-releasing hormone (CRH) mRNA levels by 30-48% in the hypothalamic paraventricular nucleus (PVN). The decreases occurred at 8 weeks but not at 2 weeks. In light of findings that CRH appears to be hypersecreted in depression, the time-dependent decrease in CRH mRNA levels in the PVN may be the common element relevant to the therapeutic efficacy of antidepressant drugs in treating major depression. We propose that the therapeutic efficacy of activating drugs in atypical depression may reflect a capacity to increase the expression of TH mRNA in the LC, an effect that could theoretically be utilized in the screening of pharmacologic agents for the treatment of this disorder.

395.7

DECREASED SEROTONIN (5-HT) SYNTHESIS METABOLISM AND 5-HT1A RECEPTOR DENSITY IN VARIOUS BRAIN REGIONS OF INFLAMMATORY DISEASE SUSCEPTIBLE LEWIS RATS. Philip W.J. Burnet*, Ivan Mefford[1] Craig C. Smith*, George P. Chrousos [2], Philip W. Gold* and Esther M. Sternberg. Unit of Neuroendocrine Immunology and Behaviour, Clinical Neuroendocrinology Branch, NIMH; [1]Clinical Neuroscience Branch NIMH; [2]Pediatric Endocrinology Section, NICHD, Bethesda, MD 20892.

We have previously suggested that susceptibility of the Lewis (LEW/N) rat to inflammatory disease is related to a hyporesponsive hypothalamic-pituitary-adrenal (HPA) axis. In contrast, the histocompatible Fischer (F344/N) rat's relative resistance to inflammatory disease is due to a hyper-responsive HPA axis. Since serotonin (5-HT) is a major stimulator of the HPA axis we have investigated whether the activity of the axis reflects the activity of the central serotonergic system by measuring 5-HT and 5-HIAA levels and 5-HT1A receptor densities in various brain regions of F344/N, LEW/N and outbred Harlan Sprague Dawley (HSD) rats which are the maternal strain for both the LEW/N and F344/N rats. The cortex and hippocampus of F344/N and HSD rats contained a significantly greater density of 5-HT1A binding sites (approx. 40% in cortex and 20% in hippocampus) than these areas from the LEW/N rat. 5-HT1A receptor densities in the striatum, hypothalamus and midbrain did not significantly differ between strains. Basal 5-HT and 5-HIAA levels in the cortex, striatum and midbrain of F344/N and HSD rats were also significantly greater than these areas from LEW/N rats. The density of the 5-HT1A receptor in the hippocampus but not other brain areas, from F344/N, LEW/N and HSD rats increased after adrenalectomy. In general, 5-HIAA levels in most brain areas from all three strains increased after adrenalectomy. 5-HT levels in the same areas from all three strains tended to be lower. Thus, 5-HT synthesis and metabolism and hippocampal 5-HT1A receptor expression are dependent on glucocorticoid levels. We conclude that the activity of the serotonergic system in the LEW/N rat is decreased in parallel to its low HPA axis activity. Which of these is the primary or secondary event is as yet unknown.

395.8

CRH ALTERS THE BINDING OF ALPHA 2-RECEPTORS ON LOCUS COERULEUS NEURONS. G.K. Weiss, R. Hauger, M. Brown, A. Ratner, D. Savage, and K. Lucero. Univ. of New Mexico School of Medicine, Dept. Physiology and University of Calififormia, San Diego, Dept. Psychiatry.

CRH is known to have effects on the noradrenergic neurons within the locus coeruleus (LC) and it is believed that these effects may mediate some of the behavioral responses to stress. We investigated the possibility that the CRH effects on the LC neurons includes changes in the alpha-2, auto-inhibitory receptors. Rats were injected with 25 µg CRH intracisternally once per day for 4 days while under halothane anesthesia (controls injected with saline). A second group was injected with CRH only on the 5th day (controls injected with saline) and both groups were sacrificed 1 hr. later. The brains were quickly removed, frozen, and stored at -80°C. Eight micron sections cut through the LC were mounted on glass slides. Specific binding of the α_2-adrenergic receptor antagoist H3-Idazoxan was measured using in vitro neurotransmitter receptor autoradiography techniques. The 4-day group (sacrificed 24 hrs after the last injection) showed a significant decrease in the number of α-2 receptors within the LC while the single injection group had a significant increase above its control (Scatchard analysis). Other studies of the A-2 region are in progress. It appears from these studies that one of the effects of CRH on the central noradrenergic neurons includes changes in alpha-2 receptors. This could be a direct effect on LC neurons or indirect via other hormones released in response to the CRH or an autoregulatory response to changes in the activity of the LC neurons to the CRH.
Supported by: MBRS RR08139 and NIH NS23262.

395.9

STIMULATION OF HYPOTHALAMO-PITUITARY-ADRENAL (HPA) ACTIVITY BY HYPOTHALAMIC DA NEURONS. B. Borowsky and C.M. Kuhn. Dept. of Pharmacology, Duke University Medical Center, Durham, N.C. 27710.

We have previously demonstrated a role for central DA neurons in the regulation of HPA activity. The administration of the DA uptake inhibitor GBR12909 into the third ventricle (III-V) or the paraventricular nucleus, but not into the lateral ventricle, stimulated ACTH secretion. We now present evidence that this HPA stimulation is mediated by hypothalamic DA neurons. The administration of 6-OHDA into the III-V significantly decreased DA content in the hypothalamus and the caudate and significantly attenuated the ACTH response to GBR12909, administered either i.p. or into the III-V. In contrast, 6-OHDA lesions of the medial forebrain bundle depleted 99% of DA in the caudate but did not decrease DA content in the hypothalamus, and did not significantly attenuate the ACTH response to i.p. GBR12909. Administration of GBR12909 into the III-V did not increase locomotor activity, as compared to vehicle injected controls. Further, examination of [^{3}H]DA uptake in synaptosome-enriched homogenates demonstrated that DA terminals, in the region of the hypothalamus surrounding the III-V, possess GBR12909-sensitive DA transporters. The present findings suggest that an endogenous DA neuronal system terminating in the hypothalamus mediates the effects of stimulants on HPA function and might play a role in the ongoing regulation of HPA function. Supported by NIDA P50 DA05303-01A1.

395.10

ARGININE VASOPRESSIN RESPONSE TO BENZODIAZEPINE WITHDRAWAL: POTENTIAL ROLE IN THE WITHDRAWAL SYNDROME. K.T. Kalogeras, J. R. Glowa,* G. Safos,* H. Bowen,* G. P. Chrousos and P. W. Gold.* CNE, NIMH, and DEB, NICHD, National Institutes of Health, Bethesda, MD 20892.

Arginine vasopressin (AVP) and oxytocin (OT) have been demonstrated to stimulate the pituitary-adrenal axis in a synergistic fashion with CRH, while atrial natriuretic peptide (ANP) inhibits cortisol secretion. Our earlier primate studies have shown that the triazolobenzodiazepine alprazolam suppresses plasma ACTH and cortisol in a dose-dependent fashion, by suppressing the CRH neuron, while chronic alprazolam treatment supresses ANP. More recent studies have demonstrated that alprazolam withdrawal is associated with a rebound elevation of plasma ACTH concentrations that was completely abolished by pretreatment with hydrocortisone. In this study we examined AVP, OT and ANP responses in primates during alprazolam withdrawal precipitated by benzodiazepine receptor blockade with Ro 15-1788, a specific benzodiazepine receptor antagonist. This was done by the administration of an iv bolus of Ro 15-1788 (2 mg/Kg) to 5 non-anesthetized tethered rhesus monkeys, 9 days after the initiation of an iv continuous infusion of alprazolam (2 mg/Kg/day). Plasma AVP, OT and ANP measurements were obtained before, and serially for 3 hours after the administration of Ro 15-1788 in the morning of day 10. Ro 15-1788 administration resulted in a profound increase in plasma AVP concentrations from a basal value of 1.5±0.3 pmol/L to a peak value, at 5 min, of 74.6±29.1 pmol/L, ($P<0.01$, ANOVA). No comparable increases in plasma OT and ANP concentrations were observed. In an effort to attenuate the benzodiazepine withdrawal syndrome by suppressing the CRH and AVP neurons a second group of 5 primates received an identical treatment and in addition a simultaneous iv infusion of hydrocortisone (96 mg/m^2/day). While the ACTH response to Ro 15-1788 was completely abolished and the behavioral withdrawal symptoms were significantly attenuated in this group, the AVP response was delayed but not suppressed by hydrocortisone. Plasma AVP concentrations increased from a basal value of 2.1±1.1 pmol/L to a peak value of 78.7±33.9 pmol/L, ($P<0.01$, ANOVA), 30 min after the administration of Ro 15-1788. We conclude that benzodiazepine withdrawal results in a major activation of the hypothalamic-pituitary-adrenal axis and that an AVP rebound elevation may play a role in the precipitation of the withdrawal syndrome.

395.11

INTERACTION OF THE BENZODIAZEPINE, ADINAZOLAM, WITH CRF IN RAT NEUROINTERMEDIATE LOBES: IN VITRO STUDIES. L.C. Saland, J.A. Carr, A. Samora*, D. Tejeda* and A. Rael*, Dept. of Anatomy, Univ. of New Mexico Sch. Med., Albuquerque, NM 87131.

Corticotropin-releasing factor (CRF) stimulates release of proopiomelanocortin (POMC) peptides, beta-endorphin (END) or alpha-melanocyte stimulating hormone (MSH) from rat neurointermediate lobes (NILS) in vitro. CRF-induced peptide release is significantly reduced by dopamine (Saland et al, '88, Neuropeptides 12: 59) or by the CRF-antagonist, alpha-helical CRF (Saland et al, '91, Neuropeptides, in press). The benzodiazepine drug adinazolam (ADIN) may modulate hormones which affect anxiety by suppressing CRF effects. Here, we examined the ability of ADIN to modulate CRF-induced END release from incubated rat NILS. Adult male Sprague-Dawley rats were ether anesthetized, rapidly decapitated, and the NILS incubated in vitro for 90 minutes with CRF (10^{-7}M), ADIN (10^{-10}-10^{-6}M) alone, or CRF followed by ADIN. Aliquots of media were taken for radioimmunoassay of END at 15 minute intervals. At 90 minutes, NILS were fixed and processed for light or electron microscopy (EM). Adinazolam added at 30 minutes suppressed the CRF-induced release of END, while ADIN alone showed non-specific modulation of END release. EM showed well-preserved tissue at the 90 minute end point. Adinazolam may act at the level of the pituitary to suppress "stress hormone" release induced by CRF. Supported by The Upjohn Co., NIH NS 21256, GM 08139(LCS) and NS 08447 (JAC).

395.12

INCREASED HYPOTHALAMIC [3H]FLUNITRAZEPAM BINDING IN HYPOTHALAMIC-PITUITARY-ADRENAL AXIS HYPORESPONSIVE LEWIS (LEW/N) RATS. Craig C. Smith*, Elizabeth Hauser*, Nicole K. Renaud*, Amy Leff*, Sofia Aksentijevich*, George Chrousos[1], Ronald L. Wilder[2], Philip W. Gold, and Esther M. Sternberg, Unit on Neuroendocrine Immunology and Behavior, Clinical Neuroendocrinology Branch,NIMH; [1]Pediatric Endocrinology Branch, NICHD; [2]Arthritis and Rheumatism Branch, NIAMS, NIH, Bethesda, MD 20892.

We have previously shown that relative LEW/N rat susceptibility and Fischer (F344/N) rat resistance to inflammatory disease, is related to deficient glucocorticoid counter-regulation of the immune response resulting from deficient corticotropin releasing hormone (CRH) responsiveness to inflammatory and other stress mediators. The GABA/benzodiazepine (BNZ) receptor complex is an important negative modulator of CRH responsiveness. We have examined *in vitro* binding of [3H]flunitrazepam to hypothalamic membrane preparations from LEW/N and F344/N rats. LEW/N rats had significantly more hypothalamic BNZ binding sites than F344/N rats, but there were no differences in BNZ binding affinities (Kd) between these two strains. Differences in BNZ receptor number were consistent with the respective plasma corticosterone levels in the two strains, and with previous studies indicating a negative correlation between corticosterone levels and BNZ number. BNZ binding in adrenalectomized (adx) F344/N rats increased to levels comparable to non-adx LEW/N rats, but remained unchanged in adx LEW/N rats. These findings suggest that basal BNZ receptor differences between these strains may be secondary to strain differences in corticosterone levels. Since BNZs attenuate hypothalamic CRH secretion through GABAergic inhibition, we suggest that strain differences in receptor number could therefore also augment strain differences in HPA axis function through differential sensitivity to GABA mediated feedback.

395.13

DIFFERENTIAL EFFECTS OF REPEATED OR CONTINUOUS EXPOSURE TO COCAINE ON THE RAT HYPOTHALAMO-PITUITARY-ADRENAL AXIS. German Torres and Catherine Rivier, The Salk Institute, The Clayton Foundation Laboratories for Peptide Biology, San Diego, CA 92186.

Acute exposure to cocaine is known to enhance the secretion of ACTH and corticosterone secretion, but the endocrine consequences of chronic administration of the drug are less well documented. We therefore compared the effects of repeated (1 x day) iv cocaine injections (5 mg/kg) for 6-days via implanted jugular catheters with those of continuous exposure via osmotic pumps (5 mg/kg/day), also for 6-days. ACTH and corticosterone levels were assessed by RIA. Repeated, daily injections of cocaine produced consistent increases in plasma ACTH and corticosterone levels 30 min after the challenge. This rise in hormonal secretion was maintained throughout the 6-days of treatment. In contrast, continuous exposure to cocaine failed to alter basal levels of ACTH and corticosterone, suggesting de-sensitization of the HPA axis to the constant presence of the drug. To investigate whether this lack of response was due to physiological alterations at the hypothalamic or pituitary level, rats implanted with osmotic pumps were acutely challenged with either CRF (5 ug/kg) or cocaine (5 mg/kg) 6-days after continuous infusion of the drug (1 ul/hr). Although both pharmacological treatments enhanced ACTH secretion, such increase appeared to be altered suggestive of tolerance in some component of the rat HPA axis. (Supported by DA 05602)

395.14

FUNCTIONAL ANTAGONISM OF MINERALO- AND GLUCOCORTICOID RECEPTOR-MEDIATED RESPONSES TO CORTICOSTERONE IN HIPPOCAMPUS E.R. de Kloet[1], H. Karst[2] and M. Joëls[2]
[1]Center for Bio-Pharm. Sci., Leiden Univ., The Netherlands
[2]Dept. of Exp. Zool., Univ. of Amsterdam, The Netherlands

Pyramidal neurons in the rat hippocampal CA_1 area contain mineralocorticoid (MRs) and glucocorticoid receptors (GRs), which bind the adrenal steroid hormone corticosterone (B) with different affinity. In previous studies we found that a 20-min application of B to hippocampal slices of adrenalectomized (ADX) rats suppressed the hyperpolarization of CA_1 neurons induced by $5HT_{1A}$ receptor stimulation. This effect of B on the 5HT-induced hyperpolarization appeared to be mediated by MRs and involves protein synthesis. Here we report the interaction between MR- and GR-mediated B effects on $5HT_{1A}$ responses: (i) Short GR activation with the selective agonist RU 28362 blocked the attenuating action of the MR agonist aldosterone on the response to 5HT; RU 28362 alone was not affective. (ii) Low steady state concentrations of the mixed agonist B (0.5 nM, at the k_D of MR) suppressed the 5HT response with a delay of 2 h, while a high concentration of B (5 nM, at the k_D of GR) resulted in a transient reduction. (iii) Neurons of moderately stressed rats, which probably have occupied both MR and GR, did not differ from ADX in their 5HT responses.

Summarizing, the data suggest that when the concentration of B rises, occupation of GR results in a functional antagonism of MR-mediated cellular responses. Accordingly, the MR-mediated attenuation of 5HT responses may be limited to conditions of low adrenocortical activity or pathophysiological conditions where the balance of MR- and GR-mediated effects is disturbed.

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395.15

INTERACTIONS BETWEEN CANNABINOIDS AND STEROIDS IN RAT HIPPOCAMPUS. J.C. Eldridge[1], H-Y Hu*[1], P.J. Extrom[1]* and P.W. Landfield[2]. *Department of Physiology and Pharmacology, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC 27157-1083[1], and Department of Pharmacology, University of Kentucky School of Medicine, Lexington, KY 40536[2].*

In previous papers, we have examined hypotheses that cannabinoids (THC) and corticosterone (CORT) share common loci of action in brain. Administration of THC induced degenerative changes in rat hippocampus resembling those seen with high CORT administration or during normal aging (Landfield, *et al., Brain Research 443*, 1988). We have also reported that THC competes against CORT binding to cytosolic receptors in hippocampus (Eldridge and Landfield, *Brain Research 534*, 1990). With a recent description of binding sites for CP-55,940 (CP), a synthetic cannabinoid, in neuronal membranes (cf. Howlett, *et al., Trends Neurosci. 13*, 1990) we have broadened our studies to include possible interactions between steroids and THC at both sites. [3H]-CP binding was very high in rat hippocampal membranes (K_d = 87 pM, B_{max} = 3000 fmol/mg protein), at levels comparable to whole cerebellum (K_d = 98 pM, B_{max} = 2800 fmol/mg). Injection of adrenalectomized rats with 1 mg CORT per day increased hippocampal CP binding modestly (25%) after 2 days, but decreased binding 50% after 4 days. No effect was seen in cerebellum. Addition of CORT or "neurosteroids" (e.g. alphaxalone and α-pregnanolone) that have gained much recent attention (cf. Baulieu and Robel, *J. Steroid Biochem. 37*, 1990) to hippocampal membranes induced only a slight alteration of CP binding properties *in vitro*. In other experiments, [3H]-CP binding was observed in hippocampal cytosol (45 fmol/mg protein). Binding was displaced by 100-fold molar excess of unlabeled CP but was not by CORT, up to 10,000-fold excess. Furthermore, excess unlabeled CP could not displace [3H]-CORT binding in hippocampal cytosol.

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395.16

REGULATION OF THE IMMEDIATE-EARLY GENES C-FOS, C-JUN AND JUN B IN RAT ADRENAL GLAND. Å. Dagerlind[1], M. Pelto-Huikko[1,3], J. Koistinaho[3], J. M. Lundberg[2] and T. Hökfelt[1]. Dept. of [1]Histology and Neurobiology and [2]Pharmacology, Karolinska Institute, S-104 01 Stockholm, Sweden and [3]Dept. of Biomedical Sciences, Univ. of Tampere, SF-33101 Tampere, Finland.

Several factors interacts with specific elements in the promotor region of a target gene, thus regulating its mRNA synthesis. We have used in situ hybridization to study the expression of three such factors, c-fos, c-jun and junB mRNA in the rat adrenal gland. Control rats, showed very low levels of c-fos and junB mRNA in the cortical layers fasciculata and reticulata, whereas no detectable labelling was seen in the zona glomerulosa. Low levels of both c-fos and junB mRNA were detected in the medulla. In contrast, the levels of c-jun mRNA were high in zona fasciculata and reticulata, whereas no labelling was found in the zona glomerulosa with no or low levels in the medulla. Capsaicin (CAPS) treatment increased the levels of c-fos and junB mRNA especially in the zona glomerulosa, whereas all three mRNAs were increased in the medulla. After denervation of the splanchnic nerve, no levels of c-fos mRNA were found in the medulla. Denervation did not effect c-jun mRNA levels. When denervated rats were given CAPS, the increase in c-fos and c-jun mRNA seen in the medulla after CAPS treatment was totally abolished. Nicotine treatment induced a rapid increase of both c-fos mRNA and c-jun mRNA especially in the adrenal medulla. Hypophysectomy did not affect the levels of c-fos or c-jun either in the medulla or cortex. However, administration of adrenocorticotropic hormone (ACTH) increased both c-fos and c-jun in the zona glomerulosa. These results indicate that Fos and Jun, although differentially regulated according to time course, magnitude and regional distribution, may play important roles in the regulation of several genes in the adrenal gland. However, especially in the zona glomerulosa, JunB seems to cooperate with Fos. In conclusion we propose that c-fos and c-jun and probably JunB in the medulla are regulated mainly through neuronal activity via the splanchnic nerve, whereas in the adrenal cortex mainly by humoral factors.

395.17

FOS EXPRESSION IN CORTICOTROPHS OF THE RAT ANTERIOR PITUITARY. G. Kasof and S. A. DeRiemer. Dept. Biological Sciences, Columbia University, New York, NY 10027.

We are investigating factors leading to the induction of the protein product of the proto-oncogene *c-fos* in the adenocorticotropic hormone (ACTH) secreting corticotrophs. The almost universal elevation of Fos in nerve and endocrine cells suggests a possible role of Fos in either short or long term regulation of secretion. The gene for Fos contains response elements that are induced by Ca^{++}, Protein Kinase C (PKC), and cAMP. We are examining the effect of known secretagogues which act through these second messengers on Fos expression. In order to more effectively examine Fos in the corticotrophs, a 4-fold enrichment of these cells has been obtained using a continuous Percoll gradient. Localization of Fos in this enriched culture was performed by immunocytochemistry using a polyclonal antibody corresponding to a.a. 2-20 (CRB). Western blots of anterior pituitary homogenates confirm the specificity of the antibody for Fos and its ability to be blocked by the peptide to which the antibody was raised. In all cases Fos-like immunoreactivity was seen in both the cytosol and nucleus. There is a significant increase in Fos immunofluorescence following the depolarization of these cells with high external K^+. This stimulation leads to a rise in intracellular Ca^{++} as detected with fluo-3/AM. Fos staining also increased in response to the PKC activator TPA(100nM). Corticotropin-releasing factor (CRF,100nM), which is known to elevate cAMP in corticotrophs, increased Fos immunoreactivity in a number of cells equalling the calculated percentage of corticotrophs in the culture. We are currently working on getting a pure fraction of corticotropes by flow cytometry which will be used to further investigate the factors necessary for Fos induction and its possible role in ACTH secretion.

Supported by a Klingenstein Fellowship to SAD

CARDIOVASCULAR REGULATION: BRAINSTEM MECHANISMS II

396.1

MODELING REVEALS MECHANISMS OF CENTRAL COMPUTATION IN THE BARORECEPTOR VAGAL REFLEX. E.B. Graves*, J.S. Schwaber, J.F.R. Paton, K.M. Spyer†, and W.T. Rogers. Neural Computation Group, E.I. duPont Co., Wilmington, DE 19880-0352 and †Royal Free Hospital School of Medicine, London NW3 2PF.

Computational modeling provides a framework for understanding function in neural systems. We are creating a model of the baroreceptor vagal reflex. This system performs nonlinear dynamical multivariate control, and thus is ideal for comprehending underlying mechanisms for use in artificial systems. Previously (Rogers et. al., Soc. Neurosci. Abs. 16:219,1990) we reported a model of primary baroreceptor afferents which showed how pressure information is encoded and transmitted to the brain. The present work extends this to illustrate how central neurons transform this code by virtue of their intrinsic membrane properties. Model 2^{nd}-order baroreceptor neurons are constructed from modified channel kinetics which account for membrane potential trajectories observed *in vitro;* including delayed response, spike rate accommodation, rebound depolarization and state-dependent response. Baroreceptor afferents are connected to these central neurons with excitatory ligand-gated synapses. Blood pressure recordings drive afferent activity, which in turn modulates central neuronal activity. Resulting patterns are studied in relation to their inputs in order to determine the transformation that is computed centrally. Regarding the 1^{st}-order/2^{nd}-order processing, consequences of different patterns of afferent convergence and timing are being explored in simulation. Our preliminary results indicate the transformation of strongly correlated burst activity on the afferents into low-rate activity of 2^{nd}-order cells due to their intrinsic membrane properties. We are now exploring the significance of population responses for parallel processing.

396.2

ROSTRO-CAUDAL TOPOGRAPHY OF CARDIAC VAGAL INNERVATION IN THE RAT *J.A. Escardo*, J.S. Schwaber, J.F.R. Paton and R.R. Miselis, Neural Computation Group, DuPont Co., Wilm., DE 19880, & U. Penn., Phila., PA.*

Cholera Toxin-Horseradish Peroxidase was injected into four distinct cardiac sites: 1) between the superior vena cava and the aorta; 2) between the aorta and pulmonary artery; 3) at the junction of the aorta and the heart, and; 4) at the crossing of the jugular vein, left ventricle and pulmonary artery. Only the first and fourth sites displayed consistent and abundant retrograde and anterograde transport. Analysis in transverse, sagittal, and horizontal planes revealed that labeled cardiac preganglionic cells typically extend from +1.44mm to -1.68mm with ref. to the obex. Cells were present bilaterally in three distinct fields: 1) scattered cells in the dorsal motor nucleus of the vagus nerve (DMV) from +1.20mm to -1.92mm with ref. to the obex; 2) a dense, rostral group of up to 126 multipolar, stellate cells in the rostral ventrolateral nucleus ambiguus from +1.44mm to +0.48mm, and; 3) a caudal group of up to 123 larger neurons between -0.12mm and -1.68mm distributed in the field between the DMV and the NA. These caudal neurons are often closely packed and have large, tortuously shaped,often intertwined dendrites. While the first injection site preferentially labeled the caudal group, the fourth site exhibited a bias for the rostral cells.

Cardiac afferents are distributed in a continuous column within the NTS from obex to -3.2mm. Caudally, afferents distribute within the medial commissural NTS, and extend ventrally half way to the central canal. More rostrally, the terminal field migrates to include only the dorsal tier of the commissural NTS. Then, at levels of the obex, where labeling is most dense, it spreads laterally and away from the commissural subnucleus into the dorsal subnucleus capping the Tractus Solitarii dorsally and dorsomedially. This distribution appears to largely overlap that of other cardiovascular inputs, specifically that of the aortic nerve. The rostro-caudal range of aortic afferents is less extensive and is shifted somewhat rostrally, extending from +0.2mm to -1.52mm.

396.3

AUTO-ACTIVITY IN THE CARDIOVASCULAR NUCLEUS TRACTUS SOLITARII (NTS): ELECTROPHYSIOLOGICAL, NEUROPHARMACOLOGICAL AND IMMUNOCYTOCHEMICAL ANALYSIS. J.F.R. Paton, J.M. Bradd*, W.T. Rogers & J.S. Schwaber. Neural Computation Group, DuPont Co.,Wilmington, DE19880-0352.

We previously reported neuronal rhythmic activity (~5Hz) in rat brainstem slices localized to the cardiovascular NTS (Paton et al., Soc. Neurosci. Abs. 16:219,1990). This region contains a significant part of the A2 aminergic cell group. In the present study we further characterized these neurons by 1) assessing the importance of glutaminergic synaptic drives for their activity *in vitro*, and 2), testing for the presence of tyrosine hydroxylase (TH). Application of glutaminergic blockers (APV; 0.5mM) or kynurenic acid (1.0mM) produced two distinct types of response. In half of the cells ongoing discharge was either reduced by 0.5 Hz or unaffected, and these are referred to as auto-active cells (AA). In the other cells rhythmic activity was abolished and these are characterized as synaptically driven neurons (SD). During glutaminergic blockade the excitatory response to topically applied glutamate (1mM) and the synaptically evoked response following electrical stimulation of the solitary tract (2-40μA) were abolished. In addition, AA cells maintained their rhythmicity during exposure to cobalt chloride (5 mM). Characterized AA and SD neurons were labeled iontophoretically with 10% lucifer yellow (-2.4nA for 10mins) in the slice and subsequently reacted immunocytochemically for TH. Our preliminary data suggests that both AA and SD neurons appear co-localized with a population of NTS neurons containing TH. Consequently, we are investigating the possibility that either AA or SD neurons belong to the A2 cell group. In summary, the data reveal two populations of rhythmically active cells in the cardiovascular NTS; one intrinsically active and, the other requiring glutaminergic drives for its activity.

396.4

PROJECTIONS OF THE NUCLEUS RETICULARIS PARVOCELLULARIS TO CARDIOVASULAR AREAS OF THE MEDULLA OBLONGATA IN THE RAT. L.D. Mitchell and M.A. Nathan, Depts. of Physiology and Pharmacology, CUNY Med. Sch., New York, NY 10031.

Central control of the circulation by the nucleus tractus solitarii (NTS), rostral ventrolateral medulla (RVLM) and caudal ventrolateral medulla (CVLM) is well documented (Ciriello et al., Brain Res. Rev., 11:359-391, 1986). However, the importance of the nucleus reticularis parvocellularis (NRP) in cardiovascular control is less understood and controversial. Kumada et al. reported that lesions of the NRP substantially decreased mean arterial pressure (MAP) in the rabbit (Circ. Res., 45:63-70, 1979). In contrast, we found that lesions of the NRP did not decrease MAP in the rat, but that combined lesions of the NRP and RVLM caused profound hypotension (Cochrane & Nathan, The FASEB J., 2:A1488, 1988). We also noted that lesions of the RVLM generally eliminated pressor responses normally caused by electrical stimulation of the NRP. In the current study, we investigated the possible anatomical projection of the NRP to the RVLM and other cardiovascular areas of the medulla oblongata.

Projections of the middle third of the NRP (about 10.3 mm caudal to bregma) were studied in Long-Evans rats. A micropipette (20-30 μM tip) was used to inject HRP-WGA (Sigma) into the NRP (1% in 0.5 M NaCl, 12.5-25.0 nL). Survival times after the injections were 24-48 hrs. The tissue was processed according to the method of Gibson et al. (Brain Res., 298:235-241, 1984).

We found that the NRP projected to the midline raphe, contraleral NRP, facial nucleus and the IXth cranial nerve, which agrees with earlier observations of Ruggiero et al. (J. Comp. Neurol., 206:278-292, 1982). In addition, we found dense anterograde ipsilateral projections and lighter contralateral projections to the NTS. A few retrogradely labeled cells were seen in the NTS. We also found labeled terminals in the RVLM. Some terminal labeling was found in the CVLM.

This study demonstrates that cardiovascular control by the NRP may involve projections to the NTS, RVLM and CVLM, areas importantly involved in the central regulation of the circulation.

(Supported by grants from the New York City affiliate of the American Heart Association and the CUNY Research Foundation.)

396.5

CARDIOVASCULAR SYMPATHOINHIBITORY CELLS FORM AN EXTENDED LONGITUDINAL COLUMN IN CAT MEDULLA. C.W. Dempesy, D.E. Richardson, and C.J. Fontana*. Lab. of Neurosurgery, Tulane Univ. School of Medicine, New Orleans, LA 70112.

Sympathetic depressor cells have been shown in rat and rabbit to be located in the caudal ventrolateral medulla, just posterior to the plane of the obex. A similarly located area has been assumed to exist in cat, based on cardiovascular depression observed during stimulation of the caudal ventral surface of the medulla (Guertzenstein and Lopes, J. Physiol. 347:345, 1984). However, the sympathoinhibitory area we have reported for cat (Neurosci. Abst. 14: 92, 1988) lies quite anterior to the plane of the obex, in a location dorsal to the rostral ventrolateral medulla. We now report that a survey using glutamate stimulation of the medullary region joining these rostral-dorsal extremes reveals a columnar distribution of cardiovascular sympathoinhibitory cells extending from -1 to +3 mm with respect to the plane of the obex, at laterality 3 to 4 mm and depth -7 to -9 mm (re the cat brain stem atlas of Berman). The greater density of these cells lies in the anterior half of this column. Chemical inhibition of any segment of this column yields hypertension, tachycardia, and partial loss of baroreflex, with muscimol providing greater blocking effectiveness than kynurenic acid.

396.6

CARDIAC ACCELERATION AND MUSCLE CONTRACTION IN THE DECEREBRATE CAT. P.N. McWilliam* and S.E. McMahon* (SPON: European Neuroscience Association). Dept. of Cardiovascular Studies, University of Leeds, Leeds, LS2 9JT, U.K.

The immediate cardiac acceleration at the onset of exercise is mediated by a withdrawal of vagal tone. In contrast, animal studies involving hindlimb contraction elicited by ventral root stimulation have reported only sympathetically mediated increases in heart rate and the latency of these responses has led to the conclusion that a 'central' rather than a 'peripheral' signal is responsible for the immediate cardiac acceleration in the intact organism. The aim of this study was to determine if muscle contraction elicited by ventral root stimulation would lead to an immediate tachycardia mediated by a withdrawal of vagal tone. Brief hindlimb contractions were elicited by electrical stimulation of L7 ventral roots (50 Hz), in cats decerebrated under halothane anaesthesia. With low vagal tone a 5 s contraction significantly reduced R-R interval from 359 ± 25 (mean ± SE, n=8) to 336 ± 24 ms ($P < 0.005$). When vagal tone was increased this response was enhanced ($P < 0.001$, n=8, paired t-test), with contraction reducing R-R interval from 474 ± 45 to 419 ± 47 ms ($P < 0.001$). The minimum latency between the onset of L7 ventral root stimulation and the end of the first shortened R-R interval was just 687 ± 29 ms (n=5). Atropine (0.4 mg kg^{-1}, I.V) prevented a 5 s contraction from producing any change in R-R interval. These results show that 'peripheral' signals, i.e. afferent impulses originating from receptors in contracting muscle, do contribute to the initial vagally mediated heart rate response in the intact organism at the start of exercise.

396.7

RESPONSES OF LATERAL TEGMENTAL FIELD NEURONS IN THE CAT MEDULLA TO STATIC MUSCULAR CONTRACTION. G.A. Iwamoto and T.G. Waldrop. Depts. of Physiology & Biophysics and Veterinary Biosciences, Univ. of Illinois, Urbana, IL 61801

Prior studies have shown that neurons in the ventrolateral medulla are involved in mediating the cardiovascular responses to static muscular contraction; this area is known to have connections with sympathoexcitatory neurons in the lateral tegmental field (LTF). The purpose of the present study was to determine if static muscular contraction alters the discharge frequency of neurons in the LTF. Single unit responses of LTF neurons to static contraction of hindlimb muscles (evoked by stimulation of the L_7 and S_1 ventral roots) were evaluated in anesthetized cats. Computer averaging analyses were used to examine the basal discharge of the studied neurons relative to cardiovascular (cardiac cycle and/or sympathetic nerve discharge) or respiratory (phrenic nerve discharge) activity. Muscular contraction elicited increases in the discharge rate of 59% of the LTF neurons studied; 74% of these neurons had a basal discharge correlated temporally with cardiovascular activity. Thirty-four percent of the LTF neurons had a discharge related to phrenic nerve activity; only ten percent of the neurons with an inspiratory-related discharge were stimulated by muscular contraction. Baroreceptor stimulation (produced by a phenylephrine-induced increase in arterial pressure) elicited a decrease in the firing rate in 58% of the neurons. These results indicate that cardiovascular neurons in the LTF are excited by static contraction of hindlimb muscles. Thus, the lateral tegmental fields may be part of the supraspinal circuitry involved in regulating cardiovascular responses during exercise (Supported by NIH 06296; American Heart Association).

396.8

BARORECEPTOR REFLEX FUNCTION IN CHRONIC RVLM-LESIONED SPONTANEOUSLY HYPERTENSIVE RATS (SHR). E.C. Vasquez, S.J. Lewis, K.J. Varner and M.J. Brody. Dept. Pharmacology & Cardiovascular Ctr., Univ. Iowa, Iowa City, IA 52242.

Lesion of rostral ventrolateral medulla (RVLM) produces a marked hypotension in conscious SHR. This study examined the effects of these lesions on baroreceptor reflex function in conscious SHR. Lesions were produced via bilateral microinjections of 30 nmol/200 nl of N-methyl-D-aspartic acid (NMDA). Baroreceptor reflex function was assessed in conscious rats by measuring reflex-mediated changes in heart rate elicited by graded i.v. doses of phenylephrine and sodium nitroprusside. Reflex function was analyzed using exponential curve fitting analysis. One day post-lesion, the RVLM-lesioned rats showed a significant hypotension in comparison to sham rats (98±6 vs 175±5 mmHg) and was still observed 15 days post-lesion (149±5 vs 173±7 mmHg). The baroreceptor reflex gain in the lesioned rats was significantly reduced when compared to sham rats (-2.2±0.3 vs -4.1±0.5 bpm/mmHg) 1 day post-lesion, but completely recovered 6 days post-lesion. These data suggest that RVLM neurons play an important role in baroreceptor reflex function. Recovery of baroreceptor reflex function may reflect increased activity in other spinal or supraspinal networks. (Supported by HL-14388 & 44546)

396.9

LESION OF NEURONS IN ROSTRAL VENTROLATERAL MEDULLA (RVLM) ALTER CARDIOPULMONARY REFLEXES IN SPONTANEOUSLY HYPERTENSIVE RATS (SHR). K.J. Varner, E.C. Vasquez, S.J. Lewis and M.J. Brody. Dept. Pharmacology & Cardiovascular Ctr., Univ. Iowa, Iowa City, IA 52242

We previously reported that chronic N-methyl-D-aspartic acid (NMDA)-induced lesion of neurons in RVLM causes profound hypotension in conscious SHR. The aim of the present study was to examine the effects of these lesions on cardiopulmonary reflex function in conscious SHR. Lesions of RVLM were produced by bilateral microinjections of 30 nmoles (200 nl) NMDA. Cardiopulmonary reflex function was tested by intravenous (iv) injection of 5-hydroxytryptamine (5-HT). One day post lesion, low doses of 5-HT (1 μg/kg) elicited reflex tachycardia (49±9 bpm) in sham and bradycardia (-53±22 bpm) in lesion rats. The Bezold-Jarisch reflex (hypotension and bradycardia) elicited by higher doses of 5-HT (2.5-20 μg/kg) was significantly enhanced in lesion rats as compared to sham rats (heart rate: -53% vs -26%; mean arterial pressure: -28% vs -18%). No significant differences in reflex function were observed between lesion and sham rats 6 to 15 days post lesion, even though arterial pressure in the lesion rats was significantly reduced through day 15. These data indicate that neurons in RVLM 1) are responsible for the reflex tachycardia elicited by low doses of 5-HT, and 2) inhibit cardiopulmonary heart rate reflex responses to iv 5-HT. (Support HL-14388 & 44546)

396.10

MEDULLARY SITES MEDIATING SYMPATHETIC RESPONSES FROM THE PARABRACHIAL NUCLEUS IN THE RAT. H. Herbert[1], S.J. Chen[2], and D.F.Cechetto[2]. [1]Univ. of Tübingen, Dept. Animal Physiol., D-7400 Tübingen, FRG; and [2]Robarts Res. Inst., Univ. of Western Ontario, London, Ontario N6A 5K8.

The pathway mediating the prominent pressor response elicited by electrical or chemical stimulation of the parabrachial nucleus (PB) is unknown. We injected the calcium channel blocker cobaltous chloride (COB) which reversibly blocks synaptic transmission into presumed relay sites in the ventrolateral medulla to determine their role in mediating this response. Arterial blood pressure (AP), heart rate and sympathetic (renal) nerve discharge (SND) were continuously monitored in chloralose-anesthetized rats.

Pressor sites in the PB were electrically or chemically (D,L-homocysteic acid; DLH) stimulated before and after bilateral injections of 300 nl COB (10 mM) into the ventrolateral medulla (VLM) at different rostrocaudal levels. COB injections into the rostral VLM (rVLM; 1.5 to 2.5 mm rostral to obex) blocked both the AP and SND increases. The blockage had essentially disappeared by 10 min after the COB injection. COB injections rostral or caudal to the rVLM did not significantly affect the AP and SND responses. Bilateral lesions of the rVLM with the excitotoxin kainic acid (KA; 50 mM, 50 nl) permanently blocked the PB pressor response. DLH injections into the effective areas in the rVLM, prior to COB or KA injections, characterized them as potent pressor sites.

The pressor response originating in the lateral PB is relayed to sympathetic preganglionic neurons in the spinal cord via the pressor region in the rVLM. This suggests that the PB provides an excitatory drive to rVLM pressor cells. (Supported by the Heart and Stroke Foundation of Ontario)

396.11

SOMATOSENSORY AND AUDITORY NUCLEI PROJECT TO A DISCRETE SUBREGION OF THE ROSTRAL VENTRAL MEDULLA IN RAT. Y. Zhu, E.J. Van Bockstaele, H. Akaoka, P.H. Luppi[1], G. Luthin[2] and G. Aston-Jones. Hahnemann Univ., Dept of Mental Health Sciences, Philadelphia, PA 19102, [1] CNRS UA 1195, INSERM U52, Lyon, France, [2] Hahnemann Univ., Dept. of Physiology, Phila. PA 19102.

The rostral pole of the nucleus paragigantocellularis (PGi), termed juxtafacial PGi, lies throughout the medial extent of the facial nucleus. This narrow region of the ventral medulla contains many bulbospinal neurons which have been implicated in pain processing, cardiovascular regulation and respiratory related activities. Previous studies, in cat, examining afferents to and efferents of the juxtafacial PGi indicated that somatosensory and auditory structures, such as the dorsal column nuclei and inferior colliculus, project to the juxtafacial PGi and that neurons in this area project to the posterior nucleus of the thalamus. Although certain studies in the rat have examined afferents to the PGi, the juxtafacial PGi has not been examined in detail for its afferents and efferents. Therefore, in the present study, discrete pressure microinjections of a colloidal gold complex of wheat germ agglutinin- conjugated to inactive horseradish peroxidase were placed into the juxtafacial PGi and retrogradely labeled neurons identified throughout the brain. Anterograde transport from selected brain areas was also used to confirm and further detail the innervation patterns of the juxtafacial PGi from its afferents. The present results reveal that the juxtafacial PGi receives input from a variety of somatosensory and auditory nuclei such as dorsal column nuclei, supraolivary reticular formation, inferior colliculus and medial geniculate nucleus confirming results seen with other species. There are also afferents from the retrofacial PGi, the caudal medullary reticular formation, the periaqueductal gray and hypothalamic nuclei. These results support a role of the juxtafacial PGi in the integration of somatosympathetic events. Supported by PHS grant 24698.

396.12

DISTINCT POPULATIONS OF NEURONS IN THE SUPRAOCULOMOTOR NUCLEUS OF THE CENTRAL GRAY (SOM) PROJECT TO THE ROSTRAL VENTROLATERAL MEDULLA (RVM) AND ABDUCENS NUCLEUS (Abd) IN THE RAT BRAIN. E. J. Van Bockstaele and G. Aston-Jones. Hahnemann University, Department of Mental Health Sciences, Philadelphia PA 19102.

Retrograde transport studies have indicated that neurons in the SOM area of the rostral ventromedial periaqueductal gray project to the nucleus paragigantocellularis (PGi) in the RVM (Van Bockstaele et al., JCN, 290:561-584, '89). Anterograde tracing studies have confirmed and further detailed this projection (Van Bockstaele et al., JCN, in press), indicating a strong and specific projection to the sympathoexcitatory region of the lateral retrofacial PGi, corresponding to the nucleus rostroventrolateralis. The SOM region has been implicated in oculomotor related activities because neurons in this area have been reported to project to the Abd in the pons. To determine whether SOM neurons projecting to the PGi are part of the same population of neurons innervating the Abd, double retrograde transport experiments were conducted from the PGi and the Abd. Microinjections (50 nl) of fluorescein latex microspheres were deposited into the Abd using glass micropipettes (15 μm tip O.D.) via a controlled pneumatic pressure device. Microinjections (300 nl) of wheat germ agglutinin conjugated to inactive horseradish peroxidase coupled to 15 nm gold particles (WGA-Au) were similarly deposited into the PGi area. Retrogradely labeled neurons were observed either with epifluorescence microscopy for the fluorescein microspheres or darkfield microscopy for the WGA-Au particles. Retrogradely labeled neurons were identified in the contralateral SOM from both nuclei. Labeled neurons from the Abd were found in the more caudal aspects of the ventromedial PAG. Labeled neurons from the PGi were found more rostrally and more dorsally than Abd-projecting neurons. Few doubly labeled neurons were identified in the SOM although these neurons were interdigitated at rostral PAG levels. These results indicate that neurons in the SOM projecting to the PGi are a separate population from those projecting to the Abd and therefore are probably not preoculomotor neurons. Supported by PHS grant NS24698.

396.13

LOCALIZATION OF ANGIOTENSIN II BINDING SITES IN THE DORSAL MEDULLA WITH HIGH RESOLUTION AUTORADIOGRAPHY. E. M. Szigethy, K. L. Barnes, D. J Diz and C. M. Ferrario. Dept. of Brain & Vascular Research, Research Institute, Cleveland Clinic Fndn., Cleveland, OH 44195.

Angiotensin II (Ang II) acts in the dorsal medulla to modulate baroreflex function. Moreover, Ang II binding sites are associated with presynaptic vagal afferent fibers in the nucleus tractus solitarii (nTS) and vagal efferent neurons in the dorsal motor nucleus of the vagus (dmnX). High resolution "wet" autoradiography now documents the relationship of putative Ang II receptors to the cytoarchitecture of these nuclei. Serial 14 μm canine medulla sections were processed for film or emulsion autoradiography with 0.5-1 nM ^{125}I-Ang II. Films were quantitated with ^{125}I-Ang II standards. Fixed sections were coated with emulsion, stored (4°C) for 6-8 weeks, developed and counterstained with cresyl violet. Comparison of film and emulsion images revealed a uniform reduction in labeling after emulsion processing with no alteration in distribution. In the nTS, Ang II binding was restricted to regions with heavy vagal afferent innervation. In the dorsal nTS, label was distributed homogeneously over both small, round cell bodies and neuropil. The highest density of binding capped the dorsolateral solitary tract. In the medial nTS, moderately dense label was concentrated over medium-sized oval perikarya, with scattered grains over the intervening neuropil. Dense labeling was seen over the majority of the large, round to oval perikarya and the surrounding neuropil in the ventral dmnX. A band of label lateral to the dmnX overlaid vagal efferent fibers coursing ventrolaterally to exit the medulla. The discrete subnuclear association of Ang II binding in the dorsal medulla with vagal cells and fibers provides support for our hypothesis that Ang II receptors are present on both afferent vagal fibers and intrinsic medullary neurons. (Supported by NHLBI grants HL-6835 and HL-38535 and the AHA).

396.14

REGIONAL HEMODYNAMIC EFFECTS FOLLOWING CHEMICAL STIMULATION OF THE FELINE AREA POSTREMA (AP). P.J. Gatti. Dept. of Pharmacol., Howard Univ. Col. of Med., Washington, DC 20059.

The purpose of the present study was to determine whether chemical stimulation of the AP would evoke increases in renal (RR) and femoral (FR) arterial resistance. Experiments were performed in chloralose-anesthetized artificially respired cats. Blood flow (ml/min) was measured using a transit time blood flowmeter. Bilateral application of the excitotoxin kainic acid (KA;40 mM) to the AP increased mean arterial pressure by 80 ± 13 mmHg in 5 animals. RR increased by 51 ± 16 % and FR increased by 208 ± 24 %. Effects occurred immediately and lasted 10-15 min. The percent change in FR was significantly greater than the percent change in RR ($p<.05$). A previous study has shown that application of KA to the AP resulted in a 43 ± 13 % increase in coronary vascular resistance (CVR) [Gatti, et al. Brain Res. 448(1988)313-319]. These data indicate that the AP controls blood flow to the kidney, heart and skeletal muscle. However, it appears that its control over skeletal muscle blood flow is greater than kidney and coronary blood flow. This preferential effect is similar to the effect seen following stimulation of the subretrofacial nucleus in the ventrolateral medulla in which FR increased more than RR and superior mesenteric arterial resistance. These data raise the possibility that the AP produces its effects via the ventrolateral medulla. Supported by the American Heart Association/Nation's Capital Affiliate.

396.15

ETHANOL (EtOH) INHIBITION OF BAROREFLEX BRADYCARDIA: ROLE OF MEDULLARY $GABA_A$ AND $GABA_B$ RECEPTORS. K. Varga* and G. Kunos. LPPS, NIAAA, Bethesda MD 20892

The effects of EtOH on baroreflex bradycardia and on cardiovascular responses mediated by $GABA_A$ and $GABA_B$-receptors in the medullary dorsal vagal complex (DVC) were studied in urethane-anesthetized rats. EtOH, 1-3 g/kg administered i.v. or 25-200 nmol microinjected bilaterally into the DVC attenuated the reflex bradycardia elicited by graded i.v. doses of phenylephrine in control animals but not in rats pretreated with 3-mercaptopropionate, a GABA-depleting agent. Muscimol, 10-40 pmol intra-DVC, caused a pressor response and inhibited baroreflex bradycardia. The pressor response to muscimol was potentiated and a tachycardic response to muscimol emerged after intra-DVC EtOH. Baclofen, 20-80 pmol intra-DVC, caused pressor and tachycardic effects and inhibited baroreflex bradycardia. EtOH potentiated the pressor response to a low dose of baclofen. Bicuculline, 10 pmol intra-DVC, inhibited the responses to muscimol but not to baclofen, and reduced the baroreflex inhibitory action of EtOH. 2-Hydroxy-saclofen, 400 pmol intra-DVC, inhibited the responses to baclofen but not to muscimol, reduced the baroreflex inhibitory action of EtOH, and prevented EtOH-potentiation of the responses to muscimol. It is concluded that EtOH inhibits baroreflex bradycardia through the potentiation of the actions of endogenous GABA in the DVC. Both $GABA_A$ and $GABA_B$-receptors are involved in this action of EtOH.

396.16

EVIDENCE FOR A SYMPATHOEXCITATORY FUNCTION OF THE A5 NORADRENERGIC CELL GROUP. **M. B. Hug and J. Ciriello. Department of Physiology, University of Western Ontario, London, Canada, N6A 5C1.**

The contribution of the A5 noradrenergic cell group to the control of the circulation remains controversial. In this study a systematic exploration of the A5 region with microinjections (20nL) of L-Glutamic acid was done to investigate whether excitation of somata was responsible for the pressor responses observed during electrical stimulation. Microinjections of L-glutamic acid (1M) into the A5 region were made in chloralose anaesthetized, paralysed and artificially ventilated male Wistar rats. The ventral surface of the brainstem was exposed using a transpharyngeal approach. Increases in mean arterial pressure (22±-8mmHg) were observed at most (70%) of the sites from which cardiovascular responses could be elicited. At the remaining sites a depressor response (9±-4mmHg) was elicited. Pressor responses were associated with either no changes in heart rate or increases of 16±-9bpm (69% of sites). Depressor responses were primarily associated with decreases in heart rate (8±4bpm; 74% of sites). Pressor responses were consistently elicited from regions which overlapped the A5 noradrenergic cell group, whereas depressor responses were elicited from the dorsal aspects the A5 region and the region immediately dorsal to it. Selective destruction of noradrenergic terminals by intraventricular injections of 6-OHDA significantly attenuated the pressor responses (7±3mmHg). As A5 neurons have been shown to directly innervate the intermediolateral nucleus of the thoracic cord, these data are consistent with the proposed sympathoexcitatory role of A5 somata. (Supported by MRC of Canada and HSFO).

396.17

VENTROLATERAL MEDULLARY PROJECTIONS TO THE PARABRACHIAL COMPLEX. S. Roder and J. Ciriello, Department of Physiology, University of Western Ontario, London, Canada, N6A 5C1.

The ventrolateral medulla (VLM) and parabrachial (PBN) complex have been shown to be involved in central cardiovascular regulation. This study was done to investigate the distribution of fibers and terminals in the PBN complex originating from neurons within VLM in the rat. Fibers and terminals in PBN from the caudal (c) VLM, rostral (r) VLM and intermediate (int) VLM, were identified using the anterograde tracer Phaseolus vulgaris leucoagglutinin (PHA-L). PHA-L was iontophoresed into the cVLM, rVLM, or intVLM, and after a survival period of 7-10 days, transverse sections of the brainstem were processed immunohistochemically for the demonstration of PHA-L or tyrosine hydroxylase contained in fibers, terminals and perikarya. PHA-L labelled cells within the injection sites were observed to overlap the A1 and C1 catecholaminergic cell groups in the cVLM, rVLM or intVLM. PHA-L labelling in the PBN complex resulting from a cVLM injection was observed primarily in the caudal two third of Kölliker-Fuse nucleus (KF), and the external lateral and central subnuclei of PBN, whereas that resulting from a rVLM injection was primarily observed in the middle third of KF and the dorsal lateral subnuclei of PBN. PHA-L labelled fibers and terminals in PBN resulting from an intVLM injection were primarily found in the rostral two thirds of KF, and the external lateral, central lateral, superior lateral and medial subnuclei of PBN. Projections to the PBN complex were observed bilaterally but with an ipsilateral predominance. These data suggest a functional organization of VLM projections to PBN that may be involved in cardio-respiratory integration. (Supported by MRC and HSFO)

396.18

EFFECT OF COMBINED GLUTAMATE AND NEUROTENSIN MIRCOINJECTIONS INTO NUCLEUS AMBIGUUS ON HEART RATE. T. X. Zhang and J. Ciriello, Department of Physiology, University of Western Ontario, London, Canada, N6A 5C1.

The nucleus ambiguus (AMB) is known to contain vagal cardiomotor neurons. In a previous study we have shown that microinjection of neurotensin (NT) into AMB elicits a vagal bradycardia. In this study, the possibility that NT and L-glutamate (Glu) microinjected into AMB (10-25nl) had a synergistic effect on the heart rate (HR) response was investigated. In addition, the effect of NT or Glu microinjections into AMB on the reflex vagal bradycardia elicited during activation of baroreceptors (l-phenylephrine, 10μg/ml, i.v.) was investigated. Experiments were done in alpha-chloralose anesthetized, paralysed and artificially ventilated male Wistar rats that had their spinal cord cut at the level of C2. Microinjections of Glu (10-25nmol) or NT (50-125pmol) elicited decreases in HR of 51$\pm$7 and 31$\pm$7bpm, respectively. Combined microinjections of Glu and NT elicited a decrease in HR (82$\pm$12bpm) that was not significantly different from the algebraic sum of the individual Glu and NT responses. In addition, microinjection of Glu significantly increased (86$\pm$20bpm), whereas NT did not alter (39$\pm$4bpm), the magnitude of the reflex vagal bradycardia elicited during baroreceptor activation. Taken together, these data suggest that Glu and NT act as neurotransmitters or modulators in the baroreceptor reflex pathway to AMB and that NT may exert a selective effect on vagal cardiomotor neurons whereas Glu may also activate other neuronal systems contributing to the reflex response. (Supported by MRC of Canada and HSFO).

396.19

CAROTID BARORECEPTOR PROJECTIONS TO THE NUCLEUS OF THE SOLITARY TRACT IN THE RAT. J. Ciriello, Department of Physiology, University of Western Ontario, London, Canada, N6A 5C1.

The medullary connections of the carotid baroreceptors were studied in the rat using the transganglionic transport of cholera-toxin (CT) or wheat germ agglutinin (WGA) horseradish peroxidase (HRP). A small pledget of 0.1% CT-HRP or 2% WGA-HRP was applied into the adventitia of either carotid sinus after cauterizing the blood vessels and carotid sinus nerve (CSN) branches originating from the carotid body. In control animals 100nl of either solution was injected around the carotid bifurcation after cauterizing CSN branches to the carotid body. After a survival period of 48-72 h the animals were sacrificed and brainstem sections were processed using the tetramethyl-benzidine method. In animals with CT-HRP applications, dense terminal and fiber labelling was observed primarily in the caudal nucleus of the solitary tract (NTS); in the regions of the commissural (Com), medial (Sm), and dorsolateral (Sdl) subnuclei of the complex. Additionally, sparse labelling was observed in the area postrema (AP). In animals with WGA-HRP application, dense fiber and terminal labelling was found in the Sdl and interstitial subnuclei of NTS. Sparse labelling was also found in Sm and in Com at the level of AP. These data have provided the first anatomical demonstration of the termination site of carotid baroreceptor afferent fibers in the medulla of the rat and suggest that different types of afferent fibers originating in the carotid sinus may be selectively labelled with conjugates of HRP. (Supported by HSFO and MRC of Canada).

396.20

CARDIOVASCULAR EFFECTS OF PHOSPHATE BUFFERED SALINE MICROINJECTIONS INTO NUCLEUS TRACTUS SOLITARIUS. S. L. Hochstenbach, T. X. Zhang, M. B. Hug and J. Ciriello. Department of Physiology, University of Western Ontario, London, Canada, N6A 5C1.

The nucleus tractus solitarius (NTS) is known to play an important role in the integration of cardiovascular afferent information. In this study, NTS was systematically explored for cardiovascular responsive sites during microinjections (20nl) of phosphate buffered saline (PBS, pH 7.2) containing 142 mM, 179 mM, 254 mM or 328 mM of NaCl. Decreases in arterial pressure (AP; range of means, 21$\pm$3 to 53$\pm$9 mmHg) and heart rate (HR; range of means, 5$\pm$2 to 40$\pm$8 bpm) were consistently elicited from only the caudal medial subnucleus of NTS in a dose dependant manner. Microinjection of PBS into area postrema did not elicit cardiovascular responses. Administration of atropine methyl bromide had no significant effect on the magnitude of the AP and HR responses. However, administration of hexamethonium bromide abolished both the AP and HR responses. Control microinjections (20nl) into the caudal medial subnucleus of a hypertonic solution of mannitol (400 mM) containing no NaCl of NTS did not elicit cardiovascular responses. These data suggest that within a restricted region of NTS there exists a pool of neurons sensitive to changes in sodium concentrations of the extracellular fluid, and when activated by the sodium these neurons elicit cardiovascular responses as a result of sympathoinhibition. (Supported by MRC of Canada and HSFO).

396.21

FACTORS MEDIATING THE HINDLIMB BLOODFLOW COMPONENT OF THE DEFENCE REACTION AND THEIR SENSITIVITY TO CLONIDINE. L.L. Watkins and W. Maixner. Dept. of Pharmacology and Dental Research Center, University of North Carolina, Chapel Hill, N.C. 27514.

We previously reported that clonidine inhibits the increase in hindlimb bloodflow (HLBF) associated with the cardiovascular defence reaction elicited by periaqueductal gray (PAG) stimulation in the rat (Neurosci.Abs.16:554,1990). The present experiments were designied to examine potential mechanisms contributing to this increase in HLBF and to examine their sensitivity to inhibition by clonidine. First, the role of epinephrine in the HLBF response was evaluated by measuring the effect of β_2-receptor blockade or adrenalectomy on PAG stimulation evoked changes in HLBF. Both β_2-receptor blockade and adrenalectomy mimicked clonidine's inhibitory effect on PAG-evoked increases in HLBF and prevented the inhibitory effect of clonidine on PAG-evoked increases in HLBF. Second, an isolated hindlimb preparation was utilized to examine whether decreased vascular resistance contributes to the HLBF response to PAG stimulation. Blood withdrawn from the left carotid artery was pumped into the rat's left femoral artery at a constant rate and changes in perfusion pressure were used to derive changes in vascular resistance. Rather than producing a decrease in vascular resistance, PAG stimulation produced a marked rise in vascular resistance that was not changed by clonidine. In summary, these results indicate that the HLBF component of the defence reaction is not associated with a neurally mediated decrease in vascular resistance, but rather is more likely due to a fall in hindlimb vascular resistance mediated by an evoked increase in circulating epinephrine or other humoral factors. Furthermore, these data indicate that clonidine's inhibition of this increase in HLBF is possibly associated with inhibition of epinephrine release from the adrenal glands. (Supported by DE08013 & RR05333).

396.22

Chemical And Electrical Stimulation Of The Rostral Ventrolateral Medulla (RVL) Increases Plasma ANF. J.H. Jiao*, P. Guyenet and A.J. Baertschi. Physiol. and Pharmacol. Depts., Univ. of Virginia, Charlottesville, VA 22908

Previous studies indicated that neural reflexes may control ANF secretion (Baertschi et al., AJP, '89,'90). The purpose of the present studies was to investigate whether the rostral ventrolateral medulla (RVL), a source of central sympatho-excitatory drive, can modulate the plasma level of atrial natriuretic factor (ANF). In anesthetized, artificially ventilated rats, electrical stimulations were stereotaxically applied to RVL. Unilateral electrical stimulation of RVL caused a 94 - 186% increase in plasma ANF over baseline ($P < 0.01$, $n = 11$), whereas sham stimulations and stimulations rostral and dorsal to RVL had no effect on plasma ANF. Unilateral microinjections of L- glutamate into RVL of artificially ventilated rats also induced a 81 $\pm$ 23% ($P < 0.01$, n=9) increase in plasma ANF compared to baseline and vehicle control injections. This indicates that cell bodies in the RVL and not fibers of passage mediated the ANF response. Glutamate injections into RVL elicited identical ANF responses in scopolamine-pretreated (n=5) and vagotomized (n=6) artificially ventilated rats, which implies that activation of the parasympathetic system does not stimulate ANF secretion. In contrast, injection of L-glutamate into the caudal ventrolateral medulla (CVL), known to inhibit RVL, elicited a 44 $\pm$ 5% decrease in plasma ANF ($P < 0.05$, n= 6). These results suggest that RVL may regulate cardiac ANF secretion via the sympathetic system. (Supported by AHA grant VA-89-F-9 and VA-90-G-8.)

397.1

HYPOTHALAMIC TRANSPORT OF ANGIOTENSIN-(1-7). C.H. Block and H.Vilsack*. Cleveland Clinic Foundation, Cleveland, OH 44195-5070.

Several studies by our group suggest a close anatomical association of angiotensin (Ang)-(1-7) with the hypothalamic vasopressin system. Specifically, Ang-(1-7) is localized by immunocytochemistry in neurons of the paraventricular (PVN) and supraoptic nuclei. Varicose immunoreactive fibers emanate from the PVN in the paraventriculo-neurohypophysial tract (PVT) and project to the neurohypophysis. In this hypothalamo-neurohypophysial system, anatomical changes suggestive of transport of Ang-(1-7) occur in response to 24 hour-dehydration in a manner similar to vasopressin, and Ang-(1-7) is not visualized in the homozygous Brattleboro rat. The current studies address whether Ang-(1-7) is transported from the PVN to the neurohypophysis. Stereotaxic knife-cuts were placed through the PVT in pentobarbital-anesthetized rats. The animals were perfused transcardially 24, 48, or 72 hours later and brain tissue was processed for immunocytochemical localization of Ang-(1-7).

Unilateral knife-cuts placed lateral to the PVN, severing part or all of the PVT, resulted in fibers engorged with Ang-(1-7) immunoreactivity approximately 20-50 μm distal to the knife-cut. The appearance of the fibers beyond 50 μm was similar to the control side. Ang-(1-7)-neurons were diffusely spread throughout the magnocellular PVN and were decreased in number on the side ipsilateral to the knife-cut after the 72-hour survival period. While Ang-(1-7) immunoreactivity in the neurohypophysis was less intense from control animals, no anatomical changes in the peptide distribution were observed.

The data from this study supports the hypothesis that Ang-(1-7) is transported in the PVT to the neural lobe. However, the possibility that angiotensin precursors are taken up and processed to Ang-(1-7) by the neural lobe cannot be excluded. The lack of changes in the neurohypophysis is not surprising since much of the fiber input arises from the supraoptic nucleus.

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397.2

BAROREFLEX AND VASOPRESSIN RESPONSES IN GUANETHIDINE-TREATED RATS. EE Benarroch, JD Schmelzer*, PA Low. Neurophysiology Lab, Mayo Clinic, Rochester, MN 55905

Guanethidine (Gu) produces chemical sympathectomy in rats (GuSx). We explored baroreflex (BR) and vasopressin (AVP) responses as potential mechanisms for maintenance of arterial pressure (AP) in GuSx rats. Rats (350-450 gr) received Gu (40 mg/kg i.p. for 5-8 weeks) or saline. After 1-4 weeks of treatment, they were cannulated under ether anesthesia for monitoring of AP and heart rate (HR), for IV bolus of phenylephrine (Phe, 0.5-2 ug/kg), and for plasma AVP sampling. In conscious rats, BR was measured as the maximum decrease in HR (ΔHR) in response to increases in AP (ΔAP) induced by Phe. AVP was measured at rest and after 25% blood volume loss. As compared to controls, GuSx showed (a) similar resting AP and AVP (b) decreased BR responses to Phe (Δ HR/Δ AP: -1.10 ± 0.16 vs. -1.89 ± 0.13 beats per min/mmHg, $p<0.01$), and (c) exaggerated AVP response to hypovolemia (Δ AVP: 19.7 ± 2.6 vs. 12.9 ± 1.3 pg/ml, $p<0.05$). In GuSx rats, microinjection of L-glutamate (0.01-100 nmol) into the nucleus of the tractus solitarius (NTS), elicited decreases in AP and HR comparable to controls. Thus (a) GuSx rats have changes in BR and AVP responses which may contribute to maintenance of AP (b) these changes may not be due to altered NTS responsiveness to cardiovascular afferent inputs.

397.3

CENTRALLY ADMINISTERED INSULIN ALTERS PLASMA CATECHOLAMINE LEVELS BUT NOT BLOOD PRESSURE OR HEART RATE. L.E. Ohman and J.R. Haywood. Department of Pharmacology, The University of Texas Health Science Center at San Antonio, San Antonio, TX 78284-7764.

The present studies investigated the effect of insulin delivered centrally on plasma norepinephrine (pNE), and epinephrine (pEPI), blood pressure (BP), and heart rate (HR). Insulin (10 μU/min) or artificial cerebrospinal fluid (aCSF) was infused into the third ventricle of male Sprague-Dawley rats (n=6) for 60 min. Neither BP nor HR were significantly altered by aCSF or insulin. Basal levels of pNE and pEPI did not differ between treatments (aCSF: 96.5 ± 15.7, 83 ± 22.2 pg/ml; insulin: 81.3 ± 16.3, 80.6 ± 15.6 pg/ml). The ventricular infusion of insulin increased pNE 88 ± 59 %, and pEPI by 108 ± 63 % ($p=.07$); aCSF produced a 0.05 ± 11 % increase in pNE, and 27 ± 19 % elevation in pEPI. A second study was performed to localize the site of the sympathoexcitatory action of insulin. Insulin (0.1 μU/min, n=5) or aCSF (5 nl/min, n=4) was bilaterally infused into the paraventricular nucleus (PVN) for 30 min. Again, neither BP or HR were significantly altered by aCSF or insulin. Basal pNE did not differ between aCSF and insulin groups (201.7 ± 44.2; 160.2 ± 19.1 pg/ml), but was decreased by both treatments (aCSF: -30 ± 6.5 %; insulin: -19 ± 11 %). Resting pEPI was similar (aCSF: 166 ± 24.6; insulin: 217.6 ± 42.8 pg/ml), and was slightly altered by aCSF (-13 ± 9 %). Insulin significantly decreased pEPI (-36 ± 2.4 %; $p<.01$). Plasma glucose was slightly elevated by both treatments. These results suggest that insulin delivered into the ventricular system has a sympathoexcitatory effect, but appears to be sympathoinhibitory when administered into the PVN. (Supported by American Diabetes Association)

397.4

Cardiovascular and Behavioral Effects of Fetal Hypothalamic Grafts in Hypertensive and Normotensive Rats C. A. Murphy, B. G. Yongue, J. Cheng and L. Skaredoff. New York State Psychiatric Institute, Columbia University, New York, NY 10032.

Spontaneously hypertensive (SHR) rats exhibit higher blood pressures, heart rates, salt appetite and activity levels, and lower body weights compared to inbred normotensive Wistar-Kyoto (WKY) rats. To examine the suggested involvement of intra-hypothalamic factors in phenotypic cardiovascular and behavioral traits in these strains, we grafted fetal hypothalamic tissue from SHRs and WKYs into the third ventricle of hypertensive and normotensive hosts. Donor tissue was implanted into the antero-ventral 3rd ventricle of male SHR, WKY or outbred Wistar hosts at either 25 or 90 days of age. Systolic blood pressure, heart rate, body weight, and locomotor activity were monitored at 3-4 week intervals following transplantation. At 5 months of age, intakes of water and .9% saline were recorded in all subjects in sodium-replete and sodium-depleted conditions. Implants of SHR hypothalamus produced higher blood pressures at 30 and 60 days of age in Wistar hosts grafted at 25, but not at 90 days of age. This effect did not persist at later ages. Wistar hosts implanted with SHR hypothalamus drank significantly more water than rats receiving SHR cortex or WKY tissue. Need-free salt intake was decreased in Wistar hosts grafted with WKY hypothalamus compared to Wistar hosts grafted with either SHR cortex or hypothalamus. In contrast, WKY hypothalamic or cortical grafts did not differentially affect SHR hosts for any variable tested. Moreover, heart rate, body weight, body temperature and activity level did not vary as function of graft type in any host. These results indicate that blood pressure and fluid-regulating behaviors can be altered by grafted tissue, in a way that is determined not only by the neuroanatomical source of the graft but by genetic factors in both the donor and host as well. We believe that this manipulation will prove useful for studying neural factors controlling consummatory behaviors and cardiovascular regulation. (BRSG 903-E004X, NIMH 00803, NIMH 45951)

397.5

CORTICOSTEROID BINDING IN LIMBIC BRAIN REGIONS OF NORMOTENSIVE AND HYPERTENSIVE SYRIAN HAMSTERS. **X. Chen and B. B. Turner. Department of Physiology, James H. Quillen College of Medicine, East Tennessee State University, Johnson City, TN 37614-0002.**

The Golden Syrian hamster differs from the rat with respect to the distribution of corticosteroid receptor types in limbic brain regions. In this study we used in vitro autoradiography to determine whether differences in either the number of Type I (mineralocorticoid) or Type II (glucocorticoid) receptors in specific brain regions are associated with hypertension in the hamster. Five outbred normotensive and five inbred hypertensive male hamsters were used. Resting blood pressures (hind limb) were 100 ± 4 mmHg for the normotensive and 138 ± 3 mmHg for the hypertensive hamsters. Hamsters were sacrificed (intra-cardiac perfusion) 12 hr after adrenalectomy. Adjacent 20 sections were incubated for 30 min with either 5 nM ^{3}H-corticosterone (B) or ^{3}H-B plus 1.25 μM RU28362; 5 μM B was used for non-specific binding. Sections were exposed for 12 weeks to LKB Ultrofilm. Binding in sixteen hippocampal regions, lateral septum, and central nucleus of the amygdala (Ce) were measured with a MCID image analysis system. Hypertensive hamsters had only approximately one-half the Type I and Type II binding of controls in both the lateral septum and in the region of the Ce. These differences closely approached, but did not reach statistical significance. No differences in binding between normotensive and hypertensive animals were found in any of the hippocampal regions. If these observations are confirmed, the possibility that region specific reductions in receptor number contribute to the development of hypertension will need to be evaluated. Supported by NIH RR05959.

397.6

HYPOTHALAMIC DEFENSE AREA MODULATION OF THE BARORECEPTOR REFLEX IN RABBITS. P.M. McCabe, Y-F. Duan, R.W. Winters*, E.J. Green, and N. Schneiderman. Dept. Psychology, Univ. of Miami, Coral Gables, FL 33124.

Previous work from our laboratory has identified the rabbit hypothalamic defense area (HDA). Electrical stimulation of this region elicits tachycardia, a pressor response, increased hindlimb blood flow, increased ventilatory rate (Markgraf et al., 1991), and escape responses (unpublished observations). Some investigators report that activation of the HDA suppresses both the cardiomotor and vasomotor components of the baroreceptor reflex (BRR), whereas others report inhibition of the cardiomotor component only. The present study assessed HDA modulation of the BRR in the rabbit.

New Zealand albino rabbits were anesthetized with isoflurane, and monopolar stainless steel electrodes were implanted in the HDA. The aortic nerve was then exposed and placed on a monopolar silver hook electrode. The HDA was electrically stimulated with a continuous train (125-300 μA, 100 pps) alone for 10 sec, followed by concurrent stimulation of the HDA and aortic nerve (100-150 μA, 32 pps) for 10 sec. After the HDA stimulation was terminated, the aortic nerve was stimulated alone for 10 sec. Histological examination of the hypothalamus revealed that stimulation sites were in the dorsal posterior hypothalamus, medial to the mammilothalamic tract and the descending column of the fornix, previously defined as the HDA.

Stimulation of the HDA produced tachycardia (+12.3 bpm) and a pressor response (+10.6 mmHG). Concurrent stimulation of HDA and the aortic nerve elicited a bradycardiac/depressor response (-8.2 bpm, -15.4 mmHG), whereas stimulation of the aortic nerve alone led to a larger bradycardiac response (-14.1 bpm) but a smaller depressor response (-10.8 mmHG). The data suggest that HDA stimulation attenuates the cardiomotor component of the BRR, but augments the vasomotor component. (Supported by HL07426 and HL36588).

397.7

SALT-INDUCED HYPERTENSION IN DAHL RATS IS ASSOCIATED WITH ENHANCED EXPRESSION OF VASOPRESSIN mRNA WHICH IS MODULATED BY ANGIOTENSIN II AND ALPHA-2 ADRENERGIC RECEPTORS. Bang H. Hwang, Timothy Peters* and Prince Chan*. Terre Haute Center for Medical Education, Indiana Univ. Sch. of Med., Terre Haute, IN 47809.

Central mechanisms for salt-induced hypertension are not clear. Dahl salt-resistant and salt-sensitive (SS) rats were fed with high-salt diets. We used ^{35}S-vasopressin (VP) oligonucleotide probe to study VP mRNA after in situ hybridization, and ^{125}I-angiotensin II (AII) and -iodoclonidine ligands to label AII receptors and alpha-2 adrenergic (A2) receptors respectively. VP mRNA, AII and A2 receptors were measured using quantitative autoradiography. Results showed that hypertension was developed in SS rats whose hypothalamic AII receptors and VP mRNAs were significantly increased, whereas A2 receptors were also up-regulated. This study suggests that salt-induced hypertension is associated with enhanced VP mRNA expression which is controlled by AII receptors and modulated by A2 receptors. Supported by PHS grant NS25087.

397.8

NEUROTENSINERGIC MODULATION OF NEURONAL RESPONSES IN THE LATERAL HYPOTHALAMIC AREA (LHA) TO STIMULATION OF CARDIOVASCULAR SITES IN THE INSULAR CORTEX (IC). G.V. Allen and D.F. Cechetto. Robarts Research Institute, University of Western Ontario, London, Ontario, Canada, N6A 5K8.

Sympathetic responses elicited from the IC involve a synaptic relay in the LHA (Cechetto and Chen,'90). This study examines, in the anesthetized rat (chloral hydrate-urethane), the effects of iontophoresis of neurotensin (NT) on extracellularly recorded responses of LHA neurons responding to electrical stimulation of the IC (500 μA, 0.5-0.7 Hz). Of 82 spontaneously firing neurons, 32 units (39%) responded to electrical stimulation of cardiovascular pressor or depressor sites in the IC. Of these, 9 units (28%) were excited, 11 units (34%) showed excitation followed by inhibition, 9 units (28%) were inhibited and 3 units (9%) showed inhibition followed by excitation. Iontophoresis of 0.1-1.0 mM NT (25-100 nA) potentiated 11 (34%) of the excitatory responses and attenuated 2 (6%) of the inhibitory responses. Three of the neurons that responded to IC stimulation and six of the neurons that did not respond to IC stimulation exhibited an increased firing rate to NT iontophoresis. These findings indicate that NT may play an important role in the neuromodulation of cardiovascular regulatory commands from the insular cortex. Supported by the Heart and Stroke Foundation of Canada.

397.9

LESION OF THE MEDIAL SEPTAL AREA IMPAIRS THE PRESSOR AND NATRIURETIC RESPONSES INDUCED BY CENTRAL CHOLINERGIC ACTIVATION IN RATS. A.C. Luiz*; Wilson A. Saad; L.A.A. Camargo*; J.E.N. Silveira*; A. Renzi*; L.A. De Luca Jr.*; Willian A. Saad* and J.V. Menani*. Department of Physiology, Dentistry School, UNESP, Araraquara 14800 SP, Brazil.

Intracerebroventricular (ICV) injection of the cholinergic agonist carbachol (CARB) in rats produces natriuresis, kaliuresis, water intake and hypertension. In this study we investigated the effect of lesion of the medial septal area (MSA) in these responses. Male rats with sham or electrolytic lesion of the MSA were implanted with chronic stainless steel cannula into the lateral ventricle. ICV CARB (8 nmol) in sham rats induced pressor (35 ± 5 mmHg), dipsogenic (10.2 ± 1.5 ml/h), natriuretic (551 ± 83 uEq/120 min) and kaliuretic (170 ± 17 uEq/120 min) responses. Acute (1-7 days) MSA lesions reduced the pressor (18 ± 3 mmHg), natriuretic (178 ± 58 uEq/120 min) and dipsogenic (5 ± 1.3 ml/h) responses. Chronic lesions (14-18 days) reduced only pressor and natriuretic responses. Kaliuresis was not changed by MSA lesions. These results suggest that MSA plays an important role on the pressor and natriuretic responses induced by central cholinergic activation in rats.
(Research supported by FAPESP).

397.10

LESION OF THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS AFFECTS THE DEVELOPMENT OF THE PRESSOR RESPONSE TO COMMON CAROTID OCCLUSION IN CONSCIOUS RATS. M. I. B. Bedran de Castro* and J. C. Bedran de Castro. Department of Physiology, Dentistry School, UNESP, Araçatuba SP 16015, Brazil.

Pressor response to common carotid occlusion (cco) produced by implanted occlusion cuffs during 60 seconds in conscious rat has two components: an initial peak (IP) of carotid reflex origin and a maintained response (MR), that is, a plateau of lower intensity observed during the last 30 seconds of occlusion, which does not depend on carotid innervation and is probably of central ischemic origin. The present study was performed to investigate the role of the paraventricular nucleus of the hypothalamus (PVH) to the development of the pressor response to cco. Acute lesion of PVH (6 hours, MAP = 132 ± 3 mm Hg) decreased IP by 24% (38 ± 3 vs 50 ± 3 mm Hg) and MR by 28% (23 ± 3 vs 32 ± 2 mm Hg). Chronic PVH lesion (6 days) produced similar reductions (MAP = 122 ± 3, IP = 37 ± 5, MR = 20 ± 3 mm Hg). Aortic denervation, performed 6 hours before cco increased the depressed responses observed in chronic PVH lesioned rats (MAP = 125 ± 5, IP = 45 ± 4, MR = 32 ± 5 mm Hg). These data suggest that PVH plays a role in the integration of the pressor response to cco and that the important buffer effect of the aortic baroreceptors on the cco response is still present after PVH lesion.

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397.11

HYPOTHALAMIC GABAERGIC INFLUENCES ON TREADMILL EXERCISE RESPONSES IN RATS. J.M. Overton*, M.W. Redding*, S.L. Yancey*, and R.W. Stremel. Dept. Physiology & Biophysics and Exercise Physiology, University of Louisville, Louisville, KY 40292.

To test the hypothesis that a GABAergic mechanism within the posterior hypothalamus (PH) mediates the cardiovascular adjustments to dynamic exercise in conscious animals, Sprague-Dawley rats were chronically instrumented with bilateral guide cannula directed at the PH, a carotid cannula, and Doppler flow probes on the mesenteric and iliac arteries. Saline (SAL, 100 nl/side) or the GABA agonist, muscimol (MUS, 125 ng/100 nl/side), were injected into the PH during steady-state treadmill exercise (20 m/min). SAL had no effect on mean arterial pressure (MAP), heart rate (HR), mesenteric resistance (MR) or iliac resistance (IR) during exercise. MUS had no consistent effect on MAP (2 ± 1 % ▲), HR (2 ± 4 % ▲), or MR (-3 ± 4 % ▲), but produced a significant increase (22 ± 7 %) in IR. In addition, hypothalamic muscimol reduced exercise run time and induced behavioral changes (e.g., sedation) most evident post-exercise. The data suggests hypothalamic GABAergic modulation of exercise hyperemia in conscious animals. Supported by Univ. of Louisville Graduate School.

397.12

HYPOTHALAMIC STIMULATION MAY INCREASE ADRENO-LUMBAR VENOUS FLOW AND EPINEPHRINE SECRETION IN THE CAT. V.K. Bergdall* and S.L. Stoddard. Dept. of Anatomy, Indiana Univ. Sch. of Med., Ft. Wayne, IN 46805.

This study was designed to determine the effect of electrical stimulation of the preoptic region and hypothalamus on adreno-lumbar (AL) venous flow and catecholamine (CA) secretion in the cat. Electrode sites (N=75) were chosen in awake, restrained cats (N=16) that elicited signs of sympathoadrenal activation (*e.g.*, hissing, pupillary dilatation). Later, under methoxy-flurane anesthesia, cannulae were placed in the left jugular and AL veins and femoral artery. AL venous samples were collected by gravity and the volume measured during 3 consecutive 1-min periods: 2 prior to stimulation and 1 commencing with stimulus onset (square-wave, biphasic pulses; 60 Hz; 0.6 mA; 30 sec). CA levels were determined by radioenzymatic assay using COMT and S-adenosyl methionine [methyl-^{3}H]; secretion was expressed as ng/min. Hypothalamic stimulation most frequently increased the secretion of both NE and EPI, although evidence of preferential secretion was observed. Stimulation of 17 sites, primarily in the medial hypothalamus and nucleus of the diagonal band, increased AL flow > 12% baseline and elicited significantly greater increases in EPI secretion. These data are consistent with the hypothesis that secreted EPI acts locally to increase blood flow through the adrenal medulla.

397.13

CALCITONIN GENE-RELATED PEPTIDE (CGRP) MODULATION OF VISCERAL AND NOCICEPTIVE INPUTS TO THE VENTRAL BASAL THALAMUS (VBT). R.Z. Liang* and D.F. Cechetto. Robarts Research Institute, Univiversity of Western Ontario, N6A 5K8.

Visceral and nociceptive afferents terminate in the VBT. CGRP has been demonstrated in visceral relay neurons from the parabrachial nucleus to the VBT. The effects of iontophoretic application of CGRP on the responses of extracellularly recorded thalamic neurons to activation of visceral and nociceptive receptors was examined in chloralose-anesthetized rats. Few neurons (39/800, 5%) were observed to change their spontaneously firing rate in response to application of CGRP. CGRP enhanced the responses of: 12/20 neurons responsive to baroreceptor activation (phenylphrine i.v.) of a total of 55 tested; 11/22 to chemoreceptor activation (NaCN i.v.) of a total of 53 tested; 6/18 to chemoreceptor activation (10% CO_2 in inspired air) of a total of 48 tested; 6/15 to gastric mechanoreceptor activation (gastric balloon inflation) of a total of 33 tested; 5/12 to taste stimuli (NaCl on the tongue) of a total of 31 tested; 11/18 to pain stimuli (cutaneous pinch) of a total of 59 tested. In addition, nociceptive inputs converged on a large proportion of the visceral responsive neurons. These results suggest that CGRP serves as a major modulator of visceral and nociceptive inputs to neurons in the ventral basal thalamus.
Supported by the Heart and Stroke Foundation of Ontario.

397.14

FOREBRAIN 5-HT_1 RECEPTORS CAUSE SYMPATHOEXCITATION IN RATS. A G Ramage, I K Anderson* and G R Martin[1]*. Depts. Pharmacology, Royal Free Hospital School of Medicine, London, NW3 2PF and [1]Wellcome Laboratories, Kent, UK

Lateral ventricular (i.c.v.) application of 5-HT causes a rise in blood pressure which is thought to be due to activation of 5-HT receptors located in the anterior hypothalamus/preoptic region. The nature of the receptors mediating this pressor effect were investigated.

In α-chloralose anaesthetized rats recordings were made of BP, HR, renal (RNA) and phrenic nerve activity (PNA). 5-HT (40, 120 nmol kg^{-1}), 5-CT (3 nmol kg^{-1}), DP-5-CT (0.3, 3 nmol kg^{-1}), 8-OH-DPAT (40, 120 nmol kg^{-1}) and saline were given by microinjection (5μl over 20s) i.c.v.

5-HT, DP-5-CT and 5-CT all caused rises in BP, HR and RNA of 24 ± 3 mmHg, 63 ± 13 beats min^{-1}, 126 ± 33 % and 24 ± 4 mmHg, 63 ± 9 beats min^{-1}, 112 ± 29 % for the highest dose of 5-HT and DP-5-CT respectively, and 14 ± 4 mmHg, 42 ± 4 beats min^{-1}, 142 ± 43 % for 5-CT. 8-OH-DPAT 40 and 120 nmol kg^{-1} only caused a rise in HR of 28±4 and 50 ± 13 beats min^{-1} respectively. 5-HT differed from DP-5-CT and 5-CT in that the rise in blood pressure was associated with an initial decrease in HR and RNA. PNA was only increase by 8-OH-DPAT. Pretreatment with methiothepin attenuated the effects of DP-5-CT on all variables.

These results suggest that activation of 5-HT_1 receptors are responsible for the sympathoexcitation and pressor effects observed when 5-HT is injected i.c.v.

397.15

HYPOTHALAMIC EXCITATORY AMINO ACID (EAA) RECEPTORS MEDIATE STRESS-INDUCED TACHYCARDIA. R.P. Soltis and J.A. DiMicco. Dept. of Pharmacology and Toxicology, Indiana Univ. School of Medicine, Indianapolis, IN 46202.

Microinjection of the GABA-A receptor agonist muscimol into the dorsomedial hypothalamus (DMH) in rats prevents the tachycardia and hypertension seen in an air-stress paradigm. Here, we examined the effect of injecting EAA antagonists into the DMH on heart rate (HR) and blood pressure during air stress. Conscious chronically instrumented rats received bilateral injections (100 nl/side) of either artificial CSF (ACSF, Groups I and II, n=12 each), the non-selective EAA receptor antagonist kynurenate (KYN, Group I, n=4 each dose), the NMDA receptor antagonist AP5 100 pmol, the non-NMDA EAA receptor antagonist CNQX 50 pmol or both AP5 and CNQX (Group II, n=4 each treatment). Below are means ± SEM of basal HR (beats/min) and changes from basal HR 3 min after start of stress (5 min post injection).

Group I	Basal	Change	Group II	Basal	Change
ACSF	345±9	+153±6	ACSF	343±7	+154±4
KYN 1 nmol	327±12	+102±15*	AP5	341±5	+104±6*
KYN 3 nmol	330±17	+58±4*	CNQX	342±17	+110±14*
KYN 10 nmol	341±14	+18±9*	AP5+CNQX	338±12	+58±13*

* different from ACSF treatment by ANOVA ($P < 0.05$)

These results suggest that activity at EAA receptors in the DMH is required for the generation of stress-induced tachycardia. (Supported by USPHS grants 5 SO7 RR 5371 and NS 19883, and the American Heart Assoc., Ind. Affil.)

397.16

HYPOTHALAMIC PEPTIDERGIC NEUROMODULATION OF THE SPECIFIC CARDIAC EFFECTS OF INSULAR CORTEX PHASIC MICROSTIMULATION: S.M.Oppenheimer, T.Saleh* and D.F.Cechetto. Robarts Research Institute/University of Western Ontario London, Canada N6A 5K8.

Microstimulation of the rat posterior insular cortex in phase with the ECG R wave elicits pure cardiac effects and has demonstrated insular cardiac chronotropic organisation (Oppenheimer and Cechetto'90). As the lateral hypothalamus (LHA) mediates insular pressor effects (Cechetto and Chen '90), insular tachycardia was anticipated to be responsive to LHA manipulations. Insular tachycardia sites were phasically stimulated once during each cardiac cycle with 500 μA for 1 minute before and after microinjections into the LHA. The insular tachycardia response was abolished in chloralose-anesthetised rats by LHA microinjection of the synaptic blocker cobaltous chloride (4mM). LHA microinjection of kynurenic acid (250mM) attenuated insular tachycardia by 95%. Microinjection of naloxone (2.5mg/ml) attenuated the tachycardia by 95%; met-enkephalin (2mg/ml) was without effect on this response. Leu-enkephalin (2mg/ml) and neuropeptide Y (NPY) (0.01mM) doubled the magnitude of the tachycardia response. We suggest that the LHA contains an obligatory synapse mediating insular tachycardia and that glutamate is the likely neurotransmitter at this site. Neuromodulation of insular tachycardia may be effected by opiate and NPY receptors, a finding of considerable clinical relevance.
(Supported by the Heart and Stroke Foundation of Ontario).

397.17

STRESS-INDUCED HYPERTENSION IN THE BHR: DURATION OF STRESSOR. D. Hatton, J. DeMerritt*, & D. McCarron*, Oregon Health Sciences University, Portland, OR 97201

Daily exposure to 2 hours of shock-shock conflict or 2 hours of air-jet stress (AJS) has been shown to cause sustained elevations of blood pressure in borderline hypertensive rats (BHR). It is of interest to determine whether shorter stress sessions would be effective in causing comparable increases in blood pressure. In the current study, the effect of 20 min of daily AJS on blood pressure was contrasted with the effects of 120 min of AJS. At eight weeks of age, male BHRs were restrained in plexiglass tubes and exposed to either 20 or 120 min of AJS for 5 days/week for five weeks. Air-jet stress consisted of a stream of air directed toward the top of the head. Maturational controls were left undisturbed in the home cage except for weekly measurement of blood pressure. Systolic blood pressure was taken weekly using a tail-cuff method (NARCO). The 20 min group had significantly higher systolic BP than the control group ($p<.001$), and the 120 min group had significantly higher BP than the 20 min group ($p<.001$). By the 5th week systolic BP was 143, 167 and 174 mmHg in the control, 20 and 120 min groups respectively. However, direct measurement of BP in the home cage at the end of the 5 week period showed no difference between the 20 and 120 min groups (117, 130, 127 mmHg for control, 20 and 120 min). Thus, it is questionable whether 120 min of AJS causes a proportionately greater increase in BP than 20 min of AJS in the BHR.

397.18

CHOLINERGIC AND ADRENERGIC MECHANISMS IN THE CARDIOVASCULAR RESPONSE TO STARTLE B.K. Taylor and M.P. Printz. Pharmacology 0636, Univ. Cal. San Diego, La Jolla, CA 92093.

Complex cardiovascular (CV) responses consisting of pressor, bradycardia and tachycardia result from airpuff stimuli delivered to conscious, unrestrained, catheterized Wistar-Kyoto rats. To investigate neurotransmitter modulation of the CV response, we used intracerebroventricular (ICV) administration of the acetycholine depletor hemicholinium-3 (HC-3), muscarinic-receptor antagonists scopolamine (Scopol) & 4-DAMP and clonidine. Saline or drug (in 5 μl) was infused via ICV cannula placed one week earlier and responses measured after the time interval shown. Resting blood pressure and heart rate (HR) were not affected by drug treatment except after administration of clonidine (Saline:343±7 bpm, Clonidine: 315±7 bpm). Behavioral (motor) responses were dissociated from the CV responses as shown below. We conclude that for the doses of agents used, the pressor response is influenced by either α-2 adrenergic or imidazole mechanisms while the bradycardia response is modulated by both muscarinic receptor blockade and clonidine.

AGENT	DOSE (μg/kg)	TIME (min)	↓ HR* (bpm)	MOTOR*	PRESSOR* (mmHg)	↑ HR** (bpm)
Saline (9)	—	120	-55±10	49±7	49±3	28±4
HC-3	5	120	-14±6	32±8	39±3	25±3
Saline (5)		30	-61±15	72±8	33±5	36±8
Scopol.	40	30	-8±7	62±13	30±3	24±2
4-DAMP (8)	40-70	15-30	-22±6	46±10	33±3	20±4
Saline (9)		2	-44±7	55±9	42±3	33±5
Clonidine	10	2	1±4	56±10	21±4	18±3

*Trial 1 responses; **Trial 10 responses.

397.19

RANDOM LIGHT/DARK CYCLES INCREASE PLASMA CHOLESTEROL IN RATS. E.S. Halas and L.M. Klevay.* Department of Psychology, University of North Dakota and USDA, ARS, Grand Forks Human Nutrition Research Center, Grand Forks, ND 58202.

Sixty male weanling rats of the Sprague-Dawley strain were divided into four groups. Two groups were illuminated continuously for eight hours, then were exposed to darkness for 16 hours, beginning at midnight, 8 a.m. or 4 p.m. Each light/dark cycle lasted two, three or four days, with cycle duration and onset of illumination being randomized. The other two groups were housed in a standard 12-hour light/dark room. One group in each room was fed a purified diet containing 2 ppm copper (Cu) while the other two groups were fed the same diet containing 5 ppm Cu. This diet (*Am J Clin Nutr 26: 1060, 1973*) was based on sucrose (62%), egg white (20%) and corn oil (10%); it contains all known essential nutrients. The rats were killed after six weeks. Cholesterol measured by an enzymatic, colorimetric method was elevated approximately 11% ($p<0.025$) in the random light/dark cycle groups. This elevation was less than that (33%, $p<0.005$) found in a preliminary experiment done with only the lower Cu diet. The lower Cu diet depressed both hematocrit ($p<0.004$) and body weight ($p<0.009$) approximately 6%. There was no anemia (mean hematocrit > 43%); a diet marginal in Cu may decrease body size and hematocrit. The results suggest shift workers are at risk for elevated plasma cholesterol.

397.20

BED NUCLEUS OF THE STRIA TERMINALIS NEURONS RESPOND TO BOTH NOCICEPTIVE AND STRESS-RELATED STIMULI. J.H. Casada and N. Dafny. Department of Neurobiology and Anatomy, The University of Texas Medical School at Houston, Houston, TX 77225.

The bed nucleus of the stria terminalis (BNST) has been shown to modulate the expression of many components of the stress response including corticosterone secretion, autonomic activity, and increases in agonistic and locomotor behavior. The present experiment was designed to determine the degree to which stress is generalized in BNST by determining if BNST neurons would respond similarly to two different stress-related stimuli. In acute experiments, 20 male Sprague Dawley rats (240-350 g) were anesthetized with 1.25 g/kg urethane. A bipolar stimulating electrode was placed in the amygdala and a recording glass microelectrode was placed in BNST. Eighty-four spontaneously active neurons were recorded from BNST. 66% of these cells responded to electrical stimulation of the amygdala, predominantly with driven activity (latency = 17.25 ± 5.41 ms). 33% responded to a noxious stimulus, tail pinch (TP), predominantly with an increase in firing rate, but only 4% responded to cutaneous stroke, a control somatosensory stimulus. A chi-square test of the interaction between amygdala stimulation and TP revealed that the proportion of neurons responsive to each are independent of one another ($p=0.25$). Thus, while the neurons of BNST are responsive to at least two different stress-related stimuli, the pathways by which these reach BNST appear to differ.

397.21

IDENTIFICATION OF ANGIOTENSINERGIC PATHWAYS IN THE PVN. K.A. Roberts, J.W. Harding, L.L. Jensen, J. Hanesworth* and J.W. Wright. Departments of Psychology and VCAPP, Washington State University, Pullman, WA 99164-4820.

Previous studies have indicated that the paraventricular nucleus (PVN) is important in the regulation of body water balance and cardiovascular activity. The PVN receives afferent input from the subfornical organ (SFO) (Lind et al., 1985; Gutman et al., 1986) and is important in the regulation of cardiovascular activity (Ishibashi and Nicolaidis, 1981). In an attempt to verify these previous studies, we have utilized the retrograde transport of fluorescent microspheres to map afferent pathways from the SFO to PVN. The use of latex microspheres offered several advantages including low toxicity and minimal diffusion from the injection site. Eight spontaneously hypertensive rats (SHRs) were injected with rodamine labelled latex microspheres. Small volumes, 30nl, of the beads were injected using a 10 ωl Hamilton syringe, via a 33 g injector into the PVN in alert animals. The animals were sacrificed after 48 hrs and brains were extracted, fixed and mounted. The sections were then observed under a fluorescent microscope and slides were taken of the injection site and SFO. Analysis of 30 micron frozen sections confirmed the existence of a strong SPO-PVN pathway. Although we have not yet demonstrated the angiotensinergic nature of this pathway, push-pull cannula studies demonstrated that direct injection of AII into the SFO resulted in massive release of angiotensins in the PVN. In addition, similar levels of release could be observed in the PVN following 48 hours of water deprivation.

REGULATION OF AUTONOMIC FUNCTIONS: GENITO-URINARY CONTROL

398.1

NEURAL REGULATION OF "PSYCHOGENIC" ERECTION: EXPOSURE TO RECEPTIVE FEMALES FACILITATES REFLEXIVE ERECTION IN RATS, EVEN AFTER HYPOGASTRIC NERVE SECTION. B. D. Sachs and Y.-C. Liu. Psychology and Biobehavioral Sciences Degree Programs, Univ. of Connecticut, Storrs, CT 06269-1020.

Sexually experienced male rats received bilateral transections of their hypogastric nerves (HGNx) or sham operations (SHAM). Three weeks later the males were placed for two minutes in an empty cage or in a cage with two inaccessible receptive females, and then were immediately tested for reflexive penile erections (supine position, penile sheath retracted, no further penile stimulation). Exposure to females reduced the erection latency by about 40% ($p<.01$), and other measures of erectile response revealed similar facilitation, irrespective of prior HGNx or SHAM surgery. We infer that (a) stimuli received by receptors in the head of male rats can facilitate reflexive penile erection, and (b) this psychogenic facilitation of reflexive erections is not mediated by the hypogastric nerves, the only nerves previously proven to mediate psychogenic erection.

398.2

PHYSIOLOGY OF MUSCLE AFFERENTS IN THE BULBOSPONGIOSUS AND ISCHIOCAVERNOSUS OF RATS. R.D. Johnson and V.P. Dugan*. Department of Physiological Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL 32610.

The bulbospongiosus and ischiocavernosus are perineal muscles that have a role in rodent male sexual behavior, specifically functioning in penile glans engorgement (cups), dorsal penile movements (flips), and ejaculation. It has been suggested that sensory receptors in these muscles play a role in these events and possibly also mediate sensations associated with sexual climax. To investigate the physiological responses of these afferents, we recorded from single afferent fibers in urethane anesthetized rats. Single fiber recordings were made from either dorsal root filaments or from the motor branch (MB) of the pudendal nerve. The muscles were exposed for stimulation. Receptors were found in the ischiocavernosus muscle and in all parts of the bulbospongiosus muscle. Conduction velocity measurements indicated they were primarily Group II myelinated afferent fibers. Almost all of the afferents were typical muscle stretch receptors exhibiting a resting discharge, a regular slowly adapting discharge rate, an increased discharge to muscle stretch, and a short silent period during muscle contraction. They also responded to penile extension and preputial retraction (ie. supine penile reflex test). Some afferents were rapidly adapting, located in tendinous tissue, and increased their discharge during muscle contraction. We have recorded from dorsal horn neurons driven by MB afferents. These results suggest that sensory receptors in the ischiocavernosus and bulbospongiosus muscles are activated during penile events and may play a role in the neural control of sexual behavior. Supported by NS27511.

398.3

AUTONOMIC NEURONS ACQUIRE INNERVATION IN THE ABSENCE OF PREGANGLIONIC INPUT: A STUDY USING SYNAPSIN AND MET-ENKEPHALIN IMMUNOCYTOCHEMISTRY. N.M. Minorsky and W.G. Dail. Anatomy Dept., Sch of Med., Univ. of New Mexico, Albuquerque, New Mexico 87131

Neurons within the Major Pelvic Ganglion (MPG) supply autonomic innervation to the corpora cavernosa penis in the rat. Previous studies have shown that penile neurons receive the majority of their presynaptic input from preganglionic fibers which are immunoreactive for met-enkephalin (mENK). Whereas mENK innervation of penile neurons is greatly reduced following acute decentralization of the MPG, it is partially recovered in chronically decentralized ganglia. In order to determine whether other fiber populations contribute to penile neuron innervation following decentralization, nerve terminals were immunolabelled with antibodies to synapsin as well as mENK. As expected, the level of synapsin staining was greatly reduced in the acutely decentralized ganglia and the number of innervated neurons was comparable to that seen by mENK immunocytochemistry (approximately 12%). In the chronically decentralized ganglia however, approximately 60% of the penile neurons were innervated by synapsin-IR fibers, compared to 30% described by mENK immunocytochemistry. These findings demonstrate a remarkable capacity for neurons to receive innervation in the absence of preganglionic input. This potential may be important for understanding autonomic control of pelvic visceral tissue following spinal cord injury.

398.4

DISTRIBUTION OF 5-HT AND ITS COLOCALIZATION WITH TRH, SP AND M-ENK IN THE CAT MEDULLA. C. Dean, L. Marson, C. Hermes*, C. Polchow* and J.P. Kampine. Depts. Anesth. and Physiol., Med. Coll. Wis. and VA Med. Center, Milwaukee WI 53295 and Dept. Physiol., Northwestern Univ. Med. School, Chicago, IL 60611.

Medullary neurons containing 5-HT have long since been implicated in the control of the circulation and their distribution has been well described. This immunohistochemical study investigated the co-localization of peptides with 5-HT within the medullary raphe and reticular formation. Cats were anesthetized with sodium pentobarbitol and a laminectomy performed to expose T9-T10 segments of the spinal cord. Rhodamine latex microspheres were injected into the vicinity of the IML using a Hamilton syringe. Cats were allowed to recover for 4-5 wks then reanesthetized and colchicine (100 ug) was injected into the fourth ventricle. Cats were perfused 24 hrs later with paraformaldehyde/acrolein fixative (3/0.5%). Spinal cord sections (20 um) were examined microscopically to identify injection sites. Sections of the medulla (40 um) were processed for 5-HT and TRH or 5-HT and SP or SP and TRH or 5-HT and M-ENK. 5-HT containing neurons were located in raphe pallidus (RP), obscurus (RO) and magnus (RM), and the ventral medullary reticular formation (VLM). In RP and RO 60-90% of cells containing 5-HT also contained both TRH and SP. In VLM 36 and 71% of cells which contained 5-HT also contained SP and TRH respectively. In addition, in these regions M-ENK was found exclusively in cells containing 5-HT. Retrograde labelling was identified in RO, RP and VLM neurons which contained 5-HT. This study demonstrates that spinally-projecting 5-HT neurons in the cat medulla also contain TRH, SP and M-ENK. (Supported by the NSF).

398.5

PROJECTIONS OF THE ROSTRAL NPGI: A REGION INVOLVED IN MODULATION OF SEXUAL FUNCTION. *A.M. Peternel*, L. Marson and K.E. McKenna.* Northwestern University Medical School, Chicago IL 60611

The rostral nucleus paragigantocellularis (nPGi) modulates the tonic inhibition of spinal sexual reflexes (Marson & McKenna, 1990). Mapping the projections to and from the nPGi will provide a neuroanatomical basis for understanding these reflexes.

The anterograde tracer PHA-L, retrograde fluorescent tracers rhodamine and Fluorogold, and the anterograde/retrograde tracer HRP-WGA were microinjected into the rostral nPGi. Rats were perfused with a fixative appropriate for the tracing technique and sections through the brainstem and spinal cord (T13-S1) were processed. Anterogradely labelled fibers and terminals were found mainly ipsilateral at L5-L6 in the region of the medial DL motoneurons of the urethral sphincter, as well as in the lateral portion of this nucleus, near the motoneurons of the ischiocavernosus muscle. Fibers were also labelled in the DM nucleus at L5-L6. The motoneurons of the anal sphincter and the bulbospongiosus are interspersed within this nucleus. Fibers were also labelled in the IML and CA of T13-L2, the region of the lateral motoneurons of L4, and the sacral IML. Retrogradely labelled cells were found mainly contralateral in lamina X of T13-L3, lamina IV and V of T13-L1, and lamina IV of L2. Cells were also found in laminae IV, VIII, and X of L4-L5 and in the medial grey and lamina IV of L6. Cells were also found in the laminae IV, V, and X of S1 and the ventral grey of S2. Anterogradely labelled fibers were found in medullary nuclei including the contralateral nPGi, NTS, nGi, facial nucleus and caudal medullary reticular formation. In addition, fibers were found in the raphe, LC and subLC.

398.6

CNS CELL GROUPS PROJECTING TO THE PENIS LABELLED WITH PSEUDORABIES VIRUS (PRV). *L. Marson, K.B. Platt and K.E. McKenna.* Northwestern University Medical School, Chicago IL 60611

Transneuronal retrograde cell body labelling in the spinal cord and brainstem was identified after injection of Bartha's K strain PRV into the penis of 25 rats. Two days later rats were perfused with 4% paraformaldehyde and the spinal cord, pelvic ganglia and brain removed. Sections of the spinal cord, ganglia and medulla were cut (30-50μm) and processed with swine polyclonal antibody to PRV and visualized with avidin-biotin HRP complex. Retrogradely labelled cells were found in a restricted region of the pelvic ganglia. Transneuronally labelled cells were found in sympathetic and parasympathetic preganglionic regions in T13-L2 and L5-L6 of the spinal cord, respectively. In addition, cells were found in intraneuronal areas throughout the lumbosacral cord with cell labelling also in L3/L4. Quantitative analysis of cell distribution was performed. In the medulla cells were labelled in the nucleus paragigantocellularis, parapyramidal region, A5, raphe and in the region of the subcoeruleus. Labelling was consistent with known anatomical organization of pelvic systems. This procedure is a valuable tool in examination of spinal cord circuitry and neural systems involved in sexual function.

398.7

ELECTROPHYSIOLOGICAL EVIDENCE FOR BRANCHED PREGANGLIONIC PATHWAYS INNERVATING BOTH THE URINARY BLADDER AND THE URETHRA IN RATS. B.Conte*, M.Kawatani and W.C.de Groat. Depts. Pharmacol., Menarini Richerche Sud.,Pomezia,Rome,Italy & Univ. Pittsburgh,PA 15261.

In d-tubocurarine pretreated rats electrical stimulation (0.5-15V, 1Hz) of the central end of an acutely transected penile nerve or stimulation of the urethra at the level of the external urethral sphincter elicited discharges (mean latency 13.8±2.4) on postganglionic nerves (PBN) to the urinary bladder (UB). The responses were potentiated (20-80%) by repetitive stimulation (7-10 Hz) and by i.v. administration of DMPP (100 ug/kg) or tetramethyl-ammonium (50 ug/kg). Ganglionic blocking agents (hexametho-nium, trimethaphan, i.v.) or tetrodotoxin applied to the nerve abolished the responses. The responses were not eliminated by acute destruction of the lumbosacral spinal cord, or chronic (1 week) removal of the contralateral major pelvic ganglion or the hypogastric nerve. However, chronic transection (1 week) of the ipsilateral pelvic nerve eliminated the evoked discharges on the PBN. Electrical stimulation of the penile nerve (2-10 V, 3 Hz, 30-60 sec duration) produced UB contractions that were blocked by hexamethonium. These observations provide evidence for the existence of a population of preganglionic axons in the pelvic nerve which give rise to two peripheral branches: one providing an input to MPG ganglion cells innervating the UB and another projecting distally to the wall of the urethra. This population of preganglionic axons might function to coordinate UB and urethra function during micturition.

398.8

THE EFFECTS OF GLUTAMATE RECEPTOR ANTAGONISTS ON THE MICTURITION REFLEX IN THE RAT. M. Yoshiyama, J.R. Roppolo & W.C. de Groat. Univ. of Pittsburgh, Dept. of Pharmacol. Pittsburgh, PA. 15261.

Previous studies from this laboratory have shown that MK-801, a non-competitive NMDA receptor antagonist, inhibits rhythmic bladder activity in the intact urethane-anesthetized (UA) rat, while having little effect in the decerebrate animal. The present study extends those initial observations to: (1) examine the effects of MK-801 on bladder cystometrograms (CMG) and external urethral sphincter (EUS) EMG (electromyogram) activity, (2) compare MK-801 to CNQX, an AMPA receptor antagonist and (3) determine the effects of UA on MK-801 inhibition of the micturition reflex. In UA (1.2 g/kg) intact rats MK-801 (0.001-3 mg/kg, iv) reduced bladder contraction amplitude, capacity and EUS activity in a dose dependent fashion, with EUS being somewhat more sensitive than the bladder. In decerebrate rats, MK-801 (0.001-3 mg/kg, iv) had little effect on bladder amplitude but increased capacity and reduced EMG activity by 70%. The effects of MK-801 (0.1-1 mg/kg, iv) administered to UA (0.6 g/kg) decerebrate rats reduced bladder contractions in a dose dependent fashion as with intact animals. CNQX (0.2-1 mg/kg, iv) had no effect on bladder activity in either the intact, UA or the decerebrate rat.

Three conclusions are suggested by these data: (1) EUS reflexes are more sensitive to the effects of MK-801, (2) the AMPA glutamate receptor does not play a major role in micturition, (3) urethane enhances the effect of MK-801 on the micturition reflex in the rat.

398.9

BLADDER AND EXTERNAL URETHRAL SPHINCTER ACTIVITY EVOKED BY MICROSTIMULATION OF THE SACRAL SPINAL CORD. J.R. Roppolo, S. Smerin, A.M. Booth, I. Nadlehaft, W.C. de Groat, Dept. of Pharmacology, U. of Pittsburgh, PA 15261.

Our previous studies demonstrate that microstimulation of the sacral spinal cord (SSC) produces large amplitude (20-50cm H_2O) bladder contractions (BC). This study extends those initial observations to include: (1) an analysis of evoked external urethral sphincter(EUS) activity (2) detailed maps of stimulus sites in SSC and (3) possible neural mechanisms underlying the evoked responses. In intact chloralose anesthetized cats, the bladder and urethra underlying the EUS muscle were cannulated for pressure recording. A laminectomy exposed the SSC and roots. Ventral root (VR) stimulation identified the segments eliciting the largest bladder (S_2-S_3; 60-100cm H_2O) and EUS (S_1-S_2; 50-120cm H_2O) pressure changes. Using "activated" iridium microelectrodes (tip area: 200μm^2 and stimuli of 50-150μA at 15 Hz, 0.2 msec) microstimulation in the S_2 and S_3 segments along penetrations starting near the dorsal root entry zone, produced BC (10-60cm H_2O) at three depths along a 3000μm track: (1) ~250μm from the cord surface, (2) at the level of the preganglionic (PG) neurons (~800μm) and (3) near the PG axons along the lateral edge of the ventral horn (~2000μm). The EUS responses were large during stimulation at the cord surface and diminished at the level of PG neurons and axons. Transection of the spinal cord (T_9), of dorsal roots (S_1-S_3) bilaterally and contralateral VRs (S_1-S_3) did not change BC. However, ipsilateral VR (S_2-S_3) section abolished the BC. These studies suggest that BC can be evoked by microstimulation of the cord in the absence of major descending and afferent input and that stimulation of ipsilateral SSC does not produce contralateral efferent outflow. (Supported by NIH contract NO1-NS-9-2366)

398.10

EFFECTS OF MK-801, NPC 12626 AND HALOPERIDOL ON MICTURITION IN AWAKE AND ANESTHETIZED RATS. P.L. Vera & I. Nadelhaft, VA Medical Center, University of Pittsburgh, Depts. of Pharmacology & Neurosurgery, Pittsburgh, PA.

Female Sprague-Dawley rats were instrumented with chronic intravesical and intravenous catheters under halothane anesthesia. On the next day, saline infusion (0.2 ml/min) into the urinary bladder elicited micturition in the awake rats. NMDA-receptor antagonists, MK-801 (60-120 ug/kg iv) and NPC 12626 (10-100 mg/kg iv), increased the micturition frequency and concomitantly decreased the volume threshold (VT). MK-801 decreased VT to 30% of control whereas NPC 12626 reduced it to 70%. In anesthetized rats (urethane;1.0 mg/kg iv), MK-801 completely inhibited bladder contractions; however, NPC 12626 still decreased VT to 60% of control. MK-801 also abolished external urethral sphincter activity (EMG). Thus the effects of MK-801 are anesthetic dependent. The effects of MK-801 and to some extent also NPC 12626, in both the awake and the anesthetized animals, were reversed by haloperidol (0.4-1.2 mg/kg iv). Therefore, excitatory amino acids acting via NMDA receptors may play a role in modulating micturition. Dopaminergic or sigma receptors may also be involved since haloperidol antagonized the effects of MK-801.

398.11

NEURAL CONTROL OF RAT LOWER URINARY TRACT FOLLOWING SPINAL CORD INJURY. M.N. Kruse, S.L. Erdman*, W.C. de Groat. Dept. Pharmacology, U. of Pittsburgh, PA 15261.

Changes in urinary bladder (UB) and external urethral sphincter (EUS) activity following spinal cord injury (SCI) were studied in decerebrate neonatal rats (P10-P28) and urethane anesthetized adult rats by recording UB pressure and EUS-EMG. In intact rats, the EUS was inactive during bladder filling but exhibited burst activity during voiding. In contrast, 10-20 days following SCI at ~T9 in adult or neonatal rats, voiding was accompanied by a tonic increase in EUS-EMG (no bursting) and incomplete voiding, indicating that SCI rats exhibit bladder-sphincter dyssynergia. Following block of the EUS with pancuronium in intact rats, the amplitude of UB contractions decreased and residual urine volume increased indicating that the EUS burst activity may facilitate voiding. Immunohistochemistry revealed a dense collection of nerve terminals containing corticotrophin releasing factor (CRF) in the L_6-S_1 spinal parasympathetic nucleus (SPN) of adult rats and in neonatal rats older than day 5. These terminals were absent in SCI rats and in 0-2 day neonatal rats. These results indicate that CRF terminals in the SPN may be a marker for the post-natal development of the descending pathway of the spinobulbo-spinal micturition reflex and the elimination of this pathway by SCI. The present findings also indicate that the SCI rat may be a useful preparation for studying bladder-sphincter dyssynergia. This work was supported by grants from the American Paralysis Association and the Spinal Cord Research Foundation.

398.12

MOTONEURONS AND AFFERENT NEURONS INNERVATING THE BULBOCAVERNOSUS MUSCLE: A TRACING STUDY IN THE CAT. S. Smerin, J.R. Roppolo, R. Stewart*, V. Erickson*, W.C. de Groat, Dept. Pharmacol., Univ. Pittsburgh, Pittsburgh, PA 15261.

The afferent and efferent pathways to the cat bulbocavernosus muscle (BCM) were identified using the retrograde tracer cholera toxin B-HRP injected into the BCM under nitrous-oxide/halothane anesthesia. Ninety six hours later anesthetized cats were perfused with fixative. Spinal cord and dorsal root ganglia (DRG) from L_6 to S_3 were processed with TMB to reveal HRP. Motoneurons projecting to the BCM (average, 125) were present predominantly at the S_1 level and almost exclusively in Onuf's nucleus (ON). The neurons were typically elongate, about 30 x 50 microns in size. While neurons were labeled in equal numbers in the dorsomedial and ventrolateral divisions of ON (ON_{DM} and ON_{VL}), neurons in ON_{DM} gave rise to thick dendrites that extended to the intermediolateral (IML) region and to the ventrolateral funiculus. Thin processes extended to the IML, to the central grey, and to the medial, ventrolateral, and lateral funiculi. Labeled DRG cells (~500 per cat) were present mainly at S_2 and gave rise to abundant fibers that extended through the medial and lateral collateral pathways to the dorsal gray commissure. Two main conclusions can be made. First, the ischiocavernosus muscle (ICM) (studied previously) and the BCM motoneurons do not differ in location or dendritic projection, but in the origin of their prominent dendrites; those for BCM being from ON_{DM} and those for ICM being from ON_{VL}. Second, afferent pathways of BCM and ICM are located primarily one segment caudal (S_2) to the location of the motoneurons (S_1) innervating these muscles, indicating that the afferents may interact with visceral reflex mechanisms in this region of the cord. **(Supported by NIH contract N01-NS-9-2366)**

398.13

HYPERTROPHY OF BLADDER PELVIC GANGLION CELLS FOLLOWING CHRONIC PARASYMPATHETIC DECENTRALIZATION. W. DelGaudio*, A.M. Booth, R. Stewart*, S. Smerin and W.C. de Groat. Depts. of Pharmacology and Behavioral Neuroscience, Univ of Pittsburgh, Pittsburgh PA 15261

Previous studies have revealed reorganized synaptic connections in parasympathetic bladder ganglia and increased (2-3X) volume of the rat bladder following chronic parasympathetic decentralizations. This study was directed toward the involvement of ganglion cells in these events.

Unilateral resection of the L_6-S_2 dorsal and ventral roots of halothane anesthetized male rats was preformed and the animals allowed to recover for at least 3 months. Both lesioned and control animals were anesthetized and fluorogold and/or fast blue (10μl 4% W/V) injected into the urinary bladder wall. After 1-4 weeks animals were perfused with fixative, the major pelvic ganglion was removed, whole-mounted and the area of dye labeled cells calculated.

Dye labeled bladder cells on the lesioned side averaged 50% bigger in cross-sectional area (652±32.5 vs. 438±6.7 um^2, mean±sem). This appears to be an increase in the number of cells having a cross sectional area of between 700 and 2000 um^2 ($p < 0.05$, ANOVA) rather than a general increase in the cell size. Unilateral dye injections established the degree of contralateral bladder innervation from normal ganglia (25%) and following chronic interruption of the hypogastric (sympathetic) or pelvic (parasympathetic) nerve or both.

In summary, interruption of the lumbosacral preganglionic outflow to the bladder leads to hypertrophy of MPG neurons. It is possible that trophic factors released from the target organ may play a key role in the neuronal hypertrophy.

398.14

ELECTROPHYSIOLOGICAL STUDIES OF THE EFFECTS OF GLUTAMATE ANTAGONISTS (MK801 AND CNQX) ON THE MICTURITION REFLEX IN THE RAT. T.Suzuki, L.A.Birder, M.Yoshiyama, J.R.Roppolo, W.C.de Groat. Dept. Pharmacology, Univ. Pittsburgh, PA 15261.

Previous studies revealed that MK801, a non-competitive NMDA receptor antagonist, depressed reflex contractions of the urinary bladder (UB). The present experiments have analyzed the effects of MK801 and CNQX, a competitive AMPA receptor antagonist, on reflex firing in bladder postganglionic nerves (BPN) in urethane anesthetized rats. Rats with an intact neuraxis, as well as acute (5-48 hrs) and chronic spinal (T8) rats were studied. Electrical stimulation of afferent fibers in the pelvic nerve elicited short latency spinal (SR; 50-70 msec) and long latency spinobulbospinal (SBSR; 80-150 msec) reflex discharges on BPN. In intact rats, MK801 in low doses (0.1-0.3 mg/kg, iv) reduced and in high doses (1-3 mg/kg, iv) blocked the SBSR. High doses of MK801 reduced the SR in some intact and spinal rats. CNQX (1-3 mg/kg, iv) had no effect on SR and SBSR. MK801 (0.1-1 mg/kg, iv) reduced the asynchronous firing on BPN induced by bladder distension or injection of a irritant (1% acetic acid) into the bladder lumen.

These data indicate that NMDA but not AMPA glutaminergic receptor mechanisms have an important role in the neural control of micturition. Furthermore, since the SBSR in intact animals is considerably more sensitive than the SR to the depressant effects of MK801 it would appear that glutaminergic transmission is only prominent in the supraspinal reflex pathway to the urinary bladder.

398.15

ROLE OF DESCENDING SEROTONERGIC AND NORADRENERGIC PATHWAYS IN THE CONTROL OF MICTURITION IN AWAKE CATS. M.J. Espey, J.W. Downie, and A. Fine. Depts. of Pharmacol. and Physiol. & Biophys., Dalhousie Univ., Halifax, NS B3H 4H7.

The role of spinal serotonin (5-HT) and noradrenaline (NA) in micturition was assessed using neurotoxins and acute antagonists. Cystometrograms were performed in awake cats with chronic indwelling i.t. and bladder cannulae. Parameters followed were: volume threshold for micturition (V_T), postvoid residual volume, voiding contraction amplitude, contraction time and bladder stiffness during filling. Immunohistochemistry for tyrosine hydroxylase (TH) and 5-HT was performed to verify toxin actions. Methysergide (2.5-20μg it) dose-dependently decreased V_T. 5,7-dihydroxytryptamine (1800μg it) depleted 5-HT in the lumbosacral cord and reduced V_T. Other parameters were unaffected. Prazosin (100μg it) was ineffective, suggesting that alpha$_1$-adrenoceptors are not involved in normal micturition (cf. Yoshimura et al, J Urol 139: 423, 1988). 6-hydroxydopamine (500μg it) depleted TH from thoracic to sacral levels and increased V_T and contraction time. The data imply a spinal inhibitory role for 5-HT in modulation of micturition. Although NA may be facilitatory to micturition, it appears not to be required for normal micturition in conscious cats. (Supported by Rick Hansen Man in Motion World Tour Society)

398.16

EFFECT OF 8-OH-DPAT ON THE SUPRASPINAL MICTURITION REFLEX (SMR) IN ANESTHETIZED RATS. A. Lecci, S. Giuliani, A. Giachetti and C.A. Maggi. "Menarini" Pharmacol. Res. Dept., via Sette Santi 3, 50131 Florence, Italy.

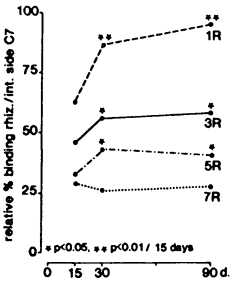
The effect of 8-OH-DPAT, a selective 5-HT$_{1A}$ receptor agonist, on SMR has been investigated in urethane-anesthetizd rats. Intravenous administration of 8-OH-DPAT (3.3 μg/kg) induced activation of SMR (bladder contractions > 16 mm Hg) in 67% of rats which bladders were filled with a volume just below that required for evoking SMR. The effect of 8-OH-DPAT was antagonized by hexamethonium. Both intracerebroventricular (icv) and intrathecal (it) administration of 8-OH-DPAT induced activation of SMR. After icv administration, 8-OH-DPAT was about 10 times more potent than after the it route (minimal effective dose, MED icv: 3.3 ng/rat). The activation of SMR induced by icv 8-OH-DPAT was completely abolished by guanethidine and the MED was shifted to 330 ng/rat by 5,7-dihydroxytryptamine (5,7-DHT) administered icv in presence of desipramine. The activation of SMR by it 8-OH-DPAT was prevented by guanethidine, but was unaffected after 5,7-DHT icv administration. These findings indicate that stimulation of 5-HT$_{1A}$ receptor facilitate micturition reflex at both spinal and supraspinal sites. In both cases, removal of sympathetic inibitory mechanisms prevented the action of 8-OH-DPAT suggesting that suppression of sympathetic outflow to the bladder could be involved in the effect of 8-OH-DPAT. In addition, 5-HT neurons seem to be important in the facilitatory action of 8-OH-DPAT on the SMR at supraspinal level.

SOMATIC AND VISCERAL AFFERENTS: CENTRAL PROJECTIONS

399.1

PLASTICITY OF MU OPIOID RECEPTORS IN THE SUPERFICIAL DORSAL HORN OF THE RAT SPINAL CORD AFTER VARYING DELAYS FOLLOWING DORSAL RHIZOTOMIES. D. Besse*, M.C. Lombard and J.M. Besson, INSERM U161 and EPHE, 2 rue d'Alésia, 75014 Paris, France.

Autoradiography of μ receptors (μ rec.) by [^{3}H]DAMGO was performed in laminae I-II at cervical levels following unilateral dorsal rhizotomies of 1, 3, 5 or 7 roots (R) around C7, at 8, 15, 30 and 90 days post-lesion (PL). At 15 days PL, following section of C7 alone, a 37% decrease of binding was observed in the rhizotomized side compared to the intact side of the C7 segment. Sectioning up to 5 and 7 roots increased the loss to a maximum of 70%. The 30% residual binding is attributed to the postsynaptic component of μ rec. (Besse et al. Brain Res, 521, 1990) which remains stable over 3 months. From 15 to 90 days PL after cuts of 1 to 5 roots, a "recovery" of binding occurs which is maximal when only C7 is cut. Because the more deprived the C7 segment is of afferents, the less pronounced is the recovery, the recovery is attributed to the presynaptic μ rec. This neuroplasticity indirectly suggests the occurence of collateral sprouting of fine primary afferent fibers because μ rec. are essentially located on these fibers.



399.2

NEUROPEPTIDE CONTENT AND INTERACTIONS WITH GABA-IMMUNOREACTIVE PROFILES OF PHYSIOLOGICALLY IDENTIFIED LAMINA I AND II MONKEY PRIMARY AFFERENT TERMINALS. F.J. Alvarez, A.M. Kavookjian* and A.R. Light. Dept Physiology. University of North Carolina, College of Medicine, Chapel Hill NC 27599.

Intra-axonally labeled A High Threshold Mechanoreceptors (HTM) terminals identified physiologically had boutons which resemble the Dense Sinusoid Afferents (DSA) previously described in monkey lamina I and II. Using colloidal gold post-embedding immunocytochemical techniques we located CGRP-immunoreactivity (-IR) in some dense-core vesicles found in DSA terminals, but we were not able to unequivocally demonstrate CGRP or SP inside A HTM terminals. GABA-IR was found in vesicle containing profiles that were presynaptic to A HTM terminals and to other non-identified DSAs. CGRP- and SP-IR were predominantly located inside presumed primary afferent profiles which contain numerous large dense-core vesicles (LDCV). GABA-IR profiles were in apposition to LDCV-containing terminals but synaptic contacts were rare. The terminals of two physiologically identifed C fibers also contained CGRP-IR dense-core vesicles. One C-fiber was a polymodal nociceptor. *Supported by grants DA04420, NS16433 and NS14014899 from NIH.*

399.3

ULTRASTRUCTURAL CHANGES IN TERMINALS IN THE DORSAL COLUMN NUCLEI OF DIABETIC RATS B.C. Albright, Dept. of Physical Therapy, East Carolina University, Greenville, NC 27858, M.R. Brown* and H.R. Knull*, Dept. of Biochemistry & Molecular Biology, School of Medicine, Univ. of North Dakota, Grand Forks, ND 58202.

The ultrastructure of terminals in the gracile and cuneate nuclei of streptozotocin (STZ) diabetic rats were examined at 1,3,10 and 30 days after the onset of diabetes. Terminals in the gracile nucleus were observed to exhibit a morphology characteristic of neuroaxonal dystrophy in all experimental groups. Only the 30 day diabetic group showed dystrophic terminals in the cuneate nucleus. The dystrophic response was characterized by the decrease in population of synaptic vesicles, the presence and increased accumulation of tubulovesicular smooth endoplasmic reticulum, the increased presence of various forms of cellular debris, and the decrease of synaptic complexes. The proliferation of glial processes was evident in all groups but most notable in the 14 and 30 day groups. Occasionally, terminals which appeared dystrophic were observed in normal and control animals. These findings show that severe diabetes as modeled by the STZ diabetic rat has an early and progressive dystrophic affect on terminals in a somatosensory nucleus of the central nervous system. Many of the dystrophic terminals had a morphology and synaptic configuration similar to primary afferents of the dorsal column nuclei. These observations are in agreement with clinical and experimental findings which often show the initial impairment of long sensory fibers and introduces the concept that the impairment of central transmission may be a contributing factor in the sensory deficits seen in diabetic neuropathy.

399.4

CALCITONIN GENE-RELATED PEPTIDE (CGRP) IMMUNOREACTIVITY IN THE CAT SACROCAUDAL SPINAL CORD. C.R. Murray* and L.A. Ritz. Depts. of Neuroscience and Neurosurgery, Univ. of Florida College of Medicine, Gainesville, Fl. 32610.

Our laboratory has been investigating the anatomical, physiological and behavioral organization of the cat sacrocaudal spinal cord, the portion of the neuraxis that innervates the tail. Recently, we have been concerned with the distribution of primary afferent fibers that exhibit CGRP immunoreactivity. CGRP labeling was found, as expected, within the superficial dorsal horn. A dense band formed across the entire mediolateral extent of the superficial portion of the fused dorsal horns. Labeling was localized throughout laminae I-V of the dorsal gray commissure. These fibers expanded rostrocaudally within lamina V. Labeling was also observed in an extensive mediolateral distribution at the level of the dorsal commissure (laminae IV/V). Horizontal sections revealed a 200-300 μm periodicity to the dorsal commissural bundles. There appears to be a greater distribution of CGRP within the deeper portions of the sacrocaudal dorsal horn than in other regions of the spinal cord.

Research supported by grant NS27511.

399.5

THE DISTRIBUTION OF C-FOS POSITIVE NEURONS IN THE LUMBOSACRAL SPINAL CORD OF THE CAT FOLLOWING ELECTRICAL STIMULATION OF THE PELVIC AND PUDENDAL NERVES. L.Birder, J.R.Roppolo, S.Smerin, V.Erickson*, W.de Groat, Dept. of Pharmacology, U. of Pittsburgh, PA 15261.

The pelvic (PN) and pudendal (PUDN) nerves contain afferent fibers which are involved in sexual and excretory functions. The present study examined the central projections of these afferents by detecting C-FOS in spinal cord interneurons following unilateral electrical stimulation of the PN and PUDN in either decerebrated or urethane (1.2 gm/kg) anesthetized cats. The PN and PUDN were stimulated at 2.5 times the threshold to produce bladder or perineal muscle contractions, respectively (usually 4-8 volts, 0.05 msec, 20 HZ, 10 sec on, 10 sec off, for 1 hour). One hour following the end of stimulation animals were perfused and tissue processed. PN stimulation produced the largest number of C-FOS cells in S_1 and S_2 segments, with fewer cells extending into L_7 and S_3. C-FOS positive neurons were found in the lateral lamina V at the base of the dorsal horn (DH), in the intermedio-lateral grey, in the superficial layers of the DH, and in the dorsal grey commissure (DGC). PUDN stimulation produced a wider distribution of C-FOS positive cells in L_6-S_3 with the densest labelling in S_1-S_2. The superficial lamina as well as laminae IV-VI and DGC contained positive neurons.

This study demonstrates similarities in the distribution of PN and PUDN afferent projections to the spinal cord and identifies possible interneuronal sites involved in the coordination of visceral and somatic components of urogenital function.

(Supported by NIH contract N01-NS-9-2366)

399.6

GENE DELIVERY TO RAT SENSORY GANGLIA AND SPINAL CORD CELLS USING HERPES VIRUS VECTORS. G.Davar, C. Meaney*, J.K. Andersen, M. Kramer*, W. Bebrin*, B.P. Vos, R. Burstein, D. Garber*, W. Rosenberg, R. Maciewicz, D. Coen*, X.O. Breakefield, Neuroscience. Center., Mass. General Hosp. East, Charlestown, MA 02129; and Neuroscience Program and Genetics Dept., Harvard Med. Sch., Boston MA 02115.

The delivery of genes, to alter the physiology of somatosensory neurons, may be possible using herpes simplex virus (HSV) type 1 vectors mutated by insertion of DNA elements into the thymidine kinase (TK) gene. To achieve gene delivery to peripheral and central neurons, we tested thymidine kinase-negative (HSV TK$^-$) vectors containing the marker gene, lacZ, under the control of different mammalian and viral promoters. Regulatory elements included: HSV promoters classified as immediate-early (alpha 4 in mutant RH105), early (beta 8 in RH116; both from Drs. Ho and Mocarski, Stanford Univ.), and late (gE in vgE26; from Dr. Weir, Walter Reed Hosp.), as well as the putative latency promoter (in tkLATZ1); and non-HSV promoters, the Moloney murine leukemia virus LTR (in tkLTRZ1) and the neuron-specific enolase promoter (in NSE-lacZ). Vectors were applied at an MOI of 0.001-0.1 to neuron-enriched neonatal rat dorsal root ganglion (DRG) cultures and $3x10^8$ PFU (RH116) into rat dorsal spinal cord or snout. In DRG cultures large numbers of positively-staining cells were observed at 4 and 11d after inoculation with all vectors except tkLATZ1. In spinal cord numerous positively-staining cells were observed in the dorsolateral funiculus adjacent to the injection site at 2d after inoculation. At 3d after inoculation onto the snout, a punctate perinuclear staining pattern was observed in a few cells in the ipsilateral trigeminal ganglion. Immunostaining with antibodies to neurofilament and S100 is being used to determine which cell types are expressing beta-galactosidase. Differences in the staining pattern following inoculation into spinal cord or snout may depend on the number of viral particles that entered the cell nuclei, and/or on the relative potency of the different promoters in different cell types.

399.7

GLUTAMATE IMMMUNOSTAINING IN THE DORSAL COLUMN SYSTEM OF RATS. R. Giuffrida, S. De Biasi, M. Bellomo and A. Rustioni, Ist. Fisiologia Umana, Univ. Catania; Dip. Fisiol. Biochim. gen, Univ. Milano; Department. of Cell Biology & Anatomy, University of North Carolina, Chapel Hill, NC 27514.

Immunocytochemical experiments have shown that glutamate-positive neuronal somata in dorsal root ganglia are mostly small. Terminals of unmyelinated and small myelinated fibers in the superficial laminae of the spinal cord were also immunostained by the same glutamate antiserum. In the present experiments, we have investigated whether comparably good correlation exists with another class of primary afferent neurons and their terminals—those ascending in the dorsal columns and terminating in the dorsal column nuclei. At least 75% of terminals identified by anterograde tracing as endings of primary fibers ascending in the dorsal columns are glutamate-immunopositive. However, the majority of dorsal root ganglion neurons with an ascending branch to the the dorsal column nuclei have large somata, and only 20 to 30% of projecting cells are glutamate-immunopositive. The difference in soma size between most dorsal root ganglion neurons that are glutamate-positive and those that project to the dorsal column nuclei suggests that glutamate may be present in cell bodies of large neurons of the dorsal root ganglia in a concentration insufficient to be detected with our present experimental conditions. This and alternative explanations for the apparent discrepancy are currently being explored.

399.8

COEXISTENCE OF GLUTAMATE AND ASPARTATE IN TERMINALS OF PRIMARY AFFERENTS TO THE SUPERFICIAL LAMINAE OF THE DORSAL HORN. K.D. Phend, R.J. Weinberg, and A. Rustioni, Depts. of Cell Biology & Anatomy and. of Physiology, U. of North Carolina, Chapel Hill, NC 27599.

In previous reports we have shown that terminals of dorsal root ganglion afferents in superficial laminae of the dorsal horn are enriched with glutamate, a probable neurotransmitter for these primary afferents. More recently, we have shown that presumed primary afferent terminals may be also enriched in aspartate although it is uncertain whether aspartate is released as neurotransmitter. We have combined double-staining immunocytochemistry with anterograde transport to verify the simultaneous presence of elevated levels of both amino acids in primary afferent terminals. WGA-HRP was injected into the sciatic nerve of anesthetized rats. After 2-7 days, animals were perfused with 2.5% glutaraldehyde/ 0.5% paraformaldehyde/ 0.2% picric acid. Fifty mm Vibratome sections of L4 were cut, reacted for TMB with tungstate stabilizer and embedded in plastic. Nickel mesh grids with thin sections were stained for glutamate and aspartate separately or in combination using different sizes of gold particles (Phend et al., Neurosci. Abst., 1990). Double staining for glutamate and aspartate was commonly observed among terminals of dorsal root afferents likely to be associated with unmyelinated and small myelinated primary afferents. A relation between these observations and the release of glutamate and aspartate from the same terminal of dorsal root fibers remains to be established.

399.9

SPROUTING OF THINLY MYELINATED AND UNMYELINATED FIBERS IN THE PARTIALLY DENERVATED PREPARATION. J.X. Bao, J.B. Munson and P.J. Reier Dept. of Neuroscience and Neurosurgery, Univ. of FL, Gainesville, FL 32610.

The goal of this study is to use established CGRP immunocytochemical staining in combination with electrophysiological mapping techniques to examine further the possibility of sprouting of thinly myelinated and unmyelinated fibers in the partially denervated preparation.

Adult Sprague-Dawley rats received unilateral dorsal root ganglionectomies of L1-L4 on the right or left side followed three weeks later by contralateral ganglionectomies. Terminal electrophysiological and subsequent immunocytochemical analyses were performed one week after the second procedure. Recording tracks were made through the dorsal horn with a tungsten microelectrode. The electrode was advanced dorsoventrally while the ipsilateral sural nerve was stimulated at Aαβ, Aδ and C fiber strengths. A series of symmetric tracks was made on the chronically denervated side and on the control side in the same animal.

Preliminary results indicate that the area of CGRP staining fibers was much larger on the chronically denervated side than on the side deafferented one week prior to physiological evaluation. Functionally, more units responded to sural nerve stimulation on the long-term denervated side. These results suggest possible sprouting of thinly myelinated and unmyelinated afferent fibers in the partially denervated rat spinal cord. Supported in part by NS15913 and NS72300.

400.1

WINDUP AND SYNAPTIC FACILITATION IN THE NEONATAL RAT SPINAL CORD IN VITRO. C.J. Woolf*, S.W.N.Thompson* and L.Sivilotti. SPON: Brain Research Association, Anatomy Dept., University College, Gower St., London WC 1E 6BT.

Low frequency repetitive stimulation of high threshold primary afferents elicits sustained depolarization and action potential windup in flexor motoneurons in vitro (Thompson et al., Eur.J.Neurosci. 2, 638, 1990). We have now examined the synaptic facilitation associated with the production of this pattern of progressive response increase. Intracellular recordings were obtained with K acetate electrodes from the young rat hemisected spinal cord preparation. In dorsal horn neurons in vitro, windup in response to high intensity stimulation of a lumbar dorsal root (0.5-1 Hz, 20 s) was observed less frequently than in ventral horn neurons in vitro or in dorsal horn cells in vivo. This phenomenon was associated with a facilitation of the synaptic input elicited by activation of low threshold afferents from both the conditioned and the adjacent (test) root, which could not be accounted purely by the cumulative membrane depolarization induced by conditioning. While neurons which generated windup had more prolonged synaptic potentials in response to single shock afferent stimulation, no correlation could be established between the occurrence of windup and the resting membrane potential, input resistance or I/V plot characteristics of a cell. Manipulation of the cell membrane potential by current injection during conditioning or increases in the frequency of the conditioning train failed to elicit windup in cells that did not respond to the control conditioning protocol. These data suggest that both pre and postsynaptic components contribute to afferent-induced sensitization in the spinal cord and that this is a network-related property of spinal responses.

400.2

EFFECTS OF ENDOGENOUS GABA ON AXONAL CONDUCTION IN THE NEONATAL RAT DORSAL COLUMN. M. Lee*, K. Sakatani, A.Z. Hassan*, M. Chesler, and W. Young. Dept. of Neurosurgery, NYU Medical Center, New York, NY 10016

We have previously shown that GABA-A receptors activation can modulate axonal conduction in isolated dorsal column of the neonatal rat spinal cord *in vitro* (Sakatani, et al. Brain Res., 542: 273-279, 1991). However, it is not known whether the GABA-A receptors in the dorsal column are activated by endogenous GABA under physiological conditions. We consequently studied the effect of a GABA-uptake inhibitor (nipecotic acid; NPA) and a GABA-A antagonist (picrotoxin; PTX) on axonal conduction in the dorsal column, using whole neonatal (1-5 day-old) rat spinal cord (below T1) *in vitro*. The dorsal column was stimulated (0.5 msec, 2 mA,, 0.2 Hz) by a bipolar platinum electrode, placed on L5 dorsal root entry zone. Orthodromic dorsal column compound action potentials (CAPs) were recorded at two points with glass micropipettes (1 M NaCl).

NPA (1 mM) decreased CAP amplitudes by 12.5 ± 9.6 % of control (mean ± SD, n=4). This effect of NPA was blocked by PTX (1 mM). GABA at 0.1 mM mimicked the effect of NPA. However, the time course of NPA action was slower than that of GABA action, suggesting that NPA increased background extracellular GABA levels which would activate GABA-A receptors in the dorsal column. PTX (1 mM), alone, increased population conduction velocities, calculated from latencies of negative peaks, by 18.7 ± 3.2 % of control (n=3), suggesting that PTX blocks tonic endogenous GABA influences on the dorsal column axons.

These results suggest that pre-myelinated dorsal column axons of the neonatal rat spinal cord are tonically affected by endogenous GABA under physiological conditions. While the biological role of interaction of GABA and GABA-A receptors in the dorsal column is not apparent, these results support our hypothesis that axonal conduction in long tracts of the spinal cord is subject to non-synaptic modulation. Supported in part by NIH grant NS10164.

400.3

DEVELOPMENT OF THE SOMATOTOPIC ORGANIZATION OF THE PRESYNAPTIC NEUROPIL IN RAT SPINAL DORSAL HORN. K. Mirnics and H.R. Koerber. Dept. of Neurobiology, Anatomy and Cell Science, University of Pittsburgh, School of Medicine, Pittsburgh, PA 15261.

In order to examine the development of the topography of primary afferents in the dorsal horn, fibers were labeled in embryos fixed at different stages (E15-P0) with either DiO, DiI or DiA. Dye crystals were placed on isolated dorsal root ganglia (ventral roots cut). Combinations of dyes were used so that the spatial relationships of the central projections of different spinal dorsal roots (L3 and L4) could be examined within the same embryo. The reduced preparations were stored in fixative at 37°C for 2 - 7 days depending upon the diffusion distance. Vibratome sections (50 microns) of the spinal cord were cut in either the transverse, horizontal or sagittal planes. At about E15 primary afferent collaterals extended in the primordium of the dorsal funiculus at least two segments rostral and caudal to their entry zone. In addition, at the level of the entry zone collaterals could be seen coursing medially across the surface of the gray matter. These fibers in turn supported collaterals which penetrated into the medial dorsal horn. Although substantial overlap of fibers from adjacent dorsal roots was observed in the primordium of the dorsal funiculus very little overlap in afferent projections was observed in the gray matter at this stage. The rostrocaudal and mediolateral extent of collaterals in the gray matter increased in successive stages until about E20 when the central projection zones and the degree of overlap was similar to that seen in postnatal animals. These results suggest that although primary afferent fibers have collaterals covering several segments in the dorsal funiculus they first enter the dorsal horn at the level of their entry zone and later expand into their somatotopically appropriate locations. Supported by NS-23725 (HRK).

400.4

SYNAPTIC CONNECTIONS OF FUNCTIONALLY IDENTIFIED INTERNEURONS IN HAMSTER SPINAL NUCLEUS PROPRIUS IN VITRO. S.P. Schneider and D.R. Sandiford*. Department of Physiology, Univ. of N. Carolina, Chapel Hill, NC 27599-7545.

To investigate anatomical substrates underlying synaptic integration in the nucleus proprius (NP), we studied the ultrastructure of synaptic connections of NP interneurons in an isolated spinal cord preparation with intact sensory innervation.

NP interneurons were physiologically characterized and stained intracellularly with horseradish peroxidase (HRP). Terminal axon branches labeled with HRP were unmyelinated or thinly myelinated. Labeled synaptic boutons that were located in proximity to the parent cell body (i.e. within an area comparable to one spinal segment) contained small, round vesicles and contacted small-to-medium sized dendrites (0.3 to 1.5 um in diameter). Labeled boutons that were sectioned serially were sometimes presynaptic to more than one dendrite. Axosomatic contacts were rarely observed. In some cases labeled boutons were postsynaptic to unlabeled axon terminals.

These findings suggest that axonal connections between NP neurons within a spinal segment are established mainly on dendrites, as are the central terminals of cutaneous afferent fibers. Thus, the activity of NP neurons is influenced by both primary afferent and local interneuronal input.

Supported by grant NS25771 from the NINDS.

400.5

RELATIVE NUMBERS OF SPINOTHALAMIC, SUPRASPINAL PROJECTION, PROPRIOSPINAL AND LOCAL CIRCUIT NEURONS IN THE SUPERFICIAL DORSAL HORN (SDH) OF THE RAT LUMBAR SPINAL CORD. T.N. Bala,* D.S. Knight, and J.A. Beal. Dept. of Cell Biol. and Anatomy, LSU Medical Ctr., Shreveport, LA 71130.

SDH neurons in the lumbar spinal cord (segment L1) which project to different levels of the neuraxis were labelled via retrograde transported fluorescent tracers delivered at the following sites: 1) the dorsal thalamus to label spinothalamic neurons, 2) a spinal hemisection at spinal cord segment C3 to label all supraspinal projection neurons and, 3) the dorsal gray in spinal segment T12 to label propriospinal neurons with short ascending connections. Cells were counted in each group and numerical estimates of neurons with dual projections, e.g., spinothalamic neurons with propriospinal connections, were determined by comparing the temporal neurogenic pattern of neurons in each group using tritiated thymidine autoradiography. Cell counts indicate that approximately 10% of the neurons in lamina I are supraspinal projection neurons and 50% of these project to the dorsal thalamus. In contrast, less than 1.0% of the neurons in lamina II have supraspinal projections. Short ascending propriospinal neurons are nearly equal in number in laminae I & II and represent 10 to 15% and 2.0 to 3.0% of the total cell populations in laminae I & II, respectively. The remaining SDH neurons, 75% of the lamina I neurons and 95% of the lamina II neurons, are presumed to be local circuit neurons. Supported by NSF grant #BNS-8908601.

400.6

SYNAPTIC ACTIVITY OF FROG SPINAL DORSAL HORN NEURONS. J.A. Meade, H. Tan and V. Miletic. Dept. Comp. Biosci., Sch. Vet. Med., Univ. of Wisconsin, Madison, Wi. 53706.

Dorsal horn neurons were studied in intracellular recordings from isolated hemisected spinal cords of adult frogs (R. pipiens). The average resting membrane potential was -68± 12 mV (S.D.) (n=118, range= -50 to -98 mV). The average membrane input resistance was 117± 68 MΩ (S.D.) (n=61, range=20 to 340 MΩ). Preliminary experiments suggest that the neurons have a membrane time constant of approximately 20 msec. Spontaneous action potentials were observed in 20 of 160 neurons. These action potentials occurred tonically (18/20) or in bursts (2/20). Excitatory postsynaptic potentials (n=77/160), inhibitory postsynaptic potentials (n=7/160), and a combination of both (n=4/160) were observed to occur spontaneously.

Experiments were also conducted in which the dorsal root was stimulated by a suction electrode while the postsynaptic response was monitored in a dorsal horn neuron. The threshold for evoking a response in the dorsal horn neuron was 129± 60 μA (S.D.) (n=78, range=54 to 424 μA). The average latency of response was 7± 11 msec (S.D.) (n=42, range=0.5 to 68 msec). Compound postsynaptic potentials were observed in a few experiments. Preliminary experiments on the reversal potential suggest that the ionic conductances of the evoked postsynaptic potential are similar to those reported for mammalian dorsal horn neurons. (Supported by NIH NS21278).

400.7

OUTFLOW OF ENDOGENOUS GLUTAMATE AND ASPARTATE FROM THE RAT SPINAL DORSAL HORN IN VITRO BY SELECTIVE ACTIVATION OF SMALL PRIMARY SENSORY NEURONS. I. Kangrga, R. Cerne and M. Randić. Dept. Vet. Physiol. Pharmacol. Iowa State University, Ames, IA 50010.

Whereas excitatory amino acids glutamate (Glu) and aspartate (Asp) have been implicated as neurotransmitters of the primary afferent fibers (PAF) the contribution of different categories of PAF to the release of Glu and Asp, and the regulation of this release by endogenous opioid peptides at the spinal level have not been characterized as yet. We have investigated whether the rate of outflow of Glu, Asp and glutamine (Gln) from the spinal dorsal horn (DH) is modified in response to selective chemical activation of the small unmyelinated PAF by resiniferatoxin (RTX) and during the perfusion of the slice with a δ-opioid receptor agonist (D-Pen^2,D-Pen^5)enkephalin (DPDPE). In superfused rat spinal cord slice - dorsal root ganglion (DRG) preparation, the DRG were superfused with RTX and the slice superfusate was collected and analyzed for amino acid content by HPLC with fluorimetric detection. RTX (0.1-10 nM for 3-6 min) produced a prolonged (10-15 min), up to three-fold increase in the rate of basal outflow of Glu, Asp and Gln (n=6). Marked desensitization of the RTX effect was observed with the second RTX challenge. Preincubation of slices with DPDPE (10 μM, 12-18 min) effectively attenuated the RTX (2 nM)-evoked outflow of all the three amino acids. Superfusion with DPDPE resulted in a decreased rate of basal outflow of Glu and Asp in a proportion of slices (n=3). It is suggested that a population of small unmyelinated PAF may be an important neuronal source of released Glu and Asp in the spinal DH. A role for δ-opioid receptors in modulation of somatosensory processing at the spinal level is indicated. Supported by NS 26352 and BNS 841 8042.

400.8

L-GLUTAMATE AND ADENOSINE-5'-TRIPHOSPHATE ACTIONS ON SUBSTANTIA GELATINOSA NEURONS. J. Li* and E. R. Perl
Physiology, Univ. No. Carolina-CH, Chapel Hill, N.C. 27599.

We sought evidence on whether ATP may be a synaptic transmitter for primary afferent fibers, by comparing actions of L-glutamate (GLU) and adenosine-5'-triphosphate (ATP) on substantia gelatinosa (SG) neurons in superfused 400-500 μm transverse slices of the spinal cord from young hamsters. Whole-cell patch recordings were made with Cs^+ or K^+ filled electrodes. Both bath-applied GLU and ATP (0.4 to 3 mM) caused similar fast depolarizations and generated action potentials in the majority of 70 neurons. TTX blocked the action potentials, but left fast GLU and ATP inward currents unaffected. All but one of 45 cells showing fast excitation by ATP was also excited by GLU; however, 10 of 54 neurons excited by GLU were unresponsive to ATP. ATP-γ-S, a non-hydrolysable ATP analogue, was at least an order of magnitude more potent than ATP in evoking fast inward current while ADP, AMP and adenosine had no effect or were much less potent than ATP. A few neurons exhibited a prolonged depolarizing current to GLU after the initial fast component; a larger number showed prolonged inward current after ATP and ATP-γ-S. Since the SG receives most of its primary afferent input from thin fibers, these observations appear consistent with an ATP-like molecule acting as an excitatory synaptic transmitter for some of these fibers. (Supported by research grant NS-10321 from the NINDS.)

400.9

$5HT_3$ RECEPTOR ACTIVATION DEPRESSSES SYNAPTIC TRANSMISSION BETWEEN CUTANEOUS AFFERENTS AND SENSORY INTERNEURONES IN XENOPUS TADPOLE SPINAL CORD. **K.T. Sillar, A.J. Simmers* & J.F.S. Wedderburn.* Gatty Marine Lab., University of St. Andrews, Fife KY16 8LB, SCOTLAND.**

Serotonin acting at the $5HT_3$ receptor subtype may be involved in the modulation of sensory pathway function (Lei & Wilcox, Soc. Neurosci. Abstr, 16,97, 1990). In hatchling Xenopus tadpoles exogenous 5HT suppresses skin sensory activation of locomotor rhythmicity and we have explored the pharmacology and site of action of 5HT in mediating this effect.

5HT (0.5- 5μM) reversibly increases the stimulus threshold for activation of fictive swimming by up to 1000%. Impulses in the primary afferents are not blocked by 5HT, indicating that its effects are mediated centrally. Intracellular recordings from postsynaptic sensory interneurones show that synaptic transmission is reversibly reduced by 5HT with no apparent change in conductance, suggesting a presynaptic site of action. The depression by 5HT is reduced by the specific $5HT_3$ receptor antagonist, MDL72222, but not by methysergide or cyproheptadine. We conclude that in this simple developing vertebrate nervous system $5HT_3$ receptors are involved in the presynaptic inhibition of cutaneous sensory synaptic transmission.
Supported by the Royal Society and SERC (UK).

400.10

FACILITATION OF THE TAIL-FLICK REFLEX BY INTRATHECAL ADMINISTRATION OF SUBSTANCE P AND BY NOXIOUS CUTANEOUS STIMULATION IS BLOCKED BY CP-96,345, A NONPEPTIDE SUBSTANCE P (NK-1) RECEPTOR ANTAGONIST. J.L. Henry, V. Radhakrishnan and K. Yashpal, Departments of Psychiatry and Physiology, McGill University, Montreal (Quebec)

Substance P has been proposed to regulate the excitability of nociceptive neurons in the dorsal horn of the spinal cord and to facilitate the nociceptive tail-flick reflex. Therefore, we examined the effects of CP-96,345 on facilitation of the tail-flick reflex 1) by intrathecal administration of 6.5 nmol of substance P at the lumbar spinal level in awake rats and 2) by noxious cutaneous stimulation in anaesthetized rats by immersing the tip of the tail in water at 55°C for 1.5 min. Reaction time was decreased by about 70% by intrathecal administration of substance P and by about 40% by tail immersion. Systemic administration of CP-96,345 (0.5 i.v. or 5 s.c. mg/kg), but not of saline, blocked the facilitation induced by intrathecal administration of substance P and by tail immersion. However, neither CP-96,345 nor CSF altered baseline reaction time. These results indicate that while NK-1 receptors do not appear to be involved in mediating the tail flick reflex in either preparation, they may be involved in expression of the facilitation of this reflex by substance P and/or by a related peptide. (Supported by Pfizer Central Research and by the Canadian MRC)

400.11

NOXIOUS CUTANEOUS STIMULATION PRODUCES ANALGESIA IN THE TAIL-FLICK TEST AND DECREASES BINDING OF SUBSTANCE P IN THE DORSAL HORN K. Yashpal, S. Kar, R. Quirion and J.L. Henry, Department of Psychiatry, McGill University, Montreal and Douglas Hospital Research Centre, Verdun, Quebec, Canada

Substance P (SP) has been proposed to play a regulatory role in nociception and both SP-like immunoreactivity and receptors are present in high quantities in the dorsal horn of the cord. However, direct evidence supporting this porposal is still lacking. Therefore a series of experiments was undertaken to examine this proposal. Sprague Dawley rats were anaesthetized i.p. with Na-pentobarbital (20 mg/kg) and chloral hydrate (120 mg/kg). After baseline readings were taken in the tail-flick test, one hindpaw was immersed in water at 55°C for 1.5 min. Readings were taken at 3 min intervals for a subsequent 20 min. For autoradiographic binding studies, rats were decapitated at 1, 10 and 60 min following immersion. The spinal cords were removed and incubated in buffer containing [^{125}]I-BH-SP as described (*Brain Res. 506, 259, 1990*). In the physiological studies, immersion induced an increase in the reaction time to 150 % of the control values at 1 min which gradually decreased over 10 min. Binding of SP was depleted in the superficial parts of the dorsal horn of the lumbar cord in the 1 min group, with partial and full recovery in the 10 and 60 min groups, respectively. Binding around the central canal remained unaltered by immersion. These results indicate that noxious cutaneous stimulation to a hind foot induces an analgesia in the tail-flick test. Furthermore, the decreased [^{125}]I-SP binding is most easily explained as being due to the occupation of NK-1 receptors by endogenously released substance P. (Supported by grants from the Canadian MRC to JLH and RQ)

400.12

RESPONSES OF DORSAL HORN NEURONES TO NOXIOUS CUTANEOUS STIMULATION AND TO IONTOPHORETIC APPLICATION OF SUBSTANCE P ARE BLOCKED BY THE SELECTIVE, NONPEPTIDE SUBSTANCE P (NK-1) ANTAGONIST, CP-96,345. V. Radhakrishnan and J.L. Henry, Departments of Physiology & Psychiatry, McGill University, Montreal, Quebec.

Until recently, the known antagonists at the substance P (NK-1) receptor were all metabolically unstable peptides. Some of them were also known to exhibit agonistic and/or neurotoxic properties. Recently, Snider *et al.* (*Science* 251:435, 1991) and McLean *et al.* (*Science* 251:437, 1991) reported a specific, nonpeptide NK-1 receptor antagonist, CP-96,345, devoid of agonistic property. We tested the effect of CP-96,345 on the responses of dorsal horn neurones to iontophoretic application of substance P (which in electrophysiological studies selectively activates nociceptive neurones) and to noxious cutaneous stimuli. In chloralose-anaesthetized cats, extracellular unit activity was recorded from neurones in segments L_5-L_7 using multibarrelled micropipettes. Neurones were first classified on the basis of their responses to natural stimuli to the skin. Six of the 8 neurones that responded to noxious thermal stimulation also responded to substance P. The slow excitation produced by substance P (80-120 nA), which lasted for 2-3 min, was effectively blocked by CP-96,345 (0.5 mg/kg, i.v.). CP-96,345 also attenuated the responses to noxious heat stimulation in these units, markedly reducing the after-discharge following stimulation. In the case of two substance P-insensitive units, the responses to noxious heat was unaffected by CP-96,345. CP-96,345 also inhibited the response of 2/4 units tested with noxious mechanical (pinch) stimulation. The results support the involvement of NK-1 receptors in the mediation of the after-excitation induced in some dorsal horn nociceptive neurones by substance P and by noxious cutaneous stimuli. (Funded by Pfizer Central Research, the Canadian MRC and NIH).

400.13

CP-96,345, A NONPEPTIDE SUBSTANCE P (NK-1) RECEPTOR ANTAGONIST, BLOCKS A SLOW EPSP ELICITED IN DORSAL HORN NEURONES *IN VIVO* BY SUSTAINED NOXIOUS AND BY REPETITIVE C-FIBRE STIMULATION, BUT DOES NOT BLOCK THE *FAST* C-FIBRE EVOKED EPSP. Y. De Koninck and J.L. Henry, Depts. Physiology and Anaesthesia Res., McGill Univ., Montréal, Qué., H3G 1Y6.

As substance P (SP) has been implicated in mediating nociceptive responses in the dorsal horn, the slow time course of action of the peptide when applied exogenously suggests that it may play a role in mediating a slow EPSP to noxious peripheral stimulation. Hence, we attempted to identify a slow nociceptive response which could be tested with a novel SP antagonist, CP-96,345, selective for the NK-1 receptor. In α-chloralose anaesthetized cats, we recorded intracellularly responses of nociceptive dorsal horn neurones to noxious stimulation of the cutaneous receptive field and electrical stimulation of afferent nerves. Noxious stimulation but not innocuous stimulation produced a slow, prolonged EPSP which outlasted the end of the stimulation by 20s to 3min. A similar slow, prolonged EPSP was obtained following a train of high intensity stimulation (10-15Hz for 4-10s) to afferents nerves at intensities recruiting C-fibres, but not at intensities recruiting only Aβ fibres. The slow EPSP in each case was associated with an increase in input resistance and was reduced with hyperpolarization and increased with depolarization as has been described in response to exogenous application of SP. This contrasted with the reversal potential of the C-fibre latency EPSP in response to a single shock stimulation, which was similar to that of excitatory amino acid EPSPs of the non-NMDA type. Administration of CP-96,345 (0.5-2.0mg/kg i.v.) specifically and reversibly blocked the slow EPSP to sustained noxious stimulation, but not the brisk response to innocuous input nor the *fast* C-fibre latency EPSP to single shock electrical stimuli. These results suggest that SP is involved in the mediation of a period of hyperexcitability characterized by a slow EPSP following noxious stimulation in the dorsal horn. This period of hyperexcitability in nociceptive dorsal horn neurones may represent the neuronal substrate underlying central components of hyperalgesia. (Funded by Pfizer Central Research, the canadian MRC and NIH. YDK was funded by the FRSQ.)

400.14

A LIGHT AND ELECTRON MICROSCOPIC ANALYSIS OF GLYCINE IMMUNOSTAINING IN THE MONKEY SPINAL CORD. G.L. Hargett and S.M. Carlton, Dept. of Anatomy and Neurosciences, Marine Biomedical Institute, University of Texas Medical Branch, Galveston, TX, 77550.

The inhibitory transmitter glycine is found throughout the mammalian central nervous system. In rat, immunohistochemical studies at the LM and EM level demonstrate glycine-immunoreactive (IR) neurons, fibers and terminals throughout the spinal cord. In the present study, four (4) monkeys (M. fascicularis) were deeply anesthetized and perfused with mixed aldehydes. Portions of the cervical and lumbar cord were removed and sectioned at 25 μm on a vibratome. Glycine-IR profiles were visualized by immunostaining with anti-glycine using the PAP technique or by post-embedding immunogold staining. LM analysis revealed glycine-IR fibers to be present in all funiculi, but most concentrated in the myelinated fibers of the ventral funiculus. Glycine-IR neurons were present in all laminae, but at much lower concentrations in LII, IX, and X. Small diameter, round or oval cells were located in the upper laminae while larger cells with branching dendrites were present in deeper layers. EM analysis of the dorsal horn showed glycine-IR present in axon terminals, cell bodies, and both myelinated and unmyelinated fibers. Glycine-IR terminals contained round, clear vesicles and occasionally dense core vesicles. Glycine-IR terminals synapsed on both glycine-IR dendrites and unlabeled dendrites. Glycine-IR terminals were observed postsynaptic to glomerular primary afferents. Glycine-IR dendrites occasionally contained vesicles and synapsed on unlabeled dendrites. In the ventral horn, several different types of terminals synapsing on motoneurons were immunoreactive for glycine. (Supported by NS11255 and NS27910).

400.15

CO-LOCALIZATION OF SUBSTANCE P AND ENKEPHALIN IMMUNOREACTIVITIES IN SYNAPTIC BOUTONS APPOSED ONTO PHYSIOLOGICALLY IDENTIFIED DORSAL HORN NEURONES: AN ULTRASTRUCTURAL MULTIPLE-LABELLING STUDY. A. Ribeiro-da-Silva, Y. De Koninck, J.L. Henry and A.C. Cuello. Depts. Pharmacology & Therapeutics, Physiology and Anaesthesia Research, McGill Univ., Montréal, Qué., H3G 1Y6

The ultrastructural distribution of substance P (SP) and enkephalin (ENK) immunoreactivities was studied in association with identified dorsal horn neurones in the cat spinal cord. In chloralose-anaesthetized cats, intracellular recordings were obtained from dorsal horn neurones at the lumbar level using HRP filled electrodes. Cells were classified on the basis of their responses to natural stimulation of the receptive field. Subsequently, HRP was injected intracellularly and the cats were perfused with an aldehyde mixture. Vibratome parasagittal sections, 50 μm-thick, were obtained and incubated, in a single step, with a mixture of a bi-specific anti-SP/anti-HRP monoclonal antibody and an internally radiolabelled anti-ENK monoclonal antibody. After the diaminobenzidine reaction, the tissue was processed for electron microscopic radioautography. As previously demonstrated in the rat (Ribeiro-da-Silva *et al.*, *J. Neurosci.* 11:1068, 1991), SP and ENK immunoreactivities were co-localized in a population of axonal varicosities, mainly in laminae I and IIo. These double-labelled varicosities contacted either the dendrites or the cell bodies of intracellularly labelled dorsal horn neurones. One enkephalin-immunoreactive, lamina V, wide dynamic range neurone responded with a marked afterexcitation to noxious stimulation of the skin. In addition, this neurone had a large number of synaptic contacts (>30%) from varicosities positive for SP but not ENK; many of these varicosities are likely to be of primary sensory origin. These findings suggest a particular interaction between peptidergic primary sensory fibres and certain physiological types dorsal horn neurones. It also indicates an important participation of neurones colocalizing SP and ENK in the integration of sensory information.

(Supported by NIH; YDK was funded by the FRSQ)

400.16

RESPONSE CHARACTERISTICS OF DORSAL HORN NEURONS ROSTRAL TO A UNILATERAL PARTIAL LESION OF THE LOWER LUMBAR SPINAL CORD IN AWAKE, DRUG FREE, CATS. H. Hirata, H. Uchida*, K. Kishikawa, Y. Yamamori* and J.G. Collins. Dept. Anesthesiology, Yale University School of Medicine, New Haven, CT 06510

There is increasing evidence that damage to the peripheral or central nervous system may change the response profile of spinal dorsal horn neurons. The purpose of this study was to examine the effect of a unilateral partial cord lesion on dorsal horn neurons (DHNs) rostral to the lesion site. Cats were implanted with a chronic recording chamber centered over the 4th lumbar vertebra. At the same time electrolytic lesions were made one to two spinal segments caudal to the recording area in the dorsal and ventral gray matter on one side of the spinal cord. Extracellular recordings from the ipsilateral dorsal horn were made, beginning one week after surgery, in awake cats.

Thus far, a total of 19 single DHNs were studied over a period of up to two months. Of these, 17 units (89%) had low rates of spontaneous activity (less than 2 impulses/sec) and most of them n = 13 (68%) fired at less then 0.5 impulses/sec. These values do not appear to be greater than those previously obtained from non-lesioned animals. Of the 15 classified DHNs, 7 units (47%) were wide dynamic range (WDR) and 8 were low threshold (LT). The percentage of WDR neurons found in the lesioned cats was somewhat higher than the values previously obtained in normal animals (11%) but similar to that in cats with complete thoracic spinal cord transection (55%). More samples are necessary to determine if this increase in WDR neurons was due to the spinal cord lesions. An increased population of WDR neurons may contribute to chronic pain syndromes observed in spinal cord injured humans. (Supported by American Paralysis Association and Paralyzed Veterans of America Grants).

400.17

EFFECTS OF SLEEP ON SPINAL CORD DORSAL HORN LOW THRESHOLD NEURONAL ACTIVITY.

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Dept. of Anesth., Yale Univ. School of Med., New Haven, CT 06510

The purpose of this study was to examine the effects of natural sleep on the response properties of spinal cord dorsal horn neurons to low intensity stimulation of their peripheral receptive fields (RFs). Extracellular recordings of single spinal dorsal horn neurons (n = 6, to date) were made in physiologically intact, awake, drug-free cats during EEG confirmed wakefulness and sleep stages (control awake, eye closed and relaxed stage, slow wave sleep, rapid eye movement (REM) sleep). The size of RF area that was sensitive to light touch and the neuronal responses to brushing the RFs were determined. There was no change in the low threshold RF size of any neuron during any sleep state. One neuron showed an increased response to brushing during REM and slow wave sleep. Two neurons presented decreased response to brushing during various sleep stages. The other three neurons demonstrated no change in response to brushing during sleep. In one of these three neurons, propofol (7.5 mg/kg) was administered intravenously after sleep effects were studied. The response to brushing in that neuron was reduced after propofol. These results indicate that natural sleep itself caused alterations in spinal cord dorsal horn neuronal activity, in a way that may be different from our previous reports of suppressive effects of pentobarbital (Brain Res 525, 189-97, '90.) and propofol (Anesthesiology 73, A 697, '90.) on spinal sensory processing. Supported in part by NIH GM-29065

400.18

ASCENDING PROPRIOSPINAL CONNECTIONS TO THE UPPER CERVICAL SPINAL CORD IN THE RAT: POSSIBLE INVOLVEMENT IN FUNCTIONAL RECOVERY FOLLOWING SPINAL CORD INJURY.

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It is well known that a large proportion of spinal neurons belong to a class of cells that interconnect different segments and levels of the spinal cord. Although these propriospinal neurons have been implicated in both sensory and motor function, little is known about the organization or function of the rat propriospinal system, a species often used in studies of the intact or injured spinal cord. The present study was carried out to evaluate the distribution of rat propriospinal neurons projecting to the upper cervical spinal cord and to study their potential involvement in the recovery of function following spinal cord injury.

Fluorescent tracers, including Fluorogold, DAPI and Fast Blue, were injected into cervical segments C2-C3; injection volumes ranged from 0.5-1.0μl. Following survival times of 5-7 days animals were perfused transcardially with 4% paraformaldehyde, tissue sectioned at 35-50μm and viewed with Epi-fluorescent illumination. The distribution of neurons with ascending propriospinal connections included the dorsal and ventral horns, the region around the central canal and the lateral spinal nucleus. Although the majority of labeled neurons were found within 5 segments of injection sites, large numbers of cells were also found throughout the lumbosacral cord. In preliminary experiments, performance on sensory and motor tasks were assessed following mechanical or neurotoxic lesions in the thoracic cord (T7-T12). In these animals functional recovery was found to correlate with the presence of propriospinal labeling from cord levels below the lesions to injection sites in the upper cervical cord. (Supported by NS28059 and The Miami Project).

400.19

LABELING OF PRIMARY AFFERENT NEURONS INNERVATING SPINAL CORD AND PERIOSTEUM FOLLOWING SPINAL CORD TRANSECTION. E.L. Hill, H.A. Dancuasse*, and V.R. Holets. Division of Endocrinology and Dept. of Neurological Surgery and The Miami Project, Univ. of Miami, Miami, FL 33136.

Osteoporosis develops after spinal cord injury even with weight-bearing (as in the neonatally lesioned rat). Changes in central and peripheral sensory innervation may affect neuropeptides (such as CGRP) implicated as modulators of bone metabolism and may be involved in bone loss after spinal cord injury. Retrograde tracing techniques were used to demonstrate that primary afferent neurons supplying periosteum in affected bones also project to areas of spinal cord known to undergo reorganization after transection.

Five day old rats received a spinal cord transection at the T8 level. Six months after transection, the retrograde tracer Fast Blue was injected under the periosteum of the right tibia. Rhodamine-labeled latex microspheres were injected into the right half of the spinal cord at L1 5 days later. Rats were euthanized 3 days later. Dorsal root ganglia (DRG) from T8-S1 were examined for labeling. Unlesioned littermates were also labeled.

Fast blue labeled cells were confined to L1-L3. In some animals labeled cells were only observed in a single DRG. Rhodamine labeling was present in T8-S1 and was greatest in T9-L1. Double labeled cells were seen in L1-L3. CGRP immunoreactivity was found in both Fast Blue and rhodamine labeled cells. Many CGRP fibers are found in periosteum and CGRP has been implicated as a modulator of bone metabolism. Primary afferent nerve fibers containing CGRP are altered in the spinal cord after injury. The peripheral processes of these DRG neurons may also innervate bone and be altered after spinal trauma. Supported by The Miami Project to Cure Paralysis and The Daniel Heumann Fund for Spinal Cord Research.

400.20

SECONDARY NEURONAL DEGENERATION IN THE RAT SPINAL CORD. S. Saporta. Department of Anatomy. University of South Florida College of Medicine, Tampa, FL 33612.

There has been considerable interest in visualizing neurons in the spinal dorsal horn that undergo secondary degeneration following damage to dorsal root afferents or their cell bodies. With modification of a newly described histochemical technique using esterification followed by ionic silver "development" of damaged neurons described by van den Pol and Gallyas (J. Comp. Neurol. 296:654-673, 1990), it has been possible to visualize both surgically damaged dorsal root afferents within the dorsal horn; and, 2 - 3 weeks after the lesion, neurons in laminae I and II that stained as if they were damaged as a result of deafferentation. It is assumed that this response is a result of the loss of primary afferent synaptic contact, and most of these neurons will continue to undergo secondary degeneration. However, the degree of synaptic loss required to allow these neurons to stain is not clear. No neuronal somata were stained during the first week after dorsal root rhizotomy. It has been possible, moreover, to quantify the distribution of these somata. Of the 103 impregnated neurons studied to date, 36 somata (35%) were located in lamina I and 67 somata (65%) in lamina II. In addition, most of these neuronal somata were sufficiently well impregnated to allow their tentative classification as lamina I type I (n= 10) or type II (n= 26) cells; or lamina II stalked (n=15) or islet (n=21) cells.

PAIN PATHWAYS: SPINAL AND TRIGEMINAL

401.1

SPINAL CORD WIDE DYNAMIC RANGE, BUT NOT NOCICEPTIVE SPECIFIC NEURONS ENCODE INTENSITY OF LONG-DURATION HEAT PAIN. R.C. Coghill[1], D.J. Mayer[1], R.L. Hayes[2], and D.D. Price[3]. Dept. [1]Physiology, [2]Neurosurgery, and [3]Anesthesiology, Medical College of Virginia, Virginia Commonwealth University 23298-0551

A common feature of both clinical and experimental pain is the absence of habituation during prolonged exposure to noxious stimuli. The central nervous system mechanisms coding these prolonged sensations of pain, however, remain poorly understood. The present investigation examines the involvement of single spinal cord wide dynamic range (WDR) and nociceptive specific (NS) neurons to prolonged (20 minute) noxious thermal stimulation and relates them to psychophysical ratings of identical stimuli. Electrophysiological experiments were conduced in paralyzed, T-2 spinal cord transected rats. Neurons whose receptive fields included the distal portion of the hind limb were stimulated by cyclical immersion (5 sec in, 5 sec out) of the hind paw in a heated water bath. The receptive field of each cell was stimulated with a neutral (35° C; 2.5 min), a warm (43° C; 2.5 min), and one noxious temperature (45°, 46°, 47°, 48°, or 49° C; 20 min.). Only one cell was examined per rat.

NS neurons exhibited a temporal response pattern dramatically different from that of WDR neurons. NS neurons stimulated with 49° C temperatures reached a peak discharge frequency during the first two minutes of stimulation and habituated to baseline (35° C) levels in less than 5 minutes. Discharge frequencies of WDR neurons similarly were highest during the first two minutes of stimulation, but, importantly, failed to habituate substantially and were maintained at approximately 70% of peak levels during the subsequent 18 minutes of noxious stimulation. Psychophysical ratings of pain intensity closely parallel the temporal pattern of WDR responses, indicating that WDR neurons, but not NS neurons subserve long-term thermal nociception. Supported by PHS grant NS-24009-1A2 and FIDIA Pharmaceuticals.

401.2

ASSOCIATIVE LEARNING OF AN ANTINOCICEPTIVE RESPONSE AT THE LEVEL OF THE SPINAL CORD IN INTACT RATS. P.A. Illich, K.D. Burks*, & J.W. Grau. Dept. of Psychology, Texas A&M Univ., College Station, TX 77843.

Prior work suggests that the circuitry needed to obtain conditioned antinociception exists at the level of the spinal cord. Because our past work used a reduced preparation (spinal) it is not clear whether this type of learning normally occurs in intact subjects. The present study addresses this issue by testing whether subjects that had been trained with the cord intact will exhibit conditioned antinociception after the cord has been transected. Twelve intact rats received differential conditioning (which controls for sensitization and pseudoconditioning). One stimulus (the CS+) was paired with mild tail-shock (2-s, 0.5 mA), while the other (the CS-) was presented alone. Vibro-tactile stimuli to the left or right rear paw served as the CS+ and CS-. Which stimulus served as the CS+ was counterbalanced across subjects.The subjects received 30 CS+ and 30 CS- trials spaced 1 min apart. One hr after training all subjects received a spinal transection at T2. Twentyfour hrs later pain reactivity during the CSs was tested with the tail-flick test. Ten of the twelve subjects exhibited longer tail-flick latencies during the CS+ relative to the CS- ($p < .05$). These results suggest spinal systems can support associative learning in intact subjects. Supported by BNS 881981 to J.W.G.

401.3

SPATIAL DISTRIBUTION OF SPINAL CORD NEURAL ACTIVITY ENCODES THE DISTINCTION BETWEEN NOCICEPTIVE AND NON-NOCICEPTIVE SENSORY EVENTS D.D. Price,[1] R.C. Coghill,[2] R.L. Hayes,[3] and D.J. Mayer,[2] Departments of Anesthesiogy,[1] Physiology,[2] and Neurosurgery,[3] Medical College of Virginia, Richmond, VA 23298

Differences between distributions of spinal neural activity evoked by noxious (45,47,48,49°C) or non-noxious (35,43°C, vigorous mechanical brushing) stimulation were analyzed by the quantitative ^{14}C-2-deoxyglucose (2-DG) technique. Experiments were conducted in unanesthetized, T-2 spinalized rats paralyzed with curare. Stimulation of the whole left hindpaw was produced by cyclical immersion (5 sec in, 5 sec out) in a water bath in the case of temperature stimuli or from a servo-driven mechanical brush stimulus (vigorous mechanical). Each of 7 groups (5/group) were stimulated with one of the above listed stimuli. 2-DG procedures were those previously employed by Coghill et. al. (J. Neurophysiol. 65(1991)133-140). As previously reported, nociceptive stimulation produced somatotopically specific increases in glucose utilization with maximum activity occurring in L4 laminae V-VI. Metabolic activity expanded rostrally and caudally (L1 - L5) within laminae V-VI but not within laminae I-IV as noxious temperature increased. In contrast, vigorous mechanical stimulation evoked significant increases in metabolic activity only within the upper dorsal horn (laminae I-IV) and this activity was restricted rostral-caudally (L3, L4). Finally, although vigorous mechanical stimulation evoked greater activity than gentle mechanical stimulation (35°C water immersion) in the upper dorsal horn, it was much less than that evoked by nociceptive temperatures (45-49°C) in the deep dorsal horn. These findings further support the role of spatial recruitment in spinal mechanisms of nociception by indicating that the distinction between nociceptive and non-nociceptive sensory events may be reflected by both large differences in intensity and spatial distribution of neural activity (supported by NINDS Grant NS-24009-2A2).

401.4

CHARACTERIZATION OF NOXIOUS HEAT-EVOKED FOS PROTEIN-LIKE IMMUNOREACTIVITY IN THE RAT SACRAL SPINAL CORD. S.L. Jones. Department of Pharmacology, University of Oklahoma, Oklahoma City, OK 73190.

The expression of fos-related proteins can be utilized as markers for neuronal activation. The objectives of the present study were: 1) to characterize the expression of fos-like immunoreactivity in the sacral spinal cord evoked by the application of noxious heat to the tail, and 2) to determine if the expression of heat-evoked fos-like immunoreactivity can be modulated by intrathecally administered alpha adrenoceptor agonists. Rats were anesthetized with pentobarbital (45mg/kg, IP) and the tail was immersed in 55-60°C water for 10sec for 30 consecutive trials at 2min intervals. Time course experiments revealed that a 2 hour survival time resulted in optimal expression of fos-like immunoreactivity. The rats were perfused with paraformaldehyde; the sacral spinal cord was removed, vibratome sectioned (50µm, transverse), and processed for fos-immunoreactivity. Neurons demonstrating heat-evoked fos-like immunoreactivity were identified in superficial and deep dorsal horn laminae. Intrathecally administered norepinephrine (60 nmole) inhibits (to 47% of control, n=3) the expression of fos-like immunoreactivity; however, norepinephrine also appears to evoke the expression of fos-like immunoreactivity in a subpopulation of neurons located in the superficial dorsal horn laminae. Supported by Presbyterian Health Fdn. and OCAST.

401.5

THE RELATIONSHIP OF AN AVERSIVE OPERANT BEHAVIOR TO THE EXPRESSION OF *C-FOS* IN THE LUMBAR SPINAL CORD OF THE RAT. C.L.Lee, H.H.Willcockson*, C.J.Vierck, and A.R.Light. Dept of Physiology, UNC-Chapel Hill, Chapel Hill, NC 27599-7545. Expression of the proto-oncogene *C-fos* in spinal cord neurons is induced by stimulation of the skin presumably through activation by primary afferents. This study cor-relates the minimum intensity of electrical stimulation that motivates a rat to turn off this stimulus with the number of spinal neurons which label for *fos* protein immu-noreactivity in the lumbar dorsal horn of an anesthetized rat model. The behaving rat turned off the stimulus by pressing a bar with its forefoot 25% of the time at the lowest stimulus intensity (0.19 mA peak-to-peak) applied through contact electrodes on the dorsal and ventral surfaces of the rat's hindfoot. This corresponded to the production of *fos* immunolabeled neurons in laminae I and II of the dorsal horn which was above background level. At 0.34 mA, the rats pressed the bar 83% of the time and the number of *fos* labeled neurons was approximately twice the number labeled following the lowest level. At still higher stimulus levels (0.57mA and 2.83mA), the rats pressed the bar nearly 100% of the time and more quickly and with more force. These higher levels corresponded to more labeled neurons in laminae I and II as well as labeled neurons in deeper laminae. Thus, the number of *fos* immunolabeled neurons appears to be correlated with the aversive operant behavior. *Supported by NIH grants DA04420, NS16433, NS14899.*

401.6

EFFECT OF SPINAL SOMATOSTATIN OR KAINIC ACID SUPERFUSION AND NOXIOUS SKIN STIMULATION ON IMMEDIATE EARLY GENE EXPRESSION IN RAT SPINAL DORSAL HORN NEURONS *H. Beck and J. Sandkühler*, II. Physiologisches Institut, Universität Heidelberg, Germany.

Noxious skin stimulation causes rapid and temporal induction of immediate early genes such as *c-fos* and releases neuropeptides including somatostatin in the spinal dorsal horn. To test whether somatostatin is involved in the induction of *c-fos* experiments were performed on deeply pentobarbital anesthetized rats. Following laminectomy a pool was formed on the dorsum of the lumbar cord by means of a specially prepared silicon rubber. The pool was filled with artificial CSF before and after superfusion with somatostatin (10 μM, for 30 min) or kainic acid (0,3 mM, for 15 min). In other rats the glabrous skin at a hindpaw was stimulated by radiant heat (twelve 54°C pulses for 20s in intervalls of 1 min). One hour after these stimulations the rats were transcardially perfused with phosphate buffered saline and 4% paraformaldehyde. The FOS protein was detected by immunocytochemistry in free floating 50 μm frontal sections through the lumbar spinal cord. The mean numbers of FOS positive cells per section were determined. No differences in mean number and pattern could be detected following superfusion with CSF and somatostatin. Noxious skin heating and kainic acid superfusion increased the number of FOS positive neurons. These results show that the controlled superfusion of the rat cord dorsum is a useful tool to study the effect of putative neurotransmitters on gene expression in the spinal cord. Somatostatin apparently failed to mimick the effect of natural noxious skin stimulation i.e. failed to induce *c-fos* which further casts doubt on its role as an excitatory neurotransmitter of primary afferent nociceptors. SUPPORTED BY A GRANT FROM THE DEUTSCHE FORSCHUNGSGEMEINSCHAFT (SA 435).

401.7

SUBNUCLEUS INTERPOLARIS NEURONS CONTRIBUTE TO THE CONTROL OF ADRENAL AND AUTONOMIC FUNCTION THAT ACCOMPANIES TRIGEMINAL NOCICEPTION. D.A.Bereiter and A.P.Benetti* Section of Neurobiology & Department of Surgery, Brown Univ./R.I. Hospital, Providence, RI 02903.

The role of neurons in rostral portions of the spinal trigeminal nucleus in mediating sensory and/or autonomic responses to noxious stimulation of orofacial regions is not well defined. To assess the influence of neurons in subnucleus interpolaris (Vi) or in the principal sensory nucleus or in subnucleus oralis (Vp/Vo) on adrenal secretion of catecholamines and on cardiovascular function, cells were stimulated directly by microinjections of L-glutamate (0.5M, 100 nl) in chloralose-anesthetized cats. Microinjections of L-glutamate into Vi evoked prompt increases in the adrenal secretion of catecholamines (CA, P<0.001), total adrenal blood flow (P<0.005), adrenal vascular conductance (P<0.001), mean arterial pressure (MAP, P<0.001) and heart rate (HR, P<0.001), whereas injections into Vp/Vo had no consistent effects. Plasma ACTH was not affected regardless of the site of injection. These results indicate that chemical activation of neurons in different trigeminal subnuclei has an unequal influence on autonomic function. To examine the influence of rostral trigeminal neurons on autonomic responses to noxious trigeminal sensory input, thermal stimuli were applied to the corneal surface before and after local blockade of the ipsilateral Vp/Vo by injections of procaine (2%, 500nl). Noxious (52°C) corneal heat evoked prompt increases in CA (P<0.001), adrenal blood flow (P<0.001), adrenal vascular conductance (P<0.05), MAP (P<0.001) and HR (P<0.05). Ipsilateral blockade of Vp/Vo by procaine did not attenuate any of the thermal-evoked responses. Innocuous (45°C) corneal heat had no significant effects. These results are consistent with a role for neurons within Vi, but not within Vp/Vo, in the integration of sensory information relevant for adrenal and autonomic responses to noxious trigeminal stimuli. Supported by NIH grant NS26137.

401.8

ANATOMICAL AND PHYSIOLOGICAL STUDIES OF THE TRIGEMINOHYPOTHALAMIC TRACT. R. Burstein, A.M. Strassman and R.J. Maciewicz. Pain Physiol. Lab., Dept of Neurol., Mass. Gen. Hosp., and the Neurosci. Prog., Harvard Med. Sch., Boston, MA 02114.

A variety of facial and intraoral stimuli can alter the activity of hypothalamic neurons that are thought to participate in the control of autonomic and endocrine responses. Recent studies reported that neurons in the trigeminal nucleus caudalis project directly to the hypothalamus. In the present study we have used anatomical and physiological techniques to describe in greater detail the locations of trigeminohypothalamic tract (THT) neurons, and their physiological characteristics. In rats, injections of the retrograde tracer Fluoro-Gold that were restricted to the hypothalamus labeled several hundered neurons in the trigeminal complex from C1 to the obex. The majority of labeled cells were located in the caudal part of the trigeminal nucleus interpolaris at levels caudal to the obex. Within the medullary and C1 dorsal horn, labeled neurons were found primarily in laminae I and V. Few neurons were found in the trigeminal complex rostral to the obex. Throughout the trigeminal nuclei, labeled neurons were predominantly contralateral (approximately 90%). The nucleus of the solitary tract, the ventrolateral medulla (dorsal and lateral to the lateral reticular nucleus), and the medullary reticular formation also contained large number of labeled neurons. To date, we have recorded from 14 trigeminal neurons that were antidromically activated (<50uA) from the hypothalamus of anesthetized rats. Recording sites were found in the superficial (1 cell) and deep (9cells) laminae of the medullary and C1 dorsal horn. Four of the characterized neurons responded to innocuous mechanical stimuli but were more strongly activated by noxious mechanical stimuli. Five neurons responded only to noxious stimuli. The receptive fields were ipsilateral and covered small areas of the face (6 cells), large areas of the face and neck (2 cells), or the entire body (1 cell). The mean conduction velocity was 7m/s (range=2-13; n=12). In some cases (4), neurons were antidromically activated from both sides of the hypothalamus. The physiological data indicate that THT neurons in the deep dorsal horn carry sensory and nociceptive information directly to the hypothalamus. The anatomical data suggest that THT neurons are located in areas implicated in nociceptive processing, and that the number of these neurons in the trigeminal complex caudal to the obex is similar to the number of spinohypothalamic tract neurons previously demonstrated in individual spinal cord segments.

401.9

RETROGRADE LABELING OF NEURONS IN THE SPINAL CORD THAT PROJECT DIRECTLY TO THE AMYGDALA AND THE ORBITOFRONTAL CORTEX. S. Potrebic, R. Burstein and R.J. Maciewicz. Pain Physiology Lab., Dept. of Neurology, Mass. General Hospital, and the Neuroscience Program Harvard Medical School, Boston, MA 02114.

The amygdala and the orbitofrontal cortex have been implicated in motivational-affective responses to noxious stimulation. Nociceptive information is generally believed to reach the amygdala primarily through a relay in the parabrachial nucleus, and the orbitofrontal cortex through a relay in the thalamic nucleus submedius. In a recent study, however, injections of the anterograde tracer PHA-L into the spinal cord labeled fibers and varicosities in both the central nucleus of the amygdala and the medial orbital cortex. In the present study we further pursued these findings by using retrograde tracing techniques to describe the locations in the spinal cord of neurons that project directly to the amygdala and orbital cortex. In 7 cases, restricted injections (200nl) of the retrograde tracer Fluoro-Gold (FG) into the amygdala labeled several hundred neurons bilaterally (60% contra., 40% ipsi.) throughout the length of the spinal cord. About half of the labeled neurons were located in the deep dorsal horn (DDH), 25% in the lateral spinal nucleus, 13% in the gray matter surrounding the central canal (ACC) and 10% in the intermediate zone/ventral horn. More than half of the labeled cells were found in lumbar (L1-3, 32%) and cervical (C1-2, 25%) segments. The lower cervical (C4,5,7,8), thoracic (T4,6,8,12), and sacral (L6-S2) segments contained 18, 16 and 8% of the labeled neurons, respectively. In 3 cases, small injections of FG (100nl) into the orbital cortex labeled a very small number of neurons (approx. 50) in the spinal cord. More than 70% of the neurons were located within the DDH and ACC. About half of the cells labeled from the orbital cortex were found in the upper lumbar cord. Marginal zone neurons were not found to project directly into either the amygdala or the orbital cortex. These findings demonstrate an additional, direct pathway over which sensory and nociceptive information from the spinal cord may reach these regions.

401.10

A DOUBLE-LABELING STUDY OF THE CELLS OF ORIGIN OF THE SPINOHYPOTHALAMIC AND SPINOTHALAMIC TRACTS IN RATS. J.T. Katter, R.J. Dado and G.J. Giesler, Jr. Dept. of Cell Biol. and Neuroanat., Grad. Prog. in Neurosci., Univ. of Minn., Minneapolis, MN 55455.

Results of recent studies in rats have demonstrated the existence of neurons in the spinal cord that project directly to the hypothalamus. These spinohypothalamic tract (SHT) neurons are found in many of the same regions of the spinal gray matter as spinothalamic tract (STT) neurons. In the present study, a double-labeling strategy was used to determine whether SHT and STT neurons represent entirely distinct populations of spinal neurons or whether some spinal neurons project to both the hypothalamus and thalamus. Fluoro-Gold (FG) and cholera toxin B (CTB) were injected unilaterally into the hypothalamus and thalamus, respectively, of adult rats. Sections through 18 identified spinal segments were examined for neurons retrogradely labeled with either or both tracers. CTB was visualized using indirect immunocytochemistry. Neurons labeled with only FG, only CTB and with both FG and CTB were observed in each segment examined. Of the total number of neurons observed that were labeled with either (or both) tracers, roughly 40% were SHT neurons, 50% were STT neurons and 10% were neurons that projected to both the hypothalamus and thalamus. Doubly-labeled neurons comprised about 30% of the total number of SHT neurons and about 20% of the total number of STT neurons that were counted. The proportion of SHT or STT neurons that were doubly-labeled and their locations within the spinal gray matter varied with the spinal level. In general, most of the doubly-labeled neurons were located in the superficial dorsal horn, the lateral reticulated area and the lateral spinal nucleus. These results indicate that some spinal neurons project to both the thalamus and the hypothalamus. Supported by NS25932, DA07234 and MH10059.

402.1

PHARMACOGENETIC STUDY OF THE ANTINOCICEPTIVE RESPONSIVENESS OF C57BL/6 AND DBA/2 PROGENITOR AND DERIVED MOUSE STRAINS TO NITROUS OXIDE (N_2O). R.M. Quock, J. Mueller*, L.K. Ruppert* and L.K. Vaughn. Univ. Illinois Col. Med. at Rockford, IL 61107 and Marquette Univ., Milwaukee, WI 53233.

We have found that DBA/2 mice are resistant to N_2O antinociception in comparison to various other inbred mouse strains (Quock et al., IBRO 3rd World Congr. Abst., in press). In this study, we investigated the inheritance pattern for this insensitivity by measuring responsiveness to N_2O in C57BL/6 and DBA/2 parent strains, their $B6D2F_1$ offspring, and BXD/Ty recombinant inbred strains created by systemic inbreeding of an F_1 cross of B6D2. N_2O antinociception was measured as percent suppression of acetic acid abdominal constrictions (AC) in 70% N_2O compared to room air. C57BL/6 mice were highly sensitive to N_2O (60% suppression of AC), DBA/2 mice were highly resistant (<10% suppression), and $B6D2F_1$ mice were intermediate in sensitivity (49% suppression). In 19 BXD/Ty strains, responsiveness to N_2O varied from 100% to 0% suppression):

BXD-14 -100%; 25 -100%; 22 - 96%; 05 - 94%; 28 - 90%; 31 - 89%; 01 - 89%; 24 - 88%; 12 - 86%; 09 - 82%; 29 - 78%; 16 - 75%; 02 - 69%; 13 - 62%; 32 - 61%; 21 - 33%; 19 - 29%; 30 - 26%; 27 - 0%.

This pattern suggests that multiple genes may be responsible for the underlying neurobiological anomaly conveying resistance to N_2O antinociception. (Supported by N.I.H. Grant DE-06894 and grants from UIC COMCOR and CRB.)

402.2

ADDITIONAL TECHNIQUE FOR DETECTING VISCEROMOTOR RESPONSE TO COLORECTAL DISTENSION IN AWAKE AND LIGHTLY PENTOBARBITAL-ANESTHETIZED RATS. Y.Harada,* K.Nishioka,* L.M.Kitahata and J.G.Collins. Department of Anesthesiology, Yale University School of Medicine, New Haven, CT 06510

Visceromotor response to colorectal distension (CRD) has been demonstrated to be useful in visceral pain studies. While visual determination of visceromotor threshold (T) in response to CRD is easy and reliable, it is a subjective method. For the objective determination of the T, we have been using a newer technique in place of EMG employed by Gebhart and colleagues (Brain Research, 450: 153-169, 1988). In addition to a distension balloon, we insert a detection balloon that enables us to detect increases in intraabdominal pressure when the visceromotor response is evoked. The present study has examined the reliability of this technique in awake and lightly pentobarbital-anesthetized rats. This protocol was approved by the Yale Animal Care and Use Committee. Male Sprague-Dawley rats (280-430 g) were used. Both balloons were inserted intraanally under light halothane anesthesia and T were measured repeatedly every 5-10 min during 20 to 60 min after recovery from halothane. The detection balloon, 1.5 cm in length, was positioned ahead of the distension balloon. In pentobarbital anesthetized rats, T were measured repeatedly every 5-15 min for 1-3 hours after appearance of a clear visceromotor response. The balloon-detected T values in the awake (n=93) and anesthetized (n=22) state were 22.5 ± 4.1 and 23.0 ± 4.4 respectively (mmHg, mean ± SD). These values are comparable to the values reported by Gebhart and colleagues (22.4 mmHg). There was no significant difference between the balloon-detected T values and visual T values (23.3 ± 3.8 - awake, n=93; 23.4 ± 2.8 - anesthetized, n=22). Not only do the techniques seem comparable, but light pentobarbital anesthesia does not change the threshold values. (Supported by NIH grant NS-09871)

402.3

CORTICAL POTENTIALS EVOKED BY TOOTH PULP STIMULATION TO STUDY PAIN IN AWAKE RATS. P.J. Danneman[1], I.S. Zagon[4,5], T.J. Morrow[2,3]. Unit for Lab. Animal Medicine[1] and Depts. of Neurology[2] & Physiology[3], Univ. of Michigan Medical School, Ann Arbor, MI 48109 and Depts. of Neuroscience[4] & Anatomy[5], Penn State College of Medicine, Hershey, PA 17033

Behavioral tests to measure antinociception in animals are potentially affected by the sedative as well as analgesic effects of drugs. Accordingly, the cortical potential evoked by electrical stimulation of the tooth pulp is being evaluated as a nonbehavioral measure of antinociception in awake rats. Bipolar stainless steel electrodes are cemented into bur holes in the dentin of the left maxillary incisor. Recording electrodes are attached to skull screws located over the contra- and ipsi-lateral somatosensory cortex and vertex. A screw located over the cerebellum is used as reference. The best results are obtained with a 0.3 msec, 400μA stimulus and an amplifier bandpass of 0.3-90 Hz. The averaged evoked potential consists of a positive component at 34-40 msec (P35), a negative component at 93-102 msec (N100), and a positive component at 165-185 msec (P175). The effects of morphine, an analgesic with sedative effects, and droperidol, a nonanalgesic tranquilizer, were studied. Drugs were administered subcutaneously and recordings were made 30 min later. Morphine (5-20 mg/kg) produced an increased latency and decreased amplitude in P35, N100, and P175. Naloxone (0.5-2 mg/kg) antagonized these effects of morphine. Alone, naloxone had little effect on latency but increased the amplitude of P35 and N100. Droperidol (1.25 mg/kg) increased the amplitude of P175 and had little effect on latency.

These results suggest that this technique will be useful for studying the antinociceptive activity of compounds which may also have sedative or motor-inhibitory effects. (RR00052)+

402.4

DEPTH OF PENTOBARBITAL ANESTHESIA ALTERS PREPROTACHYKININ mRNA AND SUBSTANCE P LEVELS IN RAT DORSAL ROOT GANGLIA FOLLOWING CARRAGEENAN INFLAMMATION. D.B. Masters, C.T. Griggs* and C.B. Berde*. Children's Hospital and Harvard Medical School, Boston, MA., U.S.A.

Since biochemical studies of spinal nociceptive processing frequently employ pentobarbital (P)-anesthetized rats, we examined effects of P anesthetic dose on the content of substance P (SP) and preprotachykinin (PPT) mRNA in response to a peripheral inflammatory stimulus.

Male Sprague-Dawley rats (200-250g) were anesthetized by a single intraperitoneal injection of either 37.5 or 75.0 mg/kg P. Carrageenan (Cg; 6mg in 150μl) was injected into the right hind paw footpad 15 min post P injection. At 1 hr and 19hr post Cg injection, animals were sacrificed and lumbar (L4-6) and cervical (C3-5) dorsal root ganglia (DRG) and spinal cord (SC; L4-L6) samples were removed and frozen. Tissue samples were homogenized in urea/LiCl; supernatants were used for SP RIA and pellets were used for specific RNA quantitation by Northern blot analysis on nylon supports using a γ PPT riboprobe, a ß-actin cDNA probe and a 28S oligoprobe. Relative differences in mRNA levels were determined using a novel optical scanning technology and an improved small-sample normalization procedure based on a 28S oligonucleotide probe. Peptides were desalted on C18 columns, measured by RIA, and normalized to eluted protein levels. Data were analyzed by paired t-tests.

At 1hr, relative to contralateral control, there were significant ($p \leq 0.05$) decreases in lumbar DRG levels of PPT (-18.4±3.1%), ß-actin RNA (-27.9±1.7%) and SP (-23.1±6.9%) in 37.5 mg/kg P-anesthetized rats (n=4). No such decreases were seen in rats receiving 75.0 mg/kg P. At 19 hr post Cg, 37.5 mg/kg P-anesthetized rats showed a significant decrease in DRG SP (-23.9±6.7%) while DRG PPT levels showed no decrease (+10.7±6.4%; n=5; p=0.18). SC SP remained stable at all time points. In conclusion, changes in PPT and SP levels in models of nociception may be altered by the depth of P anesthesia. Moreover, normalization of RNA blots using ß-actin cDNA probes alone may be misleading.

402.5

STRESS-INDUCED HYPOALGESIA ON THE TAILFLICK TEST IN RESPONSE TO AUDITORY STIMULATION IS ATTENUATED BY BENZODIAZEPINES P.S. Bellgowan, P. Roberson* & F.J. Helmstetter, Department of Psychology, University of Wisconsin, Milwaukee, WI 53211

Exposure to a variety of environmental stimuli, such as a tone that has been paired with electric footshock during Pavlovian conditioning or footshock itself, can activate endogenous antinociceptive systems. The present study indicates that certain auditory stimuli alone are able to unconditionally elicit hypoalgesia. Rat subjects were exposed to a single 30 sec presentation of white noise (95 dB) while being tested for nociceptive reactivity with the radiant heat tailflick test. Noise stress resulted in a significant, time-dependent elevation in tailflick latency relative to both pre-noise baseline and non-stimulated controls. This hypoalgesia was selectively blocked by pretreatment with the benzodiazepine agonist midazolam (2.0mg/kg). Baseline tailflick latencies of groups exposed to noise did not differ from controls when animals were tested in the same apparatus 24 h later. These preliminary results indicate that exposure to white noise results in the activation of descending antinociceptive systems through a benzodiazepine-sensitive process similar to sensitization or anxiety.

402.6

POTENTIATION OF 2-DEOXY-D-GLUCOSE (2DG) ANALGESIA, BUT NOT HYPERPHAGIA BY ZOLANTIDINE, A H_2-FAMINE$_2$ (H2) RECEPTOR ANTAGONIST. J.E. Koch, L.B. Hough and R.J. Bodnar. Dept. of Psych., Queens Col. CUNY, Flushing, NY 11367, Dept. of Pharmacol. Mt. Sinai Sch. of Med. NY NY 10029 and Dept. of Pharmacol. Albany Med. Col. Albany NY 12208.

H2 receptor antagonists inhibit analgesia following morphine and an opioid form of foot shock, but potentiate opioid and nonopioid swim analgesia. 2DG analgesia and hyperphagia are each opioid-mediated, but are dissociable. The present study examined the effects of zolantidine (ZOL), an H_2 receptor antagonist upon 2DG analgesia and hyperphagia in rats. ZOL (0.1-1 mg/kg, sc) dose-dependently potentiated 2DG (450 mg/kg, ip) analgesia on the tail-flick for up to 1 h and the jump test for up to 2 h following either prior or simultaneous administration. The potentiations of 2DG analgesia (100-700 mg/kg, ip) by ZOL failed to shift the dose-response function of 2DG. In contrast, ZOL (0.1-1 mg/kg) failed to alter 2DG (700 mg/kg) hyperphagia which is consistent with previous failures of H_2 antagonists to alter intake. These data provide further evidence for dissociations between 2DG analgesia and hyperphagia, and indicate that the potentiation of this opioid-mediated form of analgesia following H_2 receptor antagonism is similar to opioid and non-opioid swim analgesia.

402.7

INHIBITION OF ANALGESIA FOLLOWING MORPHINE IN THE PERI-AQUEDUCTAL GRAY BY METHYSERGIDE IN THE VENTRAL MEDULLA OF RATS. J.M. Kiefel, M.L. Cooper* and R.J. Bodnar. Dept. of Psychology, Queens College, CUNY Flushing NY 11367.

Supraspinal opioid analgesia is mediated in part by a descending system which originates in the periaqueductal gray (PAG), synapses in the ventral medulla (nucleus raphe magnus (NRM); nucleus reticularis gigantocellularis (NRGC) and projects to the dorsal horn of the spinal cord. Little is known about the nature of the neurochemical link between the PAG and NRM/NRGC. Since a serotonergic pathway projects from the PAG to the ventral medulla, the present study evaluated intracerebral microinjection effects of the serotonin receptor antagonist methysergide (0.5-5 ug) into the NRM/NRGC upon morphine (2.5 ug) analgesia elicited from the PAG on the tail-flick and jump tests in rats. Methysergide administered into the NRM/NRGC significantly reduced morphine analgesia elicited from the PAG by 69% on the tail-flick test and by 50% on the jump test over a 1.5 h time course. In contrast, methysergide administered into adjacent medullary structures failed to alter morphine analgesia elicited from the PAG. Thus, it appears that a serotonergic synapse participates in the transmission of opioid pain-inhibitory signals from the PAG to the ventral medulla.

402.8

PHARMACOLOGIC ASPECTS OF BRAIN STEM HYPERALGESIC AND ANALGESIC PROCESSES IN THE RAT. S.R. Hamann, J.R. Holtman, Jr., S. Parvini, J.S. Fu and W.R. Martin. Dept. of Anesthesiology, Univ. of Kentucky, Lexington, KY.

Previous studies have demonstrated opioid and nicotinic hyperalgesic and analgesic processes in the dorsal posterior mesencephalic tegmentum (DPMT) and posterior medulla (PM) of the rat, respectively (FASEB J., 5:A860, 1991). The hyperalgesic processes appear to exhibit tonic activity since either naltrexone (N) or mecamylamine (M) produced dose related analgesia when microinjected into the DPMT. Using intact conscious rats fitted with indwelling cannulae we have further explored the pharmacology of these different brain stem regions. In the DPMT, three different local anesthetics [lidocaine (L), bupivacaine (B) and cocaine (C)] produced prolongation of low intensity thermally evoked tail avoidance reflex (LITETAR). A selective kappa agonist, U-50-488, produced dose related shortening of the LITETAR in the DPMT which was abolished by N. A selective 5HT-1A agonist, 8-OH-DPAT, produced prolongation of the LITETAR when administered in the PM. These findings support previous hypotheses including 1) presence of tonically active hyperalgesic processes in the DPMT, and 2) presence of opioid kappaergic hyperalgesic processes in the DPMT. Further, the results with 8-OH-DPAT indicate the presence of serotonergic analgesic processes in the PM where opioid and nicotinic analgesia has been previously demonstrated.

402.9

INHIBITION OF PGI_2 FORMATION PREVENTS THE EXCITATORY ACTIONS OF BRADYKININ IN NODOSE NEURONS OF THE GUINEA PIG *IN VITRO*. *G. M. Koschorke, D. Weinreich, G. E. Taylor*, W. C. Hubbard* and B. Undem**. Univ. Maryland Sch. Med. and Johns Hopkins Sch. Med., Baltimore, MD.

Bradykinin (BK) increases neuronal excitability in a subpopulation of nodose neurons by selectively blocking a spike induced slow afterhyperpolarization (AHP_s). This effect is dependent upon the production of a prostagladin (PG) (Europ. J. Pharmac., 132: 61-63, 1986). To identify which PG is mediating this BK effect, PG release from individual nodose ganglia was assayed using GC/MS analysis. Spontaneous PG release ranged between 1 and 20 fmole/ganglion for PGD_2, 9αIIβ PGF_2, PGE_2, $PGF_{2\alpha}$, TxB_2, and PGI_2. Of these PGs, only PGI_2 synthesis was significantly elevated by BK (100 nM) application, from 20 ± 4 to 51 ± 8 fmole (n=5). Tranylcypromine (TCP, 10 μM), a PGI_2 synthetase inhibitor, had no significant effect on spontaneous PG release but it completely abolished the BK-induced increase in PGI_2 formation.

When PGD_2, PGE_2, $PGF_{2\alpha}$, TxB_2, or PGI_2 were superfused onto nodose ganglion neurons, only PGI_2 blocked the AHP_s. TCP (10 μM) did not effect the AHP_s but its presence in the superfusate totally prevented the BK-induced block of the AHP_s (n=6). We conclude that BK blocks the AHP_s by selectively stimulating the synthesis of PGI_2. (NIH grant NS22069 to DW).

402.10

BRADYKININ STIMULATION OF DORSAL ROOT GANGLION CELL CULTURES. M.B. Bauer, M.L. Simmons, S. Murphy & G.F. Gebhart Dept. Of Pharmacology, University of Iowa, Iowa City, IA 52242.

Although prostaglandins are traditionally considered to have a peripheral involvement in nociception, they have been implicated as also having a central role. To test for a central role of prostaglandins in nociception, dorsal root ganglion cells were cultured from embryonic 15 - 19 day old rats. After 12 days in culture with Dulbecco's Modified Eagles Medium containing NGF and mitotic inhibitors, the cells appeared to be predominantly neuronal. Ionomycin (10^{-5} M) or bradykinin (BK; 10^{-5} M) stimulated the release of arachidonate (AA) metabolites from [^{3}H]AA-labelled cells. Specific RIA revealed that BK (10^{-8} to 10^{-5} M) stimulated the release of PGI_2 (prostacyclin) but did not stimulate the release of TXA_2. Additionally, it appeared that BK (10^{-6} M) stimulated the release of guanylyl cyclase-activating factor (GAF), the production of which was inhibited by NMA (N-monomethyl arginine). Overall, it appears that BK stimulates the release of prostacyclin and possibly a nitrosyl compound from cultures of embryonic dorsal root ganglia.

402.11

GLUTAMATE RECEPTOR ANTAGONIST DEPRESSION OF A CAPSAICIN-EVOKED NOCICEPTIVE REFLEX. B. Ault & L. Hildebrand. Dept. Neurosciences, Sterling Research Group, 81 Columbia Turnpike, Rensselaer, NY 12144.

The isolated spinal cord-tail preparation of the neonatal rat (Otsuka & Yanagisawa, J. Physiol. 395, 255-270, 1988) provides a model of a nociceptive reflex, in which application of capsaicin to the sensitized rat tail evokes a depolarizing response in lumbar ventral roots (CAP-VR), primarily by activation of C-fibers. Stimulation of the segmental dorsal root evokes a ventral root potential consisting of a monosynaptic reflex (MSR) and polysynaptic activity. Capsaicin (3 μM, 3s) and maximal electrical stimulation (0.4 ms, 0.2-1 mA) were presented at 15 min intervals to avoid tachyphalaxis.

Test drugs were perfused, in cumulative doses, over the spinal cord for at least 15 min. Morphine and clonidine completely suppressed the CAP-VR (IC50s of 200±80 nM, N=3, and 180±70 nM, N=3, respectively) but had little effect on the MSR or early polysynaptic responses. The effects of morphine and clonidine were antagonized by application of naloxone or efaroxan respectively. The NMDA antagonist AP5 (1-100 μM) depressed the CAP-VR, MSR and polysynaptic activity by a maximum of 31%, 31% and 33% respectively (N=3), suggesting a limited role of NMDA receptors in Mg-containing medium. An AMPA receptor antagonist CNQX (0.01-100 μM) suppressed the CAP-VR, MSR, and strongly inhibited polysynaptic responses, with respective IC50s of 0.27±0.09 uM, 1±0.3 uM and 5.2±3 uM, N=3). These data indicate that AMPA receptor-mediated synapses play a critical role in spinal transmission, including nociceptive activity. AMPA receptor subtypes may therefore provide novel analgesic targets.

402.12

INTRATHECAL PHACLOFEN ATTENUATES ANALGESIA PRODUCED BY MICROINJECTION OF GLUTAMATE INTO THE NUCLEUS RAPHE MAGNUS IN THE RAT. M.K. McGowan and D.L. Hammond. Department of Anesthesia & Critical Care, University of Chicago, Chicago, IL 60637.

This experiment examined whether the antinociception produced by chemical stimulation of neurons in the nucleus raphe magnus (NRM) is mediated by $GABA_B$ receptors in the spinal cord. Rats were prepared under general anesthesia with an intrathecal (IT) catheter and a chronic guide cannula aimed at the NRM. After a one-week recovery period, baseline nociceptive sensitivity was determined using the tail flick test. Rats were then injected IT with either 2.5 mmol phaclofen, a selective $GABA_B$ antagonist, or with vehicle (25% Molecusol). Fifteen minutes later, 30 nmol L-glutamate was microinjected into the NRM and tail flick latency (TFL) was redetermined at fixed intervals thereafter. Microinjection of glutamate significantly increased TFL in both vehicle- and phaclofen-pretreated rats ($p < 0.05$). However, TFL in phaclofen-pretreated rats was significantly shorter than that in saline-pretreated animals 1 minute ($t = 2.36$, $p < 0.05$) after glutamate administration. These results suggest that GABA in the spinal cord may modulate descending inhibitory systems from the raphe via its action at local $GABA_B$ receptors.

402.13

OPIOID THERMAL ANALGESIA IN MICE PRODUCED BY SYSTEMIC ADMINISTRATION OF A CCK-B ANTAGONIST. H. Chen* and D.A. Downs. Dept. of Pharmacology, Univ. of Michigan, and Dept. of Neuroscience, Warner-Lambert/Parke Davis Research Inst., Ann Arbor, MI 48109.

PD 134308 is a CCK-B antagonist with high selectivity (Hughes *et al.*, Proc. Natl. Acad. Sci. USA, 1990, 87:6728-6732). Its systematic name is [R-(R*,R*)]4-[[2-[[3-(1H-indol-3-yl)-2-methyl-1-oxo-2-[[(tricyclo[3,3,1,$1^{3,7}$]dec-2-yloxy)carbonyl]amino]propyl]amino]-1-phenylethyl]amino]-4-oxobutanoic acid. An analgesic effect of PD 134308 was obtained in mice using a 50° C. tail withdrawal assay. PD 134308 produced analgesia dose-dependently in a cumulative dose response procedure (10-320 mg/kg). Full effect (20-sec cutoff) was produced by 320 mg/kg. The analgesia induced by PD 134308 was antagonized by 10 mg/kg naltrexone and by 1 mg/kg naltrindole. The analgesic effect of morphine was enhanced by PD 134308, but attenuated by CCK-4; both drug interactions were modest effects.

Recent ligand binding studies (*ibid.*) have shown that PD 134308 is not a direct ligand at opioid receptors. PD 134308-induced anlagesia could be produced through an augmentation of endogenous opioids. *H.C. supported by a Warner Lambert postdoctoral fellowship.*

402.14

OPIATE AND NONOPIATE INTERACTIONS IN TWO FORMS OF STRESS INDUCED ANALGESIA. J. Grisel, M. Fleshner, L. Watkins, S. F. Maier Department of Psychology, University of Colorado, Boulder, CO 80309.

Stress induced analgesias (SIAs) have been traditionally classified as either "opioid" or "nonopioid". In 1982 Kirschgessner et al. (*Pharm, Biochem & Beh. 17:1175).* proposed that opioid and nonopioid analgesia systems did not function as independent systems, but rather interacted in a mutually inhibitory fashion. Here we report a number of experiments which further support this model. Cold water swim (4 min. at 14° C) produces a nonopioid analgesia. We exposed rats to 5 daily sessions of cold water swim and found the hypoalgesia to be potentiated by naltrexone (14 mg/kg sc, 20 min before stress). Moreover, administration of a low dose of morphine (1.5 mg/kg sc), 15 min before the swim sessions actually reduced the analgesia that resulted. The combination of morphine and cold water produced less analgesia than did either separately. A similar pattern of findings resulted from the administration of naltrexone or morphine before another stressor that produces nonopioid SIA, social defeat. Intruder rats were exposed to established colonies for 10 min on 5 consecutive days. Naltrexone or morphine was administered before the daily defeat session. Naltrexone potentiated the resulting analgesia, and animals given defeat and morphine were less analgesic than those that received only morphine. These data provide further support to the idea that there is an interactive relationship between opioid and nonopioid analgesia systems. Supported by NSF grant BNS 88-09527.

402.15

MONOSIALOGANGLIOSIDE GM1 REDUCES NOCICEPTIVE BEHAVIORS AND SPINAL CORD METABOLISM IN RATS WITH PERIPHERAL MONONEUROPATHY J. Mao[1], R. L. Hayes[2], D. D. Price[3], R. C. Coghill[1], J. Lu[1], and D. J. Mayer[1]. Dept. of [1]Physiology, [2]Neurosurgery, and [3]Anesthesiology, Medical College of Virginia, Richmond, Virginia 23298

Effects of a monosialoganglioside GM1 were examined on nociceptive behaviors and spinal cord metabolic changes in rats with a peripheral mononeuropathy produced by sciatic nerve ligation. Behavioral changes of ligated rats were assessed by the radiant heat foot-withdrawal test and by rating spontaneous hind paw guarding positions. Effects of GM1 treatment on spinal cord metabolic activity were examined by using the 2-deoxyglucose autoradiographic technique. All drug treatments were given daily for up to 9 days postsurgery. 1) Intraperitoneal (IP) GM1 (10mg/kg, n=6/group) reduced thermal hyperalgesia of the rat hind paw ipsilateral to nerve ligation, when initiated up to 24 hrs after nerve ligation (ANOVA, $P < 0.001$); 2) The hyperalgesic response also significantly decreased in a dose dependent manner in ligated rats when intrathecal (IT) GM1 was initially administered 1 hr after nerve ligation at the lumbar spinal cord (10 - 80 nmol, n=7/group), but not at the cervical spinal cord (20 nmol, n=9)(ANOVA, $P < 0.01$), indicating a lumbar spinal mechanism of GM1 action. Moreover, 80 nmol IT GM1 significantly lowered the rating score of spontaneous hind paw guarding positions ($P < 0.05$), suggesting the attenuation of spontaneous pain; 3) IP GM1 (10mg/kg, n=6) initiated 1 hr after nerve ligation significantly reduced the abnormal increase of metabolic activity seen 10 days following nerve ligation in all four sampled regions (laminae I-IV, V-VI, VII, VIII-IX) on both sides of spinal lumbar segments (L2-L5)(ANOVA, $P < 0.01$). This decrease of spinal cord neural activity may also reflect the influence of GM1 on spontaneous pain. Our data suggest that ganglioside treatment may be a promising approach for clinical management of neuropathic pain syndromes. Supported by Fidia Pharmaceuticals.

RETINA AND PHOTORECEPTORS: CIRCUITS AND SIGNALS

403.1

NBQX REVEALS A PROMINENT NMDA RESPONSE IN THE RABBIT RETINA. Ethan D. Cohen*, Tage Honore* and Robert F. Miller*, Dept. Physiol., U.of Minn.Med.School,Mpls, USA*, A/S Ferrosan, CNS Div., Soeborg, Denmark*.

NBQX (2,3-Dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline) is the most selective antagonist yet developed for non-NMDA vs. NMDA excitatory amino acid transmission. We have studied NBQX in the superfused rabbit retina using bath application of drugs and a combination of intracellular and extracellular electrophysiological techniques. Intracellular recordings from horizontal cells (cone dominant) revealed a near complete suppression of the light response using 5-30μM NBQX. These effects are rapidly reversed when NBQX is removed from the bathing medium. Recordings from depolarizing amacrine cells (AII-like units) show a transient loss of the light evoked response in the presence of NBQX, followed by some recovery. In ganglion cell recordings, the most common observation is a transient loss of the light evoked response, followed by a substantial recovery during a prolonged application of NBQX. In the presence of NBQX, the addition of D-AP7 to block NMDA receptors strongly blocks the light evoked responses. We interpret these results to indicate that NBQX blocks non-NMDA input by selectively interacting at these receptors, leaving (or possibly enhancing) a substantial NMDA mediated synaptic response.

403.2

FUNCTIONAL CONSEQUENCES OF MORPHOLOGY IN TYPES A AND B HORIZONTAL CELLS OF CAT RETINA. R.G. Smith and P.Sterling, Dept. Anatomy, Univ. of PA, Phila., PA 19104-6058.

Horizontal cells in cat retina are of 2 morphological types. Type A has thick dendrites (> 2 um), that tend to branch distally; type B has thinner dendrites that tend to branch proximally. Both receive cone synaptic input onto spine heads that connect to dendrites via thin necks (0.1 um dia., 3-5 um long).

To explore what functional differences these morphological differences imply, we simulated, using a compartmental model, isolated horizontal cells with synaptic inputs connected to. dendritic spines. We activated each synapse separately and recorded the postsynaptic potential at the soma to map "synaptic weight" vs. radial distance from soma. Synaptic weight in the type A cell was virtually constant with radial distance, whereas synaptic weight in the type B cell decreased with radial distance. Transfer of voltage from soma to peripheral dendrites was nearly 100% for both types when synapses were inactive. When all synaptic conductances were partially activated (100 pS/synapse), the space constant of synaptic transfer in the type B cell was markedly reduced.

If both cell types contribute to a Gaussian-like surround in the cone, the B type would contribute to the narrow, domed peak, whereas the A type would contribute to the wide, distal skirt. Supported by EY00828 and T32-EY07035.

403.3

SYNAPTIC APPARATUS ASSOCIATED WITH TRANSMISSION OF A SINGLE PHOTON EVENT. R. Rao and P. Sterling. Dept. of Anatomy, Univ. of PA, Philadelphia, PA 19104.

A rod in cat retina contacts 2 rod bipolar cells at a single ribbon synapse. The presynaptic membrane subjacent to the ribbon (arciform density) extends for almost 2 um, demarking an area over which ~100 synaptic vesicles can attach prior to exocytosis. The ribbon surface serves as depot for ~500 vesicles. We reconstructed from electron micrographs of serial sections the corresponding synaptic apparatus at the second stage of the circuit where 2 bipolar axons contact 5 AII amacrine cells. Individual ribbons in the rod bipolar axon are small: length of arciform density = 0.23 ± 0.09 um; surface area = 0.045 ± 0.025 um^2. However, the number of ribbons is great: 59 ± 3 ribbons/2 bipolar axons. Consequently the total area for attachment at the presynaptic membrane can accomodate ~675 vesicles, and the total ribbon surface can accomodate ~1500 vesicles. Thus, the synaptic apparatus at the second stage expands by roughly 5-fold.

The signal in a rod due to one photoisomerization is prolonged (integration time ~250 ms), but at the AII cell it quickens to about 50 ms. If the total length of the arciform density is proportional to the number of vesicle release sites, and the probability of release is the same at each site, then the number of vesicles modulated by one photoisomerization (#release sites x probability of release x integration time) would be similar at both stages. Supported by EY00828.

403.4

MORPHOLOGY AND SYNAPTIC INPUTS TO LUCIFER YELLOW INJECTED BIPOLAR CELLS IN RAT RETINAL SLICES. J.F. Muller[1,2], P.D. Lukasiewicz[1] and M.S. Silverman[2]. [1]Dept. Ophthalmol., Washington Univ. Sch. of Med., [2]Sensory Neurosci. Lab., Central Inst. for the Deaf.

The morphology and circuitry of rodent bipolar cells have not been well characterized. We have injected Lucifer yellow into visually identified bipolar cells in rat retinal slices which were lightly fixed in aldehydes. With this technique, a variety of bipolar cells can be labeled efficiently. In most cases, Lucifer yellow clearly labeled bipolar cell dendrites, axons and terminal arbors. In well-labeled cells Lucifer yellow was photoconverted with intense blue light in the presence of diaminobenzidine, to yield a dark, stable, osmiophilic reaction product. Slices were subsequently fixed in more concentrated aldehydes, post-fixed, stained and embedded, similar to Sandel *et al.*, (J. Comp. Neurol. 283:303,1989), for camera lucida drawing and ultrastructural analysis. Four general morphologies of bipolar cell have been distinguished from these preparations: Two terminate in sublamina a of the inner plexiform layer (IPL), (type a, putative off cells), and two terminate in sublamina b (type b, putative on cells). 1) The more commonly found type a bipolar cell has a fine, diffuse terminal arbor, similar to the *cb2* bipolar cell described in cat (Kolb *et al.*, Vision Res. 21:1081, 1981). Our preliminary ultrastructural findings suggest that these cells, as with *cb2* bipolar cells in cat (Nelson & Kolb, Vision Res. 23:1183, 1983), receive cone input through a non-invaginating basal junction. 2) A second morphology of type a rat bipolar cell is characterized by a single terminal swelling close to the amacrine cell layer border. 3) The most commonly found bipolar cell has been a type b cell, terminating in a small calyx near the proximal IPL border, resembling rod bipolar cells described in a number of mammalian species. 4) A second type b bipolar cell has fine, broadly stratified terminals, more distally in sublamina b, similar to *cb6* bipolar cells described by Kolb and coworkers in cat. Further ultrastructural analysis is under way to determine whether these bipolar cell types are contacted by rods or cones.

403.5

TWO DIFFERENT EXCITATORY AMINO ACID RECEPTORS IN ON-BIPOLAR CELLS OF THE MUDPUPPY RETINA. W. B. Thoreson and R. F. Miller. Dept. of Physiology, University of Minnesota, Minneapolis, MN 55455.

Whole cell recordings were obtained from ON-bipolar cells in the mudpuppy retinal slice. At a holding potential of -50 mV, L-2-amino-4-phosphonobutyric acid (L-AP4, 5-10 μM) produced an outward current and membrane conductance decrease. In contrast, AMPA (50 μM) produced an inward current accompanied by a conductance increase. A selective non-NMDA excitatory amino acid antagonist, NBQX (2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline, courtesy of T. Honoré, 30 μM), suppressed the conductance increase observed with AMPA, but had no significant effect on the response to L-AP4. Responses to L-AP4 persisted for up to 1 hr. when the patch pipette contained 0.5 mM GTP. Before washout of the L-AP4 response by prolonged dialysis, kainic acid (KA, 30 μM) typically caused a small outward current similar to L-AP4. After washout of the L-AP4 response, KA produced an inward current similar to AMPA. These results suggest that ON-bipolar cells in the amphibian retina possess both L-AP4 and KA/AMPA excitatory amino acid receptors. It is, at present, unclear what role the non-L-AP4 receptors play in normal synaptic transmission. Supported by NIH grants EY03014 and EY06213.

403.6

GLUTAMATE RECEPTOR SUBTYPES IN OFF-CENTER BIPOLAR CELLS. B.R. Maple and F.S. Werblin, Neurobiology Group, Univ. of California, Berkeley

Glutamate activated conductances were studied in bipolar cells isolated from salamander retina. A multibarrel array was used to rapidly deliver known concentrations of glutamate to voltage clamped cells. "Off" cells were selected for, based on their tendency to have relatively short Landolt clubs or displaced morphology. Responses were characterized according to the ranges of activation and desensitization with respect to glutamate concentration, and the effects of glutamate antagonists.

Initial results suggested the existence of at least three types of excitatory non-NMDA receptors, based on the following dose-response profiles observed: 1) Cells for which activation began at about 15 uM GLU, but no desensitization was observed at concentrations up to 40 uM. At concentrations above 40 uM, the initial peak response continued to grow, but the steady state response declined somewhat due to desensitization. 2) Cells with no response to 20 uM GLU, but with a CNQX sensitive response that strongly desensitized at 40 uM. 3) Cells which responded to 10 uM GLU and exhibited CNQX resistant responses that almost completely desensitized at 20 uM GLU.

Some cells displayed both "on" and "off" conductance mechanisms. Typically, in these cells 20 uM GLU elicited a slow conductance decrease associated with a reversal potential near -5 mV, but 40 uM GLU elicited a response dominated by a type 2 excitatory conductance increase. These cells could operate in an opponent fashion with respect to different photoreceptor inputs.

Earlier studies of glutamate responses and osmotically induced transmission in retinal slices (Soc.Neurosci.Abs. 16:297.2) suggest that type 1 receptors may predominate in bipolar cells with axons ramifying in the distal 1/4 of the IPL, whereas type 2 and 3 receptors may predominate in more centrally ramifying bipolar cells. Bistratified cells ramifying in both distal and central "off" sublaminae probably possess both "on" and "off" receptors, as they displayed APB sensitivity and predominantly "on" responses to osmotically induced transmission.

403.7

LOCALIZATION OF $GABA_A$ RECEPTOR IN THE OUTER RETINA OF CAT AND MONKEY. P. Masarachia*, N. Vardi, and P. Sterling. Dept. Anat., U. of Penn., Phila, PA 19104.

Horizontal cells in cat retina are thought to be GABAergic because they contain mRNA for glutamic acid decarboxylase (GAD) and endogenous GABA. However, mammalian horizontal cells do not react with antisera to GAD, nor do they accumulate exogenous GABA; monkey horizontal cells are negative for an mRNA probe for GAD. We sought additional evidence on this issue by localizing the $GABA_A$ receptor. Monoclonal antibodies to the β and α subunits (bd-17 and bd-24, kindly donated by J. G. Richards), were applied to aldehyde-fixed retinas of cat and monkey. The binding patterns were revealed as a diaminobenzidine reaction product ("stain"). As reported by others, (Hughes et al., 1991, Vis. Neurosci., 6:229-238) stain was densely distributed in the inner plexiform layer, especially in strata 4 & 5, and in somas of certain amacrine, bipolar, and ganglion cells. A new finding was that stain was consistently present (to both antibodies and in both cat and monkey) in the outer plexiform layer mainly, as a dense, punctate deposit. This stain, observed by EM, filled the extracellular space at the triad but was absent from the space surrounding the horizontal cell terminal bulbs. Stain was also distributed in fine strings of tiny, punctate deposits lateral to the pedicle bases, but rod spherules were not stained. The presence of immunoreactivity to $GABA_A$ receptor in the cone synaptic triad supports the hypothesis of GABAergic transmission at the cone synaptic complex, most likely from the horizontal cell. Supported by EY00828, EY08124.

403.8

THE COEXISTENCE OF ENKEPHALIN AND NEUROTENSIN IN AMACRINE CELLS OF THE CHICKEN RETINA: A RE-EXAMINATION USING A MORE SENSITIVE DOUBLE-LABEL PROTOCOL. C.B. Watt. Alice R. McPherson Laboratory of Retina Research, The Center for Biotechnology, Baylor College of Medicine, The Woodlands, Texas 77381.

In an earlier study, less than 50% colocalization was observed between the populations of enkephalin- and neurotensin immunoreactive-amacrine cells in the chicken retina. This lower percentage of coexistence observed in the previous study most likely resulted from the fact that 1) a less sensitive monoclonal antibody against enkephalin was used and 2) the double-label paradigm utilized a combination of a fluorescein- and peroxidase-label. For the present study, a more sensitive double-label paradigm was performed on cryosections collected throughout each of the four retinal quadrants. Primary antibodies were raised in different species, while respective secondary antibodies were conjugated to either fluorescein- or rhodamine- isothiocyanate. An examination of more than six thousand labelled cells in each of the retinal quadrants revealed that all labelled cells express both enkephalin- and neurotensin- immunoreactivity. Therefore, these results indicate the presence of a single population of chicken amacrine cells in which each member contains both of these putative neuroactive peptides.

This work was supported by NIH grant EY05622 and by the Retina Research Foundation (Houston).

403.9

CHANGES IN HORIZONTAL CELL TEMPORAL FREQUENCY SENSITIVITY RESULTING FROM STEADY ANNULAR ILLUMINATION. I.D. Cadenas and D. Tranchina. Dept. of Biology, New York University, N.Y.,N.Y. 10003.

We have examined the effects of steady annular illumination (I_A) on turtle luminosity-type horizontal cell (L-HC) responses to sinusoidal modulation of the intensity of a small spot around a mean light level (I_0). The presence of a steady concentric annulus leads to enhancement of the sensitivity to high frequency flicker of the spot, an indication of speeding up of response kinetics. Several cells also demonstrated attenuation of low-frequency senstivity under these conditions. The modifications in kinetics is accounted for by a discrete stage model in which the D.C. gain and the time constant of a single stage of low-pass filtering are changed. Across a population of cells, the degree of enhancement observed was uncorrelated with the extent of hyperpolarization when I_A goes from I_A=0 to I_A=I_0. No relationship was apparent between the magnitude of enhancement and L-HC receptive field size. A change from I_A=I_0 to I_A=0 depolarizes L-HCs, but depolarization to approximately the same extent by uniformly dimming a field in which I_A=I_0, does not affect sensitivity to high-frequency flicker of the spot. When in a series of experiments, the light intensity of the annulus is gradually increased from darkness to $2I_0$, there is accompanying increasing enhancement of the sensitivity of high-frequency responses. Repeating this experiment at lower overall light levels, however, diminishes enhancement of sensitivity. The data indicate that neither spatial contrast nor the changes in L-HC polarization produced by the presence or absence of a steady annulus is solely responsible for the changes in sensitivity observed. These results suggest the possible involvement of another neural element.

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403.10

A MODEL OF RESPONSES OF CATFISH TYPE C AMACRINE CELLS INVOLVING PARALLEL INPUTS FROM ON AND OFF BIPOLAR CELLS. [1]D. Naylor, [2]D. Tranchina, [3]K.-I. Naka. [1]Dept. of Biomath, Mt. Sinai Sch of Med,N.Y.,N.Y.,10029, [2]Dept. of Biology and [3]Dept. of Ophthal.,New York University,N.Y.,N.Y., 10003

The linear component of the type C cell response to white noise light input accounts for <10% of the power while the 2nd order Wiener kernel contributes >60%. Thus, a successful model of highly non-linear C cells should fit both 1st and 2nd order Wiener kernels. Signal processing measured in bipolar cells is highly linear and we have modelled it as a bandpass filter involving a quotient of polynomials, $B_1^{ON}(\omega)$. In our model, ON and OFF bipolar cells are assumed to have transfer functions of similar form but opposite polarity, $L_1^{ON}(\omega)$ and $L_1^{OFF}(\omega)$. $L_1^{ON}(\omega)\equiv B_1^{ON}(\omega)B_2(\omega)$ and $L_1^{OFF}(\omega)\equiv B_1^{OFF}(\omega)B_2(\omega)$, where $B_2(\omega)$ is a "lead-lag" filter. $B_2(\omega)$ is designed to represent pre-synaptic filtering in bipolar terminals and is identical in each pathway. Each of the parallel pathways then is assumed to have a static non-linearity which intervenes between the bipolar terminals and the post-synaptic ligand gated conductance in the C cell. Finally, linear filtering, $L_3(\omega)$, occurs post-synaptically in the amacrine membrane in this model. While $B_2(\omega)$ and $L_3(\omega)$ cannot be measured directly, they were justified physiologically by indirect means. This scheme enabled us to predict analytic forms for the 1st and 2nd order Wiener kernels of C cells. The parameters of the predicted analytic forms were adjusted successfully so as to fit simultaneously the actual 1st order kernel and diagonal of the 2nd order kernel. These parameters subsequently were able to reconstruct the entire 2nd order kernel as a further support for the model.

supported by NSF BNS8919993

403.11

COMPARISONS OF VOLTAGE-GATED CURRENTS IN SECOND AND THIRD ORDER NEURONS IN THE SALAMANDER RETINA Z-H. Pan* and M. M. Slaughter. Biophysics, SUNY School of Medicine, Buffalo NY 14214

Whole cell voltage clamp experiments were performed on isolated cells from the tiger salamander retina to characterize and compare the excitable currents. Bipolar cells were found to have large delayed-potassium currents that could be blocked by tetraethyl ammonium (TEA), fast activating and inactivating potassium currents blocked by 4-aminopyridine (4-AP), and an inwardly rectifying current blocked by extracellular barium or cesium, and slightly by TEA or 4-AP. Small inward calcium currents, both sustained and transient, but no sodium current, were observed. The principal voltage gated currents in the physiological range are the potassium currents. Horizontal cells also had prominent delayed potassium currents, though at only about a tenth of the bipolar cell current density. The A-type current was small or absent. These properties may explain the horizontal cell's small contribution to the ERG. There was also a prominent inward rectifying current that activated near the potassium reversal potential. A small, inward current was found that was only partially blocked by TTX. The calcium current in horizontal cells was very small, possibly reflecting the calcium-independent release of transmitter. In contrast, third order neurons have very large inward sodium currents. An A-current was usually present, with faster kinetics than that in bipolar cells, and a large delayed potassium current was found that was similar in current density to bipolar cells. No inward rectifying currents were observed. The calcium currents, both sustained and transient, were much larger than those found in other retinal neurons. Supported by NEI grant #05725.

403.12

PHYSIOLOGICAL SUBLAMINATION OF THE INNER PLEXIFORM LAYER (IPL) IN THE TURTLE RETINA. J.Ammermüller*, H.Kolb, R.Normann, R.Weiler. University of Oldenburg, Germany and University of Utah, USA.

We intracellularly recorded and stained 150 amacrine and ganglion cells in eyecup preparations of *Pseudemys* turtle. We have identified 21 of the 29 amacrine cells and 19 of the 21 ganglion cells described by Kolb (1982). This sample allows us a relatively complete description of the physiological sublamination of the IPL by correlating the light responses of the morphologically identified cell types with their stratification levels in the IPL. Thus amacrine cells with processes in distal sublamina a (S1) respond with sustained hyperpolarizations and those in proximal sublamina b (S4,5) with sustained depolarizations. Transient responses are correlated with amacrines stratifying in S2/3 and S3 on the ON-OFF sublamina a/sublamina b border. Ganglion cells mono-, bi- or tristratified from S1-S3.5, give sustained OFF responses. Similarly, ganglion cells mono- or bistratified from S3.5-S5 are sustained ON center. As expected, most bi- and tristratified ganglion cells with dendrites in both sublaminae respond in an ON/OFF fashion while diffuse ganglion cells with branches throughout the IPL gave ON center responses. With the majority of IPL branching neurons being multistratified in the turtle retina, the simple bisublamination of ON and OFF responses seen in cat retina is obviously not as clear cut. Nevertheless our results show that an ON-OFF subdivision is largely valid for amacrine cells and for ganglion cell types with dendrites restricted to one or other of the sublaminae.

VISUAL CORTEX: STRIATE MECHANISMS

404.1

SPATIAL FREQUENCY CHARACTERISTICS OF NEURONES IN FERRET VISUAL CORTEX. N. Port, I.D. Thompson*, M.P. Graham* and A.J. Parker*. University Lab. of Physiology, Oxford, OX3 1PT, U.K.

The ferret is increasingly used in visual neurobiology. We have characterised the spatial properties of the neurones in its visual cortex using drifting sinusoidal gratings. Pigmented ferrets were anaesthetised (70:30 N_2O:O_2, Sagatal 1 mg/kg/hr) and paralysed. Gratings were generated (luminance, 120 cd/m^2; contrast, 0.84) and detailed analysis of spike time and waveform was provided by BrainWave software. Surface normal penetrations were made into cortex representing central visual field.

Hand plots of receptive fields showed orientation tuning and, in the upper layers, marked end-inhibition. When stimulated with drifting gratings, some neurones showed a modulated response, others were unmodulated. A range of acuities could be observed within a single penetration. In one case, there was a change from 1.4 c/deg to 0.18 c/deg for neurones separated by less than 1 mm. It is our impression that neurones with lower acuities were found in the deeper layers. The neurones also varied in the bandwidth of their spatial tuning, from low pass to as narrow as 1.3 octaves. Orientation tuning to drifting sinusoidal gratings at peak spatial frequency yielded bandwidths at half-height that were typically around 60 degrees. Normally, adjacent neurones displayed similar peak orientation tuning but, in one pair of neurones recorded simultaneously, the peak orientations differed by 90 degrees.

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404.2

RESPONSES OF CELLS IN RABBIT AREA 17 TO DRIFTING SINUSOIDAL GRATINGS

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As part of our continuing inquiry into spatial and temporal properties of neurons in rabbit area 17, we measured their responses to sine-wave drifting gratings. Single cell activity in anesthetized and paralyzed rabbits was recorded. Units were identified as simple (S) or complex (C) with light or dark bars and stationary spots. In simple cells (S) (n=45), only 58% responded to gratings with modulated discharges. The remainder (42%) were unresponsive to the same stimuli. On the other hand, 85% of complex cells (C) (n=20) had an unmodulated discharge to sinusoidal gratings, and only two of them were unresponsive. In two C, the discharge was modulated at optimal frequency. Six other cells could not be classified as either S or C, but they responded to gratings. Most units were low-pass to spatial frequency. Few neurons (S=33%, C=20%) were band-pass. So far no high-pass have been encountered. In contrast, most cells were band-pass in the temporal domain (S:mean=3.1 ± 1.5Hz; C: mean=2.8 ± 0.7Hz). In some cells (6%), the temporal tuning curve appeared double-peaked. And another 6% exhibited a U-shape curve below the spontaneous firing level. The influence of the contrast is being evaluated. As demonstrated in other species, cells in rabbit area 17 can be differentiated on the basis of responses to gratings.

Supp. FCAR, NSERC and MRC.

404.3

RESPONSES IN STATIONARY TARGETS ARE GENERATED BY DIFFERENT MECHANISMS IN SIMPLE AND COMPLEX CELLS. C. Morin*, F. Hubert*, Y. Michaud, B. Nault, P. McKinley[1], and S. Molotchnikoff, Univ. Montreal, Biologie, C.P. 6128, Succ A, and McGill, Physical & Occupational Therapy[1] Montreal, Canada, H3C 3J7

The goal of this study was to examine the extent to which striate neurons were influenced by neighboring cells in the anaesthetized rabbit. Using single cell recordings, 84 striate units were evaluated for responses to both stationary (on/off) and moving light bars before and after injection of 300nl of either lidocaine or GABA 2mm away from the recorded cell. Surprisingly, responses to stationary spots were affected in 55% of the neurons, while the responses to moving spots were modified in only 36% of the cells ($P<0.02; x^2=7.5; df=2$). When directional index (DI) was < 50%, the on/off response was modified more often ($p<0.01; x^2=45.9$) than in units whose DI was >50%. Amongst the non-directional neurons, simple cells showed mainly decreased responses (67.7%) while the complex cells were mostly increased (62.5%). In contrast, direction selective (DI>50%) simple *and* complex cells maintained unaffected on/off responses (66.6% & 80% respectively). Results suggest that the non-directional cells are more dependent on horizontal cortico-cortical connections and on/off responses are mediated through different mechanisms in simple and complex cells. Supported by an FCAR grant to S. Molotchnikoff and P. McKinley

404.4

EFFECTS OF SUBSTANCE P IN THE CAT STRIATE CORTEX ARE SELECTIVE FOR CELL TYPE AND CORTICAL LAYER.
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In the cat striate cortex Substance P (SP) is contained in intrinsically projecting neurons of layers V and VI. The SP content is high during the first postnatal month and thereafter decreases dramatically (Wahle et al., Soc. Neurosci. Abstr., 14:745, 1988). We tested the effects of SP on single neurons in one month and one year old cortex to seek for a functional role of SP in striate cortex and for possible differences in SP effects between immature and mature cortices. Drugs were delivered microiontophoretically during extracellular recording and visual stimulation in anaesthetized and immobilized animals. SP affected about 20% of the cells tested (N=93). Its main effect was a reversible, strong enhancement of visually evoked responses (VER) and/or spontaneous activity. The affected cells either had complex receptive fields (layers V and VI) or gave sluggish responses to visual stimulation (deep layer VI or white matter). The enhancement commenced after some delay (1-3 min) and generally outlasted the administration of SP. The effect was dose-dependent and did not decrease in strength when SP administration was prolonged or repeated. Complex cells in layers II-IV and unimodal and simple cells in all layers were not affected by SP, or showed only slight changes in spontaneous activity and VER. Differences in SP effects between one month and one yaer old cortices have not been observed so far. Our findings support a role for SP in modulating synaptic transmission in cat visual cortex. The exact mechanism of this action will be elucidated by ongoing experiments.

404.5

MICROIONTOPHRETICALLY-APPLIED NORADRENALINE AND SEROTONIN HAVE FUNCTIONALLY ANTAGONISTIC EFFECTS ON RESPONSE PROPERTIES OF CAT VISUAL CORTEX NEURONS. J. Goyer and M.S. Cynader, Dept Ophthalmology, Univ. British Columbia.

The effects of microiontophoretic applications of noradrenaline (NA) and serotonin (5-HT) on some response properties of visual cortex neurons were assessed in anesthetized, paralyzed cats. Unit responses to single flashes and bidirectional sweeps of bright slits of the optimal orientation, as well as orientation tuning curves were assessed before, during and after NA or 5-HT application.

The signal-to-noise ratio of the activity evoked by single flashes (duration 400 msec) was significantly increased after NA application (16.8 vs 10.5), but was decreased after 5-HT application (4.6 vs 10.5). The direction selectivity of the assessed cells (as measured with bidirectional sweeps along the cell's preferred orientation) was also significantly increased by NA (7.8 vs 4.5), and decreased by 5-HT (1.9 vs 4.5). The sharpness of the orientation tuning curves was again increased by NA and decreased by 5-HT.

These results suggest that NA and 5-HT play functionally antagonistic, modulatory roles on the response of visual cortex cells to different stimuli, NA acting to "sharpen" the cortical cell's response, while 5-HT "smooths" cortical cell response.

404.6

THE ROLE OF GABAERGIC INHIBITION IN THE ADAPTATION OF DIRECTION SELECTIVITY IN NEURONS IN AREA 17 OF THE CAT; Stuart G. Marlin, Robert M. Douglas, and Max S. Cynader, University of British Columbia, Depts. Ophthalmology & Psychology, Vancouver, B.C. Canada.

We investigated a possible mechanism for adaptation of single cortical neurons using iontophoretically applied GABA receptor agonists and antagonists. The adaptation of responses to prolonged unidirectional visual stimulation was compared to the adaptation during iontophoretic application of the GABA antagonists bicuculline ($GABA_a$) and phaclofen ($GABA_b$) as well as the agonists GABA and Baclofen. Bicuculline increased the firing level of all cells by approximately 100%. Both preferred and nonpreferred direction of motion responses increased. In a small number of cells phaclofen increased the responses by about 33%. Neither bicuculline nor phaclofen blocked the adaptation to prolonged stimulation, and the higher responsivity under bicuculline actually increased the rate of adaptation. GABA and Baclofen dramatically decreased the firing of cortical neurons, however, adaptation to prolonged stimulation was still observed. Adaptation-induced changes in the direction selectivity of the neurons were also observed during bicuculline and phaclofen application, even in cells where the nonpreferred direction motion responses had increased to equal the preferred direction responses. Bicuculline alone (without visual stimulation) dramatically increased spontaneous activity, but did not cause an adaptation-like reduction in the response rate suggesting that pre-synaptic depletion of neurotransmitter may be an important part of the mechanism of adaptation in cortical neurons.

404.7

Optical Imaging of Cortical Responses to Moving Plaids Reveals Linear Processing of its Oriented Components, in Area 18 of Cat Visual Cortex. *D. Malonek and A. Grinvald.* The Weizmann Institute of Science, Rehovot 76100, Israel, and The Rockefeller Univ., New York, NY 10021.

The spatial organization of orientation tuning was revealed with the use of small oriented bar stimuli (Hubel & Wiesel). Recently optical imaging revealed that orientation tuning maps obtained by large gratings produced functional maps identical to those produced with small bars. Thus, lateral interactions between activities evoked by multiple bars within the gratings did not change the spatial maps.

Here we examine functional interactions among regions, tuned to different orientations, in area 18 of cat visual cortex. We employed plaids stimuli composed of two orthogonal gratings and extending over large visual angle ($>15^0$). Using optical imaging based on intrinsic signals, *in vivo*, we measured activity maps in anesthetized and paralyzed adult cats. Either moving plaids or their individual components were used as stimuli. To determine whether significant interaction exists among regions tuned to different orientations (activated simultaneously), we compared activity maps generated by the above stimuli set. We found that the maps generated by moving plaids stimuli were identical (in shape and intensity) to the sum of maps generated separately by its components.

This finding indicates that, at the level of population activity, the interactions associated with these complex stimuli do not affect the intensity or spatial activation of area 18. If perceptual direction of motion is spatially coded, plaid's motion coding should produce a map which differs from a linear sum of its components. Thus, our results are consistent with a two stage model for motion processing: Area 18 stage codes local motion of oriented components (bounded by the "aperture problem") while the stage of motion integration is performed at higher visual areas. (Supported by the Riklis Family Foundation)

404.8

CORTICAL DIRECTION SELECTIVITY AS A FUNCTION OF TEMPORAL FREQUENCY. A.B. Saul and A.L. Humphrey. Dept. of Neurobiology, Anatomy and Cell Science, U. of Pittsburgh, Pittsburgh PA 15261.

We had previously speculated that lagged X- and Y-cells in the cat lateral geniculate nucleus provide inputs to direction selective cells in cortex (*J. Neurophys.* 64:206). We noted that convergence of lagged and nonlagged inputs would be expected to produce direction selectivity only at low temporal frequencies, because the quarter-cycle response phase difference between these cells at 1 Hz grows to a half-cycle by about 4 Hz. We expected that cortical cells would resemble their nonlagged inputs at high temporal frequencies, since lagged responses have lower resolution. We have now measured temporal frequency tuning in each direction in 62 cortical neurons.

Direction selectivity in cat visual cortical neurons is indeed strongest at low temporal frequencies. As predicted, selectivity is often lost with increasing frequency even though the cell continues to respond in both directions. Responses in the nonpreferred direction are typically suppressed at 1 Hz, but become stronger by about 4 Hz and sometimes equal or exceed the responses in the preferred direction. About 25% of our sample showed clear increases in the nonpreferred direction response with increasing temporal frequency while the preferred direction response declined or remained relatively constant. We quantified this phenomenon by estimating the optimal temporal frequency in each direction. The preferred direction was tuned to lower frequencies than the nonpreferred direction in about 70% of the cells, and 40% had preferred direction optima at least an octave lower than nonpreferred direction peaks. Average optimal frequencies were 3 Hz in the preferred direction and 5 Hz in the nonpreferred direction. Temporal resolution, on the other hand, differs little between the two directions (13 Hz vs. 16 Hz on average, lower than the resolution of nonlagged cells but not of lagged cells).

In summary, a population of visual cortical cells exists that are direction selective around 1 Hz, lose selectivity around 4 Hz, and respond in both directions out to about 8-16 Hz. These phenomena can be interpreted in terms of the lagged projection from the LGN.

Supported by EY06459 and MH18273.

404.9

NON-LINEAR RESPONSES TO APPARENT MOTION IN CAT VISUAL CORTEX NEURONS. J.C.Boulton* and C.L.Baker,Jr.
Dept. of Medical Physics, Utrecht University, The Netherlands; and
Dept. of Psychology, McGill University, Montreal, Canada.

Recent human psychophysics has shown that the perception of movement from two-flash apparent motion of random Gabor kinematograms can be predicted from the stimulus spatiotemporal power spectrum *only* when the stimulus onset asynchrony (SOA) is less than 100 ms. For larger SOAs this relationship breaks down, implying nonlinearity (Boulton and Baker, ARVO 1991).

Comparable experiments on direction selective cells in Areas 17 and 18 of cat visual cortex reveal similar behaviour. Two-flash apparent motion of a Gabor stimulus was used to examine direction selectivity across a range of SOAs (0 to 200 ms) and jump sizes (0 to 1 cycle of the cell's optimal spatial frequency, lambda). For short SOAs (< 100 ms) linear behaviour is observed with peak directional responses for small jump sizes (< 1/4 lambda), often followed by reversal for larger jump sizes. However, many cells also showed strong directionally selective responses at long SOAs (> 100 ms) and large jump sizes (> 1/2 lambda). These non-linear responses are compared to human psychophysical performance.

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404.10

SPATIAL ORGANIZATION OF INHIBITORY INFLUENCES UPON NEURONS IN THE CAT'S STRIATE CORTEX. G.C. DeAngelis*, J.G. Robson†, I. Ohzawa, and R.D. Freeman. School of Optometry, Univ. of California, Berkeley, California 94720, and †Physiological Laboratory, Cambridge CB2 3EG, UK.

Intracortical inhibitory interactions are known to be involved in the generation of receptive fields of neurons in the visual cortex. In addition, there are two main types of inhibitory influences which modulate the responsiveness of striate cortex neurons: end-inhibition (or endstopping) (Hubel & Wiesel 1965) and "cross-orientation" inhibition (Morrone et. al 1982). We have studied the spatial organization of these inhibitory influences through extracellular recordings of single unit responses from the striate cortex of anesthetized and paralyzed cats.

Length-response and width-response curves are constructed using rectangular patches of drifting sinusoidal grating centered on the receptive field. Cells which exhibit an optimum in their length-response curves (end-inhibition) tend to show a similar effect in their width-response curves (side-inhibition). Tests with superimposed gratings of different spatial frequencies suggest that regions eliciting end- and side-inhibition only slightly overlap the excitatory receptive field. In similar tests, superimposed gratings of orthogonal orientations are used to measure the spatial summation of cross-orientation inhibition. For all cells tested, the summation area for cross-orientation inhibition is centered upon the excitatory receptive field and of similar or smaller extent. Thus, cross-orientation inhibition is confined within the excitatory receptive field, while end- and side-inhibition arise from surrounding regions.

End- and side-inhibition may be further distinguished from cross-orientation inhibition through dichoptic measurements. For binocular cells which show end- or side-inhibition monoptically, inhibition can also be elicited dichoptically. In contrast, we were never able to elicit cross-orientation inhibition dichoptically.

A model is proposed to explain the spatial organization of end- and side-inhibition and cross-orientation inhibition in terms of cortical circuitry. (EY01175)

404.11

MODULATION OF STEREOSCOPIC PROCESSING IN PRIMATE VISUAL CORTEX V1 BY THE DISTANCE OF FIXATION. Y. Trotter*, S. Celebrini*, S. Thorpe* and M. Imbert. IDN, Lab. Neurosc. Vision, Univ. Paris 6, France.

The question of knowing how far objects appear to be away from us is related to binocular distance perception which has to be distinguished from relative depth perception or stereopsis. Retinal horizontal disparity alone is not sufficient to allow either 1) the true distance of an object to be determined or 2) to make accurate judgments about the 3-dimensional shape of objects. This is because both types of judgments require additional information about the fixation distance which could originate mainly from the angle of vergence and/or the degree of accomodation and/or retinal vertical disparity. We have addressed the question of what happens to the response properties of individual neurons when the distance of fixation is changed. We recorded responses of neurones to static random dot stereograms in cortical area V1 of a monkey trained to perform a fixation task. We first studied 79 neurons for stereopsis at a fixation distance of 40 cm. About half of them (47%) were disparity sensitive. Thirty one neurons were also tested at other fixation distances (20 cm/40 cm/80 cm). By doubling the size of the image on the screen when the fixation distance of this screen was increased by a factor of two, it was possible to maintain the angular size of the stimulus constant. The luminance level was about 1 cd/m^2 at the 3 distances. In 22 cells, disparity selectivity emerged only at certain viewing distances. Consequently, the proportion of disparity selective units was actually higher when the neurons were tested at more than one fixation distance (71%) than when only the 40 cm distance was used. For 9 of the 31 neurones, there were changes in the spontaneous activity of the neurons related to changes of viewing distances. In 6 of these cases, the spontaneous activity was increased when the animal was fixating at shorter distances. Our findings show that retinal horizontal disparity sensitivity of neurons as early as cortical area V1 is influenced by fixation distance and thus by extraretinal factors probably related to vergence.

404.12

CHARACTERISTICS OF NEURONS IN MARKED LOCATIONS IN STRIATE CORTEX OF BEHAVING MONKEY. D. M. Snodderly and M. Gur* Eye Res. Inst. and Harvard Med. Sch., Boston MA, 02114; *Biomed. Eng., Technion, Haifa, Israel.

Localization of recording sites in behaving animals has been difficult because the visibility of electrode tracks and lesions declines rapidly with survival time. With currents producing discrete, well-localized damage (1.2 μa for 6 sec), neither electrolytic lesions nor iron deposition clearly marked recording sites. In contrast, coating the electrode with the nontoxic fluorescent carbocyanine dye, DiI, resulted in discretely labeled penetrations visible for at least 10 days.

During recording, electrode depth was monitored carefully. The cortical surface was visualized through a small slit in the dura and blood vessels were avoided to minimize damage. A biopsy was taken, fixed with glutaraldehyde/paraformaldehyde, and sectioned. Sections were photographed with a fluorescence microscope to map the electrode tracks. Then they were reacted for cytochrome oxidase to visualize the laminae, which obliterated the fluorescence. Laminar boundaries were drawn and the fluorescence photographs of the electrode tracks were projected onto the drawings to observe the trajectory of the electrode through the cortical layers. The depths determined in this way corresponded well to microdrive depth readings.

As previously reported (ARVO, 1988), cells in the superficial layers often had small receptive field activating regions with potent inhibitory surrounds. A powerful stimulus for these cells was a moving texture composed of small rectangles oriented to match the preferred orientation of the cell and configured to avoid simultaneous stimulation of the center and the surround. Natural surfaces such as those of vegetation, earth, and animals often are imaged to produce such oriented textures. These patterns could be used by cortical neurons to contribute to the discrimination of objects from backgrounds. This suggests another perceptual role for upper layer neurons, in addition to the commonly mentioned ones of border extraction and spatial frequency analysis.

404.13

High Resolution Optical Imaging of Functional Architecture in the Awake Primate. Amiram Grinvald, Ralph Siegel, Eyal Bartfeld and Ron D. Frostig. Laboratory of Neurobiology, The Rockefeller University New York, NY 10021 and The Weizmann Institute of Science, Rehovot, Israel 76100.

Optical imaging of the functional architecture of cortex, based on intrinsic signals, is an useful tool for the study of the development organization and function of the living mammalian brain.

Here we establish that this technique is also suitable for exploring the brain of awake behaving primates. We designed a chronic sealed chamber which was mounted on the monkey skull over the primary visual cortex and permitted imaging experiments through a glass window. Restriction of head position alone was sufficient to minimize movement noise in the awake animal imaging experiments. High resolution imaging of the ocular dominance, and the blobs was achieved simply by taking pictures of the exposed cortex when the awake monkey was viewing video movies. Furthermore, the functional maps could be obtained without the noise reduction procedure of synchronizing the animal respiration and the data acquisition to the EKG. The wavelength dependency and time course of the intrinsic signals was similar in anesthetized and awake monkeys indicating that the signal sources were the same.

We therefore conclude that optical imaging is well suited for exploring functional organization related to higher cognitive brain functions of the primate as well as providing a diagnostic tool for delineating functional cortical borders and assessing proper functions of human patients during neurosurgery.

Supported by IBM and the Riklis Family Foundation.

405.1

HETEROGENEITY AND COMPARTIMENTALIZATION IN THE OLFACTORY BULB OF ELASMOBRANCH FISHES.
L. Dryer and P.P.C. Graziadei. Florida State University, Department of Biological Sciences, Tallahassee, Fl 32306.

Anatomical observations reveal that the Elasmobranch olfactory bulb is an elongated structure immediately adjacent to the olfactory organ. Depending on the species, it appears as a succession of swellings (i.e. the Bonnethead shark, *Sphyrna tiburo*) or as two independant subunits (i.e. the Sharp-nosed shark, *Rhizoprionodon terranovae*). We examined several species in order to understand the functional significance of these morphological features.

The olfactory bulb of all specimens stained with Golgi method exhibit two distinct populations of mitral cells: A first type has loose dendritic arborization bearing numerous terminal boutons. The second type of mitral cell, smaller, shows an extremely dense feather-like arborization.

Localized extracellular injections of Biocytin in the olfactory epithelium result in restricted labeling of the bulb, suggesting a topographical arrangement of the olfactory projections onto the bulb. The lateral olfactory bulb would process information coming from the lateral epithelium, whereas the medial bulb would process information coming from the medial epithelium. Several sizes of glomeruli were also observed with this method. Further experiments are in progress to delimit the projection fields with accuracy and define the lateral interconnections between the subdivisions of the olfactory bulb.

405.2

CONNECTIVITY OF THE AREA POSTREMA IN THE HAMSTER (*Mesocricetus auratus*).
A.P. Knox, N.L. Strominger, L.D. Savoy* and C.B. Halsell. Departments of Anatomy and BioStructure and Function, Albany Medical College, Albany, NY 12208 and UCONN Health Center, Farmington, CT 06030.

The connectivity of the area postrema (AP) was investigated in the hamster. Iontophoretic injections of WGA-HRP were made into either the central portion of the AP or the parabrachial nucleus (PBN). After 24-48 hours survival time, animals were perfused with phosphate buffered saline followed by a 3% glutaraldehyde solution in phosphate buffer. Brains were removed *in toto*, cut at 50 μm and processed for HRP histochemistry with tetramethylbenzidine. Alternate sections were mounted unstained with the remaining set counterstained with a Nissl stain.

PBN injections resulted in the labeling of numerous neuronal perikarya throughout the AP, medial and commissural subnuclei of the solitary complex. Injections of the lateral PBN were most effective in labeling of AP neurons. Preterminal label was present in the AP but not prominent. Injections in the AP resulted in labeling of perikarya in subnuclei (medial and commissural) of the solitary complex. Afferent label from AP injections was present in the solitary complex at the level of the injection and most prominently in the lateral PBN.

The area postrema of the hamster is in a pivotal position to receive not only primary afferent information but also information from the systemic blood due to a weak blood brain barrier. The AP may play an important role in modulating visceral function by influencing pontine visceral relay centers.

405.3

POSTNATAL MATURATION OF THE MITRAL/TUFTED CELLS OF THE RAT ACCESSORY OLFACTORY BULB.
S. Takami and P.P.C. Graziadei. Dept. Biol. Sci. Florida State Univ., Tallahassee, FL 32306-3050.

The mitral/tufted cells (MTCs) of the rat accessory olfactory bulb (AOB) are morphologically different from the mitral and tufted cells of the main olfactory bulb (MOB); the MTCs have more diverse dendritic branching and unique glomerular arbors (GAs). To clarify the developmental sequence of the MTCs dendritic branching and the appearance of the GAs we have studied the MTCs in the rat AOB during the perinatal period.

Using the rapid Golgi and Golgi-Kopsch methods, impregnated MTCs were observed in the rat AOB from postnatal P1 to P29. The mitral and tufted cells of the MOB were also observed at comparable stages. At P1/P2 MTCs had dendrites branching in beaded structures, however, GAs were not present. GAs were instead often observed in the mitral and tufted cells of the MOB. At P8/P10 dendrites of the MCTs began to show branching and early formation of GAs. By P15, GAs and complex branching of the dendrites acquired more complexity; morphological details as observed in the adult were obvious by P22 to P29. Our observations indicate that the MTCs and their specific intraglomerular GAs develop postnatally and at later stages than the GAs of the mitral and tufted cells of the MOB. Supported by a grant from the NIH (NS 20699) to P.P.C.G.

405.4

ONTOGENY OF VOMERONASAL NEURONS AND ACCESSORY OLFACTORY BULB IN NEONATAL OPOSSUMS (Monodelphis domestica): A ^{3}H-THYMIDINE AUTORADIOGRAPHIC STUDY.
R. T. Wang, T. L. Soltesz*, T. Hayes* and M. Halpern[1]. Departments of Anatomy, Marshall Univ. Sch. of Med., Huntington, WV 25755 and [1]SUNY Health Sci. Ctr. at Brooklyn, NY 11203.

Five groups of neonatal South American opossums at the ages of 1, 3, 7, 14 and 28 days (N=44 total) were injected with a single dose of ^{3}H-thymidine (2 to 5 μCi., i.p.) for tracing the development of their vomeronasal (VN) system. Neonates survived an additional 1, 7, 14, 21 or 28 days following injection and were processed into light microscopic autoradiogram on slides. For 1 day survival groups, injection of ^{3}H-thymidine to neonates on Day 1 or 3 radioactively labeled stem cells throughout the primitive VN organ while injection made after Day 7 selectively labeled stem cells in the developing VN organ at the basal cell layer and some at the apical sustanticular cell (SC) layer. The SC showed a marked reduction in the number of cells to incorporate ^{3}H-thymidine after Day 14. For incorporation time longer than 7 days in all groups studied, labeled VN neurons emerged in the intermediate zone of the sensory epithelium where they sustained a life span exceeding 28 days. Furthermore, telencephalic neuroblasts generated prior to Day 3 first differentiated into mitral cells in the main olfactory bulbs (MOB) by Day 8 and in the accessory olfactory bulbs (AOB) by Day 15. Injection made on Day 7 and thereafter labeled neuroblasts which gave rise to the granule cells predominantly and very few mitral cells in the MOB and AOB. Supported by NIH RR-05870, NS-11713 and MUSOM.

405.5

FLUORESCENT TRACT-TRACING STUDIES OF DESCENDING PROJECTIONS FROM THE INSULAR CORTEX TO THE NUCLEUS TRACTUS SOLITARIUS.
T. S. Donta and J. A. London. Center for Neurological Sciences and Dept. of BioStructure and Function, Univ. CT Health Center, Farmington, CT 06030.

This laboratory has been investigating descending cortical projections to the taste-responsive rostral pole of the nucleus tractus solitarius (NTS) in golden Syrian hamsters (Mesocricetus auratus). Previous work in this lab has demonstrated that the contralateral projection is greater than the ipsilateral projection, and that the contralateral projection area extends further dorsally in layer 5 than the ipsilateral projection area. The rostral-pole of the NTS is primarily innervated by the chorda tympani nerve. Here we expand our studies to include descending cortical projections to other regions of the NTS. Injections of retrograde tracers into NTS regions were guided by multi-unit electrophysiology and confirmed by histology. Responses in the NTS were recorded after application of a search stimulus (0.03 M NaCl, 0.1 M KCl and 0.1 M sucrose) to the anterior tongue in order to map a taste active region. Once a taste active region was delimited, a tracer was injected into the center of the active region. The retrograde labels used were latex microspheres filled with either rhodamine or fluorescein. It was found that the same area of the ventral agranular insular cortex projected to NTS areas receiving chorda tympani or glossopharyngeal nerve innervation, or to more caudal areas near obex. The cortical projection cells to these different regions were highly intermingled. However, only cells projecting to the rostral pole of the NTS were found in the dorsal part of the agranular insular cortex and in the granular insular cortex. No differences in the rostral-caudal mapping of the cortex to the NTS were observed.
Supported by a UCHC Foundation grant and USPH Grant 2P01-NS16993-09.

405.6

THE ORGANIZATION OF AFFERENT FIBERS AND LOCAL SYNAPTIC CIRCUITS IN PIRIFORM CORTEX FOLLOWING MITRAL CELL LOSS.
Juan C. Bartolomei and Charles A. Greer. Sections Neurosurg. & Neurobiol., Yale Univ. Sch. Med., New Haven, CT 06510.

The mutant mouse Purkinje Cell Degeneration (PCD) loses all mitral cells (MCs) in the olfactory bulb (OB) over a period of about 30 days beginning at 4 mon. of age. Tufted cells and denervated granule cells subsequently exhibit significant local synaptic reorganization in the OB. Because a primary input of piriform cortex (PC) is derived from the MCs, our current goal is to assess changes in axonal distribution and synaptic organization in the PC after loss of MC axons in PCD mice.

In 5 to 7 mon. old PCD mice a small incision was made in the LOT and a pledget of Horseradish Peroxidase inserted. The distribution of labeled fibers in control and PCD mice was assessed with light microscopy. In control mice there was a prominent rostral to caudal distribution of labeled fibers along the PC with a well demarcated subliminar distribution limited to layer Ia. In PCD mice there was a large reduction in the rostral to caudal extent of axonal distribution. At the rostral levels the sublaminar distribution appeared relatively normal through layer Ia though the density of labeled axons was significantly reduced. Centrifugal axons in the LOT did not appear effected in PCD mice since contributing nuclei were labeled retrogradely. Radially oriented montages of electron micrographs were used as translaminar probes of ultrastructural organization of PC in PCD mice. Preliminary data revealed a substantial decrease in the number of myelinated axons contributing to the LOT. Synaptological analysis of PC showed terminal boutons that correspond to those previously attributed to intracortical and LOT axons (Friedman & Price, 1986). Preliminary counts suggested a dramatic reduction in the number of large pale profiles characteristic of LOT axons. Thus far the findings do not support the notion of extensive denervation induced collateral sprouting of tufted cell axons in the PC of PCD mice. Further studies continue to assess alterations of PC synaptic circuit organization occurring due to MC axon loss.
Supported in part by NIH DC00210 and NS10174.

405.7

PARALLEL PROCESSING OF CHEMOSENSORY INPUT IN THE BRAIN OF THE SPINY LOBSTER. M.Schmidt, E.Orona and B.W.Ache. Whitney Lab. & Departments of Zoology and Neuroscience, Univ. of Florida, St. Augustine, FL 32086

The biramous antennules (1st antennae) of decapod crustaceans are complex sensory organs bearing olfactory (aesthetasc), mechano-chemosensory, and mechanosensory sensilla. To analyze the central processing of antennular information we backfilled antennular afferents and intracellularly recorded and stained deutocerebral neurons in the spiny lobster, *Panulirus argus*. The deutocerebrum contains two, distinct pathways for processing antennular input. One pathway includes the olfactory, parolfactory, and accessory lobes (OL, PL, AL), neuropils that process olfactory and mechanosensory information originating in the lateral flagellum of the antennule. Antennular afferents terminate in columnar glomeruli in the OL, where the input is transferred to different classes of multiglomerular local interneurons, one of which connects the OL to the AL, and to pluriglomerular projection neurons. Axons of the OL projection neurons run together with axons of AL projection neurons via the olfactory-globular tract to higher protocerebral centers. The second pathway includes the lateral and medial antennular neuropils (LAN, MAN), which lack glomerular organization and receive mechano- and non-olfactory chemosensory input from both flagella and the basal segments of the antennule. A major output element of the LAN and MAN are antennular motoneurons whose neurites are interwoven with the afferent projections. The second pathway appears to mediate local antennular reflexes (LAN) and coordinate reflexes of the head appendages to antennular stimulation (MAN). The independent processing of olfactory and non-olfactory chemosensory input originating from the same appendage of the spiny lobster further argues that olfaction is a distinct chemosensory modality even in non-vertebrate, aquatic animals. (Supported by DFG grants Schm 738/1-1, 1-2 and NSF grant 88-10261).

405.8

ELECTROTONIC AND DIFFUSION MODELS OF INTERACTIONS AMONG DENDRITIC SPINES OF THE MAMMALIAN OLFACTORY BULB. T.B. Woolf, G.M. Shepherd, and C.A. Greer. Sect. of Neurobiol. & Neurosurg., Yale Univ. Sch. Med., New Haven, CT 06510.

Granule cells in the olfactory bulb mediate lateral and self inhibition of mitral/tufted cells through reciprocal dendrodendritic synapses involving spine heads (gemmules). We recently reported serial EM reconstructions of these spines (*Synapse*, 7:181) and electrotonic models of granule cells based on LM observations in the mouse (*J. Neurosci.* 1991, in press). We describe here detailed compartmental models of spines and short dendritic sections, based on EM morphology, to assess communication between spines via electrotonic spread and diffusion of putative neuroactive compounds.

To model diffusion, diffusion constants were estimated for 16 compounds from a determination of the Stokes radius and an empirical equation for cytoplasmic viscosity (*Biophys J.*, 58:31). Compounds with a relatively high diffusion constant, such as Ca, cAMP, cGMP, or IP_3, decreased to less than 10% concentration change at distances greater than 2 um following a 1 msec pulse of elevated concentration at the spine head. With slower diffusion rates, such as those for calmodulin, PKA, calcineuron, or calpain, changes were less than 10% at distances of only 0.8 um. Since spines are separated by a mean of 7.5 um, it seems unlikely that effective communication occurs by diffusion. In contrast, the electrotonic models, suggested that although voltage differences can develop within a spine, significant communication occurs between nearby spines. The results support the idea that spines form compartments limiting diffusional interactions between spines.

Supported in part by NIDCD, NINDS, ONR.

405.9

ARBORIZATION CHARACTERISTICS OF OLFACTORY RECEPTOR CELL AXONS IN OLFACTORY BULB GLOMERULI. N. Halasz[1] and C.A. Greer[2,3]. [1]Inst. Biophys., Biol. Res. Ctr., Hungarian Acad. Sci., Szeged, Hungary and Sects. [2]Neurosurg. and Neurobiol., Yale Univ. Sch. Med., New Haven, CT 06510.

To further our understanding of receptor cell projections to the olfactory bulb we studied the arborization of individual axons within glomeruli. Specifically, we wished to address the issue of whether a subglomerular organization may be established by the arborization characteristics of individual receptor cell axons within glomeruli.

Olfactory bulbs from Sprague-Dawley rats were processed with a Golgi-EM procedure chosen for its selective impregnation of individual receptor cell axons. Correlated light and electron microscopic analyses confirmed that individual axons could be resolved with the light microscope and reconstructed with camera-lucida. The terminal arbor of single axons encompassed areas of 41-1840 um^2 (mean $\pm$ S.E.M. = 880.36 $\pm$ 59.91). This accounted for only 3 - 20% of total glomerular area in any single case (mean $\pm$ S.E.M. = 12.12 $\pm$ 1.44). The number of axon bifurcations ranged from 1 - 15 (mean $\pm$ S.E.M. = 6.84 $\pm$ 0.51) and the total number of terminal enlargements or varicosities suggestive of synaptic specializations from 2 -19 (mean $\pm$ S.E.M. = 8.1 $\pm$ 0.51). Further studies revealed that a single terminal enlargement or varicosity could make synaptic contact with more than 1 postsynaptic target. These data show that olfactory receptor cell axons segregate into small subglomerular compartments. This differential innervation could provide a basis for subglomerular organization and perhaps differential synaptic organization.

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405.10

ANALYSIS OF THE STRUCTURE AND FUNCTION OF INDIVIDUAL HRP-LABELED GUSTATORY NEURONS IN THE NUCLEUS OF THE SOLITARY TRACT (NST) OF THE RAT. W.E. Renehan, S.P. Weaver*, X. Zhang* and L. Schweitzer. Division of Gastroenterology, Henry Ford Hospital, Detroit MI and Dept. of Anatomical Sciences and Neurobiology, University of Louisville.

To enhance our understanding of structure-function relationships in the NST, and as a prelude to future developmental studies, we have begun an investigation of the morphology of taste-sensitive neurons in the adult rat brainstem. Glass micropipettes filled with 6.0% HRP in Tris-KCl buffer were advanced into the rostral NST and a bipolar stimulating electrode was placed on the ipsilateral chorda tympani (CT) nerve. The response properties of neurons driven by the CT stimulating electrode were tested using 0.5 and 0.1 M NaCl, 0.5 and 0.1 M KCl, 0.01 M quinine HCl, 0.01 N HCl and 1.0 M sucrose. Following receptive field characterization the cells were impaled and injected with HRP. Cells were grouped according to soma size and shape, axonal target and dendritic field morphology. Most of the neurons recovered in this initial phase of our study have been quite small, with soma diameters in the range of 10-15 um, and have responded best to NaCl or quinine HCl. The majority of these neurons do not project to rostral targets, but instead appear to terminate in the NST or adjacent regions of the brainstem. Our results suggest that the intracellular labeling technique will permit us to relate the physiological and morphological properties of gustatory neurons in the NST.

CORTEX: ANATOMY

406.1

THE CONE ELECTRODE: ULTRASTRUCTURAL STUDY FOLLOWING LONG-TERM RECORDING. **P.R.Kennedy, S.Mirra and R.A.E.Bakay. Yerkes Research Center, Emory Univ., VA Med. Center, & Neurosci. Lab., GA Tech, Atl, GA.**

The achievement of long-term recording (LTR) of neural signals has potential clinical and investigative applications. To facilitate LTR a novel cone electrode consisting of an insulated gold wire within a hollow glass cone was developed (Kennedy, J. Neurosci. Methods, 29(1989)181-193). Sciatic nerve segments were inserted into the cones and implanted into cerebral cortex in rats and monkeys. Recordings and light microscopic preparations indicated connections between neurites within the glass cone and surrounding cortical neurons.

Electron microscopic study now further defines the structures within the electrode cones following implantation for as long as 15 months. After removal of electrodes and breaking of the glass cones, tissue within the cones was carefully removed under a dissecting microscope. EM examination revealed centrally myelinated axons, dendrites, synaptic profiles, blood vessels and glial cells. Peripheral nerve was not identified. Neuronal cell bodies were present only in the adjacent neuropil.

These studies suggest that cortical neurites grow into the tip of the cone electrode and remain viable for 15 months as shown by EM studies and by recordings of movement related activity in the same monkey. RR00165 Yerkes; Am. Paralysis Ass.

406.2

DISTRIBUTION OF NEUROFILAMENT IMMUNOREACTIVE NEURONS IN THE RHESUS MONKEY MOTOR CORTICES. M.J. Campbell and H. Barbas. Mt. Sinai Sch. Med., NY, NY 10029, and Boston Univ. and Sch. Med., Boston, MA 02215.

A monoclonal antibody to a nonphosphorylated neurofilament protein (SMI-32, Sternberger Monoclonal Inc.) labels a subpopulation of pyramidal neurons in cortex. The distribution of labeled neurons varies substantially across cortical areas (J. Comp. Neurol. 282:191-205, 1989). In this study we focused on the motor and premotor areas. The distribution of labeled neurons varied in a systematic way within architectonic subdivisions of the cortical motor system (J. Comp. Neurol. 256:211-228, 1987). Area 4 had the highest density of immunoreactive neurons both within the frontal and the entire cortex. Anterior to area 4 the density of labeled neurons was still high but decreased gradually in progressively rostral premotor areas. The dorsal premotor sector had a higher density of labeled neurons than the ventral. Within each premotor sector the central parts on the lateral surface had a higher density of immunoreactive neurons than either the supplementary area on the medial surface, or the lower part of ventral area 6. The preponderance of neurons that contain this cytoskeletal protein in the above frontal areas may be related to the specialization of the cortical motor systems.

(Supported by grants NS28571, NS24760, and BRSG955-SAR)

406.3

LAMINAR PATTERNING OF CORTICOCORTICAL CONNECTIONS IN THE RAT. D.H. Wang*, T.W. Deacon, and J. Connell* . Biological Anthropology, Harvard University, Cambridge, MA 02138

Using the tracers WGA-HRP reacted with TMB, PHA-L immunoreacted with DAB, and fluorescent dyes Fast Blue and Fluorogold we have attempted a comprehensive survey of laminar patterns of rat corticocortical connections, and contrasted them with those found in the monkey or cat.

In the rat, unlike cat and monkey, essentially all corticocortical projections include a significant portion of afferent terminations in layer 1. Additionally, labeling of afferents in deeper layers varies depending on the sites of origin and termination of the projection. Quantitative differences in the relative intensity of labeling in deep layer 3 and in layer 4 as compared to labeling in layers 1, 2, and upper layer 3 divide projections into two general classes. I. Projections originating from primary sensory areas, or targeting tertiary or polysensory areas terminate most densely in deep layer 3 and layer 4 and exhibit relatively lighter labeling in upper layer 3. This results in a bimodal distribution of label involving layer 1 and these middle layers. II. Projections from secondary areas to primary areas or from frontal to parietal areas terminate primarily in layer 1 with a decreasing density in layer 2 and upper layer 3, and sparing deeper layers. In general, labeling in infragranular layers is quite sparse, though exceptions do exist.

Placement of different fluorescent tracers into areas that receive projections from a common third area revealed laminar differences in cells of origin. Both extensive overlap and nearly complete segregation of cell groups into layers 3 and 5 were observed in different cases. For example, neurons in the lateral visual area (Oc2L) tend to be segregated according to their axonal targets, with laterally directed projections originating from supragranular and medially directed projections originating from infragranular cells. Although laminar differences are much more subtle and graded in the rat as compared to cat and monkey, it appears that there are corresponding laminar asymmetries.

406.4

A COMPARISON OF FRONTAL LOBE INPUTS TO THE PRIMARY, SUPPLEMENTARY AND CINGULATE MOTOR AREAS IN THE MONKEY. R.J. Morecraft and G.W. Van Hoesen. Depts. of Anatomy and Neurology, Univ. of Iowa, Iowa City, Iowa 52242

Although frontal lobe interconnections of the primary (area 4 or M1) and supplementary (medial area 6 or M2) motor areas are well understood, how frontal association cortex influences these or other areas that give rise to the corticospinal tract is not. We investigated the distribution of frontal lobe inputs to M1, M2 and the cingulate motor area (areas 24c and 23c or M3) in the rhesus monkey using fluorescent dyes. M1 received input from area 6, M2 and cingulate areas 24c and 23c. M2 received input from areas 46, 9, 12, 8B, 6, M1 and cingulate areas 24c and 23c. M3 received input from all cytoarchitectural types of cortex within this lobe including areas 10, 46, 9, 12, 8B, 8A, 6, M2, M1 and orbitofrontal cortex. A double labeling experiment demonstrated that less than 1% of all labeled neurons in areas 46, 9, 6, 4C, and M2 send axon collaterals to areas 24c and 23c. The heavy component of dorsolateral prefrontal input to M3 suggests a hierarchy concerning the diversity of frontal lobe inputs. M1 receives the least diverse frontal lobe input which arises exclusively from primary association cortex. M2 receives more diverse input which arises from multimodal association, primary association and primary motor cortices and M3 receives the most diverse and widespread frontal lobe input which includes paralimbic, multimodal association, primary association and primary motor cortices. These patterns of connectivity suggest that frontal association input gains preferential access to the cortical motor areas via M3. (Supported by grant NS14944)

406.5

FUNCTIONAL ACTIVITY IN THE CEREBRAL CORTEX OF THE RAT MODEL OF PARKINSON'S DISEASE MAPPED WITH ^{14}C DEOXYGLUCOSE. A. Dispenzieri and L.L. Brown, Dept. of Neurology Albert Einstein College of Medicine, Bronx NY 10461.

An ultimate target of efferent activity from caudate-putamen is the neocortex. To determine the effect of caudate-putamen dopamine loss on general neural activity in the cortex of an animal model of parkinson's disease, we used ^{14}C deoxyglucose autoradiography in rats with a unilateral 6-hydroxydopamine lesion of the substantia nigra. Animals were studied both at rest and with a sensory challenge. Primary motor, sensory and secondary motor regions all showed alterations in local cerebral glucose utilization (LCGU) associated with the lesion, but not more than 15%. Lesion animals showed an overall significant side-to-side difference in glucose utilization not shown in sham lesion controls, with a decrease in LCGU ipsilateral to the lesion. Mean cortical LCGU of all 73 regions analyzed was significantly decreased relative to untreated controls bilaterally (-14%, $p<.001$). The response of the cortex to the sensory challenge was also modified by the lesion. The results demonstrate a small but significant and widespread change in neural function related to dopamine depletion of the basal ganglia.

406.6

PREFRONTAL, PREMOTOR, MOTOR, AND HIPPOCAMPAL CONNECTIONS OF AREA 24 IN THE MACAQUE MONKEY. T. Arikuni, H. Sako*, and A. Murata*. Dept. of Anat. and Neurophysiol., Nihon Univ. Sch. of Med., Tokyo 173, Japan

The present study was undertaken to clarify the organization of transcortical connections between the dorsolateral prefrontal cortex and the hippocampal formation in the macaque monkey. Under direct vision, small amount of 10% solution of wheat germ agglutinin-conjugated horseradish peroxidase (WGA-HRP) was injected into each of several regions of area 24 of the cingulate cortex in the Macaca fuscata. The connections of area 24 with the prefrontal cortex, premotor area, motor area and the temporal cortex connected to the hippocampus were analysed. WGA-HRP-labeled cells and terminals appeared densely in areas 10, 9 and 13 and in the rostral area 46, while labeled cells and terminals appeared patchly in the caudal area 46 and areas 8A and 45. HRP-labeled cells and terminals were distributed more extensively in area 6aβ than in areas 6aα and 6b. Labeling was seen in areas 4a and 4b of the motor cortex and in the prosubiculum of the hippocampal formation. HRP-labeled cells were distributed in layer V of areas 28 (the entorhinal area) and TH, while they were distributed in layers III and V of areas 35, 36, TF, and TE. All of these areas contained HRP-labeled terminals. In summary, the prefrontal cortex and premotor area have strong reciprocal connections with area 24 which is strongly reciprocated with the prosubiculum and parahippocampal areas.

406.7

A DOUBLE LABELING STUDY ON SUBCORTICAL INPUTS TO THE SUPPLEMENTARY MOTOR AREA IN THE MONKEY. H.Tokuno*, J.Tanji, M.Kimura and H.Aizawa. Natl. Inst. Physiol. Sci. Okazaki 444, Japan.

To elucidate subcortical inputs to the supplementary motor area (SMA) precisely, we labeled pallidal or cerebellar terminals in the thalamus using wheat germ agglutinated horseradish peroxidase (WGA-HRP), and labeled thalamic neurons projecting to the SMA using Fast Blue (FB) in the same monkey.

FB was injected into the hand-arm area of the SMA after confirming the somatotopy using intracortical microstimulation. After 3-4 weeks, WGA-HRP was injected into the internal segment of the globus pallidus or the deep cerebellar nuclei. After 3 days, the animals were perfused under deep anesthesia. The sections were processed with tetramethylbenzidine and observed with bright-field and epifluorescent illumination. In pallidal injection cases, numerous FB labeled thalamic neurons were distributed in pallidal terminal areas. In contrast, few labeled neurons were found in cerebellar terminal areas.

The present results indicate that the SMA receives subcortical information mainly from the basal ganglia via the thalamus.

406.8

TOPOGRAPHY AND COLLATERALIZATION OF BRAINSTEM DOPAMINERGIC (DA) PROJECTIONS TO MOTOR CORTEX, SUPPLEMENTARY MOTOR AREA AND LATERAL PREFRONTAL CORTEX. IN OWL MONKEY. P. Gaspar*, I.Stepniewska, and J.H.Kaas. INSERM U106, Paris, France and Vanderbilt University, Nashville TN 37240.

Motor and premotor areas receive a dense DA innervation in primates. We determined the origin of this projection and showed that some neurons project via collaterals to more than one field by injecting different fluorescent dyes in parts of the prefrontal cortex, MI, and SMA, first identified by microstimulation in adult owl monkeys (*Aotus trivirgatus*). The distributions of retrogradely labeled cells were related to the locations of tyrosine hydroxylase immunolabeled (DA) neurons on the same or alternate brain sections. All the cortically projecting DA cells were located in the A8-A10 complex, largely in its dorsal components, the parabrachial pigmented n. of the ventral tegmental area (VTA), pars γ of the substantia nigra (SNc), and the dorsal part of the retrorubral area (A8). Fewer cells were in the midline groups of VTA (LR,LC) and in n. paranigralis. DA neurons projecting to MI, SMA, and prefrontal cortex were largely intermixed, with some collateralization (double or triple labeled cells). Cells projecting to MI were always more numerous than to the other cortical locations.

The topography of the cortically projecting DA cells corresponded in part to a histochemical subdivision of the A8-A10 complex defined by the presence of calbindin-immunoreactive neurons, and by neurotensin and noradrenergic terminal innervation. (Supported by grant NS 16446).

406.9

COMPARISON OF CORTICAL PROJECTIONS TO PRIMARY AND SUPPLEMENTARY MOTOR AREAS IN OWL MONKEYS (AOTUS TRIVIRGATUS). I. Stepniewska, P. Gaspar* and J.H. Kaas. Dept. of Psychology, Vanderbilt University, Nashville, TN 37240.

We used injections of two different fluorescent tracers to demonstrate that primary motor cortex (MI) and supplementary motor area (SMA) receive some inputs from separate regions of cortex, and some from the same regions, although few neurons project to both fields.

Forelimb representations in MI and SMA were first identified by intracortical microstimulation, boundaries were marked with microlesions and different tracers were injected into the same body part representation in both fields. Brains were sectioned in the coronal plane or parallel to the surface after flattening, and alternate series of sections were stained for myelin, CO or AChE. Injections revealed arrays of short intrinsic connections in both fields and interconnections between the fields. MI had major inputs from areas 3a and 1, while SMA did not; neither area received notable input from area 3b. More caudally cortex in the 2—5 region projected to both SMA and MI, although only a few double labeled neurons were detected. Almost all the projections from SII region and adjoining cortex were to MI. Foci of labeled neurons projecting to MI and SMA from lateral premotor and rostrally adjacent cortex overlapped extensively, but few cells were double labeled. Some regions of cingulate cortex projected to both fields, but rarely via the same neurons. Finally, cortex on the medial wall just rostral to the SMA and more caudal cingulate region sent projections to SMA but not MI. (Supported by NS 16446).

406.10

AREA LIP INPUT TO AREA 7A IN THE INFERIOR PARIETAL LOBULE OF MACAQUE. J.-R. Tian, J.C. Lynch, and S.G.P. Hardy. Department of Anatomy, University of Mississippi Medical Center, Jackson, MS 39216.

Studies of the sources of neural input to area 7a, a higher-order association area in the inferior parietal lobe, have been inconclusive about whether there is a significant projection to 7a from the lateral intraparietal area (LIP), a region in the lateral bank of the intraparietal sulcus that seems to have predominantly visual and oculomotor functions. This question was addressed by making small placements of the retrogradely transported tracers diamidino yellow or fast blue in area 7a and/or LIP in 3 M. fascicularis and 1 M. mulatta. Labeled neurons were counted in evenly-spaced sections through the LIP, cingulate gyrus, and the principal sulcus region of prefrontal cortex (PS). Typically, both cingulate gyrus and PS cortex contained more than twice as many neurons that projected to 7a as did LIP. Neurons in LIP that were labeled by 7a injections were restricted to a single small zone. In contrast, neurons in 7a that were labeled by LIP injections were distributed widely through the area. More neurons were labeled in 7a by the LIP placements than vice versa. These experiments confirm that LIP is a source of neural input to 7a, but suggest that, in spite of the close physical proximity of 7a and LIP, and of their functional similarities, both the cingulate gyrus and the PS region of prefrontal cortex provide more robust neural connections with 7a than does LIP. Furthermore, the data suggest that the projection from 7a to LIP may be considerably more extensive than the projection in the opposite direction. (Supported by PHS grant EY04159)

406.11

PATTERN OF PROJECTIONS FROM THE PREMOTOR AREAS ON THE MEDIAL WALL OF THE HEMISPHERE TO THE PRIMARY MOTOR CORTEX. J.W. Holsapple and P.L. Strick. VA Med. Ctr. and Depts. of Neurosurg. & Physiol., SUNY-HSC @ Syracuse, Syracuse, NY 13210.

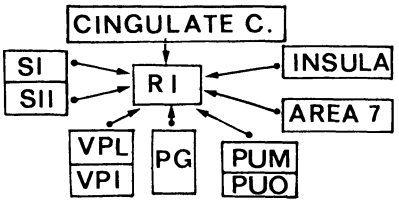
The representation of the distal forelimb in the primary motor cortex of macaques is found in 2 regions: 1) a rostral region on the crest of the precentral gyrus and 2) a caudal region in the anterior bank of the central sulcus. The results of prior studies have suggested that anatomical and physiological differences exist between the 2 regions of distal forelimb representation (e.g., Strick and Preston, '82; Holsapple et al., '91). We used retrograde transport of wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP) to examine the pattern of interconnections between the arm areas of the premotor areas on the medial wall of the hemisphere and the rostral and caudal regions of forelimb representation in the primary motor cortex of macaques (*Macaca nemestrina*). Tracer injections into the supplementary motor area (SMA, n=2) and the caudal cingulate motor area on the ventral bank of the cingulate sulcus (CMAv, n=2) labeled large numbers of neurons in the rostral region of forelimb representation on the crest of the precentral gyrus. Surprisingly, few neurons were labeled in the caudal region of the forelimb representation in the anterior bank of the central sulcus. In contrast, tracer injections into the caudal cingulate motor area on the dorsal bank of the cingulate sulcus (CMAd, n=2) labeled large numbers of neurons in the caudal region in the central sulcus, as well as in the rostral region. These results support the concept that the rostral and caudal portions of the forelimb representation in the primary motor cortex each have a unique input-output organization. Furthermore, they provide additional evidence that the CMAd is a motor field which is distinct from the SMA and CMAv. Support: VA Med.Res.Serv.; USPHS 2957, 24328, 843902.

406.12

THE INTERCONNECTIONS OF THE RETRO-INSULAR CORTEX OF THE MARMOSET MONKEY. W. Guldin, S. Mirring* and O.-J. Grüsser; Dept. of Physiology, Freie Universität Berlin, 1000 Berlin 33, Germany.

The retroinsular cortex (Ri) is a cortical representation of somatosensory and vestibular input, situated in the posterior Sylvian sulcus of the marmoset monkey (Callithrix jacchus). Different retrograde tracers were placed into the cortical regions around this sulcus. The loci of injection were identified by single unit recordings and subsequent histology.

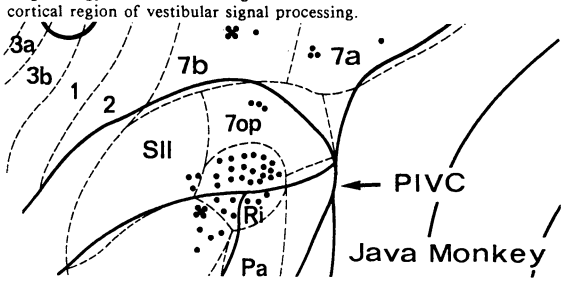
Thalamic afferences originate mainly in the oral- and medial-pulvinar nucleus (PUO, PUM) in the posterior group PG and in the lateral ventral and the inferior ventral nucleus (VPL, VPI). The cortical input to Ri of the Callithrix monkey comes from the area 7, the cingulate cortex, the somatosensory areas SI and SII and the insula. Our results support in part our former findings on the interconnections of the parieto-insular vestibular cortex (PIVC) of the Squirrel monkeys.



406.13

EFFERENCES FROM THE VESTIBULAR CORTEX TO THE VESTIBULAR NUCLEI IN PRIMATES. S. Akbarian, O.-J. Grüsser and W. Guldin. Dept. of Physiology, Freie Universität Berlin, 1000 Berlin 33, Germany

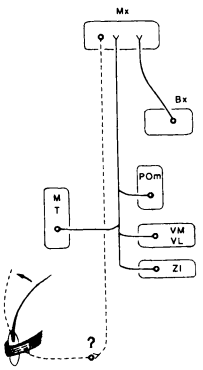
The parieto-insular vestibular cortex (PIVC) is a vestibular cortical field located in the posterior insula and the retroinsular region. Vestibular neurons were found in the PIVC of Java monkey (Macaca fascicularis) and Squirrel monkey (Saimiri sciureus). Identifying injection sites by recording single unit responses, we placed different retrograde tracers in the vestibular nuclei (VN) of anesthetised Java and Squirrel monkeys. In both species retrograde nerve cell labelling in the PIVC region could be detected. The only other cortical regions with noteworthy labelling after VN injections were area 7 and the cingulate gyrus. These findings indicate that the PIVC is the main cortical region of vestibular signal processing.



406.14

VIBRISSAL MOTOR CORTEX IN THE ADULT MOUSE; ITS CORTICAL AND THALAMIC INPUT.

Ullrich, N.*, E. Welker* and H. Van der Loos.
Institute of Anatomy, University of Lausanne, 1005 Lausanne, Switzerland.



In order to study the potential role of a somatosensory pathway in adult plasticity in a related motor pathway we have investigated their interrelationship in the vibrissal system at cortical and thalamic levels. Using platinum-iridium electrodes (kindly provided by Prof.J.Donoghue, Brown University, RI), we delineated a discrete portion of the primary motor cortex where, under light anaesthesia, movements of individual caudal vibrissae could be elicited. At this cortical locus we deposited a fluorescent retrograde tracer (rhodamine beads), while an anterograde tracer (biocytin) was injected in the barrel in SI corresponding to the stimulated whisker (as determined physiologically, in the same animal; n=10). The vibrissal motor cortex receives input from the barrel cortex and the following thalamic nuclei: ventral lateral (VL), ventral medial (VM), medial portion of the posterior complex (PO), zona incerta (ZI) and some of the medial thalamic nuclei (MT). Future analysis will further define the significance of PO as a link between the motor and sensory vibrissal pathways. Support: Swiss NSF 3100.009468.

406.15

INTERHEMISPHERIC COMPARISONS OF THE ORGANIZATION OF BRODMANN'S AREAS 44 AND 45 IN HUMAN BRAIN. T.L. Hayes and D.A. Lewis. Departments of Behavioral Neuroscience and Psychiatry, University of Pittsburgh, Pittsburgh, PA 15216.

Although motor speech functions are believed to be localized to the left hemisphere in Brodmann's areas 44 and 45, little is known about how the cyto- and chemoarchitecture of these areas compare to their homologues in the right hemisphere. We used Nissl stains to compare the cytoarchitectonic features of these regions in five control human brains. Areas 44 and 45, located in the posterior inferior frontal gyrus, were identified according to published criteria. At its most caudal extent, area 44 is dysgranular. More rostrally, in area 45, the cortex becomes granular, with unusually large pyramidal neurons present in deep layer III. At the most rostral aspect of area 45, the large layer III pyramidal neurons are not seen. These cytoarchitectonic features were identified in both hemispheres. However, measurements of the size of the large layer III pyramidal neurons in caudal area 45 revealed that they were larger in the left hemisphere than in the right ($p<.05$); the largest neurons were 10-20% larger in the left than the right. Further comparisons were made using immunohistochemical markers of non-phosphorylated neurofilament protein (NPNFP) and calbindin. As previously reported (*Soc. Neuro. Abstr. 16:240, 1990*), the large layer III pyramidal neurons in the left hemisphere were immunoreactive for NPNFP but not for calbindin. The present study revealed a similar pattern of immunoreactivity in the right hemisphere. Further investigation of possible hemispheric differences in these regions are underway. These studies may provide insight into the anatomical basis of the lateralization of motor speech function.

406.16

RELATIONSHIPS BETWEEN CONNECTIVITY AND CYTOSKELETAL PROFILE OF CORTICOCORTICALLY-PROJECTING NEURONS. J.H. Morrison, P.R. Hof, S.B. Kupferschmid, P.F. Good, W. Janssen*, and N. Archin*. Fishberg Research Center for Neurobiology and Dept of Geriatrics, Mount Sinai School of Medicine, New York, NY 10029.

Previous analyses combining retrograde transport of Fast Blue (FB) with immunohistochemistry have demonstrated that a substantial subpopulation of corticocortically-projecting neurons (CCPN) contain high concentrations of non-phosphorylated neurofilament protein (NPNFP). However, the relative proportion of a given projection that is NPNFP+ varies with its functional role and connectivity characteristics (Campbell et al, *Brain Res*, 1991:**539**). For these analyses, FB injections were placed in one of the following regions: prefrontal cortex ventral to the principal sulcus (vPS), inferior parietal cortex (vIPS) or cortex in the superior temporal sulcus (STS). The distributions of FB only and FB plus NPNFP double labeled neurons were analyzed in regions that provide cortical projections to each injection site. Quantitative analyses revealed that 1) Interconnections between these three association areas vary in the proportion of CCPN that are NPNFP+ such that connections between vIPS and STS have the lowest proportion (30-40%), vIPS and vPS intermediate (40-50%), and STS and vPS the highest (75-90%). 2) Reciprocal connections tend to have a similar proportion of double labeled cells. 3) The incidence of NPNFP+ CCPN from homologous contralateral areas varies from 25-100% depending on the system involved. 4) Analyses within the visual system suggest that CCPN within a given sensory modality may display differential typological characteristics as compared to CCPN interconnecting high-level association regions. Supported by AHAF and NIH AG06647.

406.17

INSULAR CORTEX OF THE CAT: THE RETROGRADE TRANSPORT OF HORSERADISH PEROXIDASE TO THE VENTRAL GROUP, MIDLINE NUCLEI, INTRALAMINAR COMPLEX, AND MEDIODORSAL NUCLEUS OF THE THALAMUS. F.G. Martinez-Sandoval and J. de la Rosa. Fac. de Med.,Univ. Autonoma de Guadalajara, Mex.

The cells of origin of projections from the ventromedial, ventrobasal, and intralaminar thalamic complexes, midline nuclei, and mediodorsal nucleus to the orbito-insula, insula proper and insulo-temporal cortex of the cat, were analyzed by means of the retrograde transport of horseradish peroxidase (HRP). After injections in different parts of theses cortical zones, HPRP-positive neurons were found in all experiments in the nucleus ventralis medialis (VM), interventralis (Iv), centralis medialis (Cm), parafascicularis (Pf), subparafascicularis (Spf), reuniens (Re), and mediodorsal (MD). In some experiments labelled neurons were detected in the nucleus ventralis anterior (VA), ventralis lateralis (VL), submedius (S), centre median (CM), paratenialis (Pt), and paraventricularis anterior (Pva). It seems that VA innervates the orbito-insula and most of the insula proper but not the caudal insula nor the insulo-temporal cortex. VL sends projections to the orbito- insula and most of the insula proper but not to the ventral insula nor the insulo-temporal cortex. S, Pc, CL, and CM innervate all regions of the insula except the insulo - temporal cortex. These findings suggest a multidisciplinary participation of the insula in the relay and/or control of modalities such as somato - motor, somato - sensory, visceral-motor, visceral-sensory, and an important role in the limbic system.

CORTEX: LESION AND STIMULATION

407.1

MUSCIMOL INJECTIONS IN MONKEY AREA 4 IMPAIR INDIVIDUATED FINGER MOVEMENTS, IN AREA 6 PRODUCE CONTRALATERAL NEGLECT. M.H.Schieber, L.Kim* and W.T.Thach. Depts. of Neurol. & Neurosurg., Anat. & Neurobiol., Wash. U. Sch. Med., St. Louis, MO. 63110.

What each cortical motor area--including the motor cortex in area 4 (MC), and the arcuate premotor area (APA) and supplementary motor area (SMA) in area 6--contributes to finger movements in any given behavior is uncertain. To investigate, muscimol was injected intracortically, reversibly inactivating each area as a Rhesus monkey performed a visually-cued individuated finger movement task. Muscimol was injected (1-5μg in 1μl over 1-5 min. via a 26-30g needle) at left hemisphere sites where neuron activity was well related to finger movements. MC was injected on 7, APA on 3, and SMA on 2 occasions.

MC injections were followed within 5-15 min. by deterioration of the trained contralateral finger movements. APA or SMA injections, in contrast, had no identified effect on task finger movements. Nor did they disrupt other aspects of the task, even though APA neurons responded briskly to the visual cues, and both APA and SMA neurons were well related to task finger movements. MC injections also rendered the monkey unable to extract raisins from a narrow well with the contralateral fingers, though in walking and climbing this hand appeared normal. Again, neither APA nor SMA injections affected these finger movements. Nevertheless, on bilateral simultaneous presentation of apple pieces, APA and SMA injections each decreased the likelihood both that the monkey would use his contralateral hand to retrieve bait, and that he would take contralateral bait with either hand. APA injections also decreased the likelihood that the monkey would first turn his head contralaterally to examine the bait. MC injections had no effect on laterality of bait retrieval or initial head turning.

MC thus appears necessary for all individuated finger movements, but not for finger control in walking or climbing. If APA and SMA neurons are active during visually-cued finger movements, why does inactivation of these areas not disrupt this performance, and instead produce only a mild contralateral neglect?

Support: K08NS01150, R01NS27686, R01NS12777, and McDonnell Center.

407.2

MODALITY-INDEPENDENT DEFICITS IN SPATIAL MEMORY TASKS INDUCED BY LOCAL INJECTIONS OF BICUCULLINE INTO THE PREFRONTAL CORTEX OF MONKEYS. T. Sawaguchi and P. S. Goldman-Rakic. Section of Neurobiology, Yale Univ. Sch. of Med., New Haven, CT 06510.

To examine the modality-specificity of spatial working memory mediated by the prefrontal cortex (PFC), bicuculline was injected into the dorsolateral PFC of monkeys who were trained on 3 different oculomotor tasks associated with memory-guided saccades to left or right spatial targets: 1) a standard oculomotor delayed response task cued by visual stimuli (VODR); 2) the ODR task cued by auditory stimuli (AODR); 3) a delayed alternation task where the monkeys' previous somatic (S) responses presumably guided alternatively saccades to the left and right targets (SODR). In each daily session, the monkeys performed the VODR and one or two of the other memory task(s) as well as a control task which required the monkeys to make only sensory-guided saccades. Several delays (1-5 sec) were employed in each ODR task. Local injection of bicuculline (1-3μg) into the PFC induced deficits in all of the memory tasks but not in the control task. When the VODR task was impaired, the AODR and SODR tasks were invariably impaired, and vice versa. The deficits were usually restricted to the memory-guided saccades directed toward the target contralateral to the injection site, and the impairment was dependent on the duration of the delay. Further, the effective sites of the injection were clustered in a small region of the dorsal bank of principal sulcus. The data suggest that the same region of the dorsolateral PFC is involved in visual, auditory, and somatosensory working memory for spatially guided behavior and that this region can be considered as a working memory center for spatial cognition.

407.3

MICROSTIMULATION STUDIES OF PREMOTOR AND OCULOMOTOR CORTEX IN OWL MONKEYS. T.M. Preuss, J. Stepniewska, and J.H. Kaas. Dept. of Psychology, Vanderbilt University, Nashville, TN 37240.

We carried out extensive motor mapping studies of the dorsolateral frontal lobe in seven owl monkeys (*Aotus trivirgatus*). Animals were anesthetized with ketamine and xylazine and stimulated intracortically with .5—1 MΩ microelectrodes. Under these conditions, somatic and ocular movements could be elicited from a large territory rostral to primary motor cortex (M1); only the extreme ventrolateral and polar regions were unresponsive at high currents.

A dorsal premotor region lies between the low-threshold (<40 μA) M1 and the oculomotor cortex. This region is agranular in architecture, like M1, but has smaller pyramidal cells; somatic movements could be elicited at currents of ~40—150 μA, and sometimes less An additional somatic zone is located just rostral to the M1 face representation. Forelimb and (more laterally) face movements were elicited from this zone, often with low currents (<40 μA). This zone is dysgranular, rather than agranular, and is probably homologous to the arcuate premotor area (APA) of macaques. Rostral to the premotor zone, eye movements were elicited at low thresholds from the frontal eye field (FEF) and supplementary eye field (SEF), and at higher currents from the cortex located just rostral to FEF (previously described as the frontal ventral area), and also in the territory between FEF and SEF. In the latter region, thresholds typically ranged from ~50—200 μA, and at many points, movements of the face as well as the eyes could be elicited. (Supported by NS 16446, EY 06304, and JSMF #90-35.)

407.4

LATE RESPONSES TO TRANSCRANIAL MOTOR CORTEX STIMULATION IN LOWER LIMB MUSCLES OF HEALTHY HUMANS. W.B. McKay, M.R. Dimitrijevic, M.*Kofler Division of Restorative Neurology and Human Neurobiology, Baylor College of Medicine, Houston, Texas 77030.

Short latency motor evoked potentials (MEPs) have been recorded in response to transcranial motor cortex stimulation from skeletal muscles whose spinal motor nuclei are innervated by the stimulated cortical cells. Magnetic stimulation applied at intensities of up to 2.5 Tesla with the coil centered over the vertex of the skull elicited surface EMG recorded MEPs from upper and lower limb muscles in 7 healthy subjects. The mean onset latencies for the early responses recorded from the quadriceps, hamstring, tibialis anterior and triceps surae muscles were 25,26,30 and 32 ms respectively. Second, later responses were recorded with mean latencies of 66, 60, 76 and 75 ms respectively. Two possible mechanisms for the generation of the second response are: 1. a strech reflex elicited by the early contraction or; 2. a longer additional pathway such as cortico-bulbo-spinal mediated response that would require a longer time to arrive.

407.5

DYNAMIC MODULATION OF PRIMATE MOTOR CORTEX OUTPUT DURING MOVEMENT J. P. Donoghue and J. N. Sanes Center for Neural Science, Brown Univ., Providence, RI 02912

These experiments tested the hypothesis that motor cortex output is dynamically modulated during skilled arm movements. *M. Fascicularis* monkeys performed a visually guided step-tracking task which required flexion of the wrist against a spring load. During movement preparation and execution the motor cortex was stimulated at one of 11 sites through 70 um Pt-Ir wires that were chronically implanted in areas 4 and 6. The EMG was recorded in 7 arm muscles (biceps, triceps, ECU, ECR, PL, EDC, FCU). Individual sites were stimulated with 30 ms pulse trains below 20 uA in each of 6 epochs: (1) during hold (no load), (2) during the same hold but after target location was displayed, (3) 250 ms after a go cue, (4) during movement initiation, (5) at mid movement, and (6) during hold in target zone

Modulation in the amount of evoked EMG during different epochs was site dependent. Stimulation at sites where digit movements were evoked at threshold showed only suppression or no change in EMG evoked from digit or wrist extensors. Pronounced modulation of FCU, ECU, ECR, or PL (1.5-10 fold increase of evoked EMG above epoch 1) was seen at wrist and shoulder or mixed movement sites, most frequently in epoch 3 (68% of cases). Evoked EMG during epochs 4-6 at these sites were typically equal to or below that evoked in epoch 1, even though the muscles remained active. At 2 sites evoked EMG in wrist muscles paralleled expected changes in the excitability of wrist flexor motor neuron pools. Modulation due to the instruction was uncommon (19% of the muscle observations). The occurrence of evoked EMG could depend on the stimulation epoch occurred: in 39% of 31 cases a muscle was activated from cortex only in ≤3 epochs. These findings suggest that the relationships between groups of cortical neurons and muscles may be temporarily maintained during different movement epochs. This organization may direct feedback onto selected cortical cell groups and could participate in adaptive reorganization of cortical motor maps observed after nerve transections or maintained changes in posture. Supported by NIH NS 25074, 22517, March Of Dimes 5-562, Culpeper and Whitehall Foundations

407.6

MUSCLE INHIBITION FROM TRANSCRANIAL STIMULATION IN NORMAL HUMANS. E. M. Wassermann, A. Pascual-Leone, J. Valls-Solé,* L.G. Cohen, and M. Hallett. Human Cortical Physiology Unit, Human Motor Control Section, NINDS, NIH, Bethesda, MD 20892.

We used transcranial magnetic stimulation to study 5 normals for inhibitory motor responses. Subjects activated the abductor pollicis brevis (APB) while we stimulated sites one cm apart on the contralateral scalp with a Cadwell MES-10 stimulator and an "8"-shaped coil. Rectified EMG from APB was averaged. In the area where stimulation produced the largest motor evoked potentials (MEPs), low stimulus intensities (30-35% stimulator output) elicited silent periods (SPs) on 20 trial averages without evidence of preceding facilitation. At slightly higher intensities (40-50%), MEPs followed by SPs were found at a cluster of sites on the central scalp (MEP area). At the same stimulation intensity, averages from 3 to 9 sites just anterolateral to the MEP area yielded SPs without facilitation. SPs without facilitation could not be produced at any other sites at this intensity. At sites further lateral, high intensities (60-100%) evoked robust SPs without MEPs on single trials.

Muscle suppression without preceding facilitation may be mediated by low threshold corticospinal neurons, spinal circuits excitable by weak cortical output, or inhibitory cerebral circuits. The difference between MEP and SP thresholds is greatest anterolateral to the center of the MEP area. This region may correspond to an inhibitory motor area defined by direct cortical stimulation with electrical pulse trains.

407.7

CORTICOSPINAL PROJECTIONS TO LOWER LIMB MOTONEURONS IN MAN. **B. Brouwer and P. Ashby* Dept. of Rehab. Therapy, Queen's Univ., Kingston, ON K7L 3N6 and Playfair Neuroscience Unit, Toronto Western Hospital, Toronto, ON M5T 2S8**

The relative strength of the projections from cortical neurons activated by transcranial magnetic stimulation to lower limb spinal motoneurons of the vastus medialis (VM), biceps femoris (BF), tibialis anterior (TA), medial gastrocnemius (MG), soleus (SOL), extensor digitorum brevis (EDB) and flexor digitorum longus (FDL) were examined in 34 normal subjects. The magnitudes of the postsynaptic potentials (PSPs) produced in spinal motoneurons were estimated from the changes in firing probability of single motor units. Strong short latency facilitations were observed in all TA motor units and in 67% of those innervating the small muscles of the foot. Less than 30% of the motoneurons of the triceps surae muscles were facilitated by the cortical stimulus and of those that were, the PSPs were small. The responses in other lower limb muscles were variable. It is argued that the observed pattern and the magnitude of the evoked responses are consistent with the known projections of the rapidly conducting corticospinal tract in subhuman primates.

407.8

ACTIVATION OF LOWER LIMB MUSCLES BY MAGNETIC STIMULATION IN SPINAL CORD INJURED (SCI) PATIENTS IS CONTEXT DEPENDENT. M.C.Verrier, B.E. Mustard and C.D. MacKinnon*, Departments of Rehabilitation Medicine and Physiology, University of Toronto., Lyndhurst Hospital, Toronto., CANADA M5T 1W5.

Magnetic stimulation (MS) to motor cortex activates contralateral lower limb compound motor action potentials (CMAPs) in normals during different motor tasks. MS in SCI has been used to determine integrity of the cortical spinal pathway but not as an determinant of neural recovery or therapeutic intervention. Clinically, SCI subjects with incomplete lesions demonstrate an ability to cycle with progressively more phasic and reciprocal activity during the recovery phase. We used MS to activate lower limb CMAPs (biceps femoris BF, rectus femoris RF, tibialis anterior TA, and soleus S) in these patients at rest, during selective muscle activation and during upright cycling to determine whether MS has utility as a therapeutic strategy. In studies using MS to the motor cortex (4 cm posterior; 2 cm lateral to Cz; with a Cadwell MES-10 with a 9.5 cm coil) CMAPs were repeatedly elicited in Q, and S during selective muscle activation but not routinely in BF and TA (n=1). Response latencies for Q and S were 23msec and 31msec respectively. In normals (n=10) BF, Q and TA were reliably elicited in all subjects and S in 8 out of 10 subjects with facilitation. In contrast to normal subjects, CMAPs in SCI subjects were not elicited during active cycling. A single MS stimulus interrupted cyclic S activity and elicited maintained S activity that lasted throughout the trial. The study suggests that the response to MS in SCI patients may be context dependent and indicative of existing spinal motor circuitry.

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407.9

INHIBITORY INTERACTIONS BETWEEN TRANSCRANIAL BRAIN STIMULI APPLIED OVER THE MOTOR CORTEX IN MAN. T. Kujirai*, M. Sato*, J.C. Rothwell, B.L. Day*, P.D. Thompson*, S. Wroe*, P.T. Asselman* and C.D. Marsden*. MRC Human Movement & Balance Unit, Institute of Neurology, Queen Square, London WC1N 3BG, U.K.

With ethical committee approval, we gave pairs of transcranial brain stimuli separated by intervals of 1-20ms over the motor cortex of 10 normal volunteers. Both electrical (Digitimer D180) and magnetic (Magstim 200 connected to a figure 8 coil) stimuli were used. EMG responses were recorded from surface electrodes over the contralateral first dorsal interosseous (FDI) muscle. In active muscle, responses to electrical stimulation (at 1-1.2 times threshold) were facilitated by a preceding conditioning stimulus (either electric or magnetic), whereas responses to magnetic stimulation were inhibited. This suggests that the inhibition of magnetically-evoked responses occurred at the level of the motor cortex. The amount of inhibition depended upon the intensity of the conditioning stimulus. At high intensities of conditioning shock, the amount of inhibition was reduced, and in some subjects became a facilitation. The position of the conditioning stimulus was important. Moving the conditioning shock away from the motor strip decreased the amount of inhibition. However, conditioning stimulation of the hand area of cortex was not necessary to produce inhibition of responses evoked in the FDI. For example, conditioning stimuli applied at the vertex at an intensity subthreshold for producing EMG responses in the active FDI could still inhibit test responses evoked in the same muscle by stimulation over the hand area. The inhibitory interaction between pairs of stimuli is probably produced by a combination of several mechanisms, including refractoriness of pyramidal tract cells discharged by the conditioning stimulus, recurrent inhibition from pyramidal tract collaterals, and cortico-cortical inhibitory processes.

407.10

CORTICAL ACTIVITY PRECEDING SEQUENTIAL HAND MOVEMENTS AFTER BILATERAL LESION OF THE SUPPLEMENTARY MOTOR AREA (SMA)

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We studied a patient with a chronic bilateral lesion of the SMA. Sequential hand movements (right wrist flexion-left wrist extension) revealed a mean interonset latency of 893±237 (Control 219±38 ms) when self-initiated and 912±283 ms (Controls 199±44 ms) when triggered by a visual stimulus. Back averaging the EEG activity preceding the first EMG discharge (right hand extension) showed absent readiness potential (RP). A motor potential (MP) starting some -70 ms was recorded over the primary motor cortex. Using the second EMG for triggering revealed a MP preceding left wrist extension by -120 ms. The interval between the maximal negativity of both MP was 927±202 ms. These observations indicate that cortical activity preceding sequential movements may be used to estimate the central delay for the assembling of motor programs. Such process appears to depend upon the integrity of the SMA.

SPINAL CORD AND BRAINSTEM III

408.1

INVESTIGATION OF MOTONEURONAL AND PREMOTONEURONAL MECHANISMS OF PUDENDAL REFLEX MODULATION DURING MICTURITION IN THE CAT. B. Fedirchuk, L. Song* and S.J. Shefchyk. Depts of Med. and Physiol., Univ. of Manitoba, Winnipeg, Canada, R3E 0W3.

This work investigates the mechanisms responsible for pudendal reflex suppression during micturition. Intracellular recordings were obtained from antidromically identified external urethral sphincter (EUS) or external anal sphincter (EAS) motoneurons (MNs) in decerebrate male cats. Sacral afferents from the sensory branch of the pudendal nerve and the superior perineal cutaneous nerve were electrically stimulated at $\leq 5T$ (T = minimum stimulus to elicit a cord dorsum potential) to produce postsynaptic potentials (PSPs) in the pudendal MNs during reflex distention or brainstem evoked micturition. In addition, 500μs hyperpolarizing short pulses were injected into the MN to assess MN membrane conductance changes before and during the void.

The membrane potential of EUS MNs hyperpolarized during the bladder contraction and void while EAS MNs depolarized or showed no change. PSPs produced in EUS MNs from both peripheral nerves were reduced in amplitude or abolished when the bladder pressure was elevated and voiding occurring. Preliminary evidence supports the presence of a conductance increase of the MN during voiding, however the magnitude of this conductance change is unlikely to be entirely responsible for the decrease in the PSP amplitude observed.

Recordings made from the proximal end of S2 dorsal root filaments have revealed dorsal root potentials produced by stimulation of sensory pudendal, superior perineal and pelvic afferents. The contribution of primary afferent depolarization to sphincter reflex modulation must be considered along with actions at segmental interneuron sites.

This research was supported by the Medical Research Council of Canada.

408.2

THE IDENTIFICATION OF GLYCINERGIC TRIGEMINAL PREMOTONEURONS. J.E. Turman, Jr., S.H. Chandler and R.S. Fisher. Dept. of Kinesiology, Anatomy/Psychiatry and the Brain Research Institute, UCLA, Los Angeles, CA 90024.

A glycinergic innervation of the trigeminal motor nucleus is suggested from physiological studies which investigated the inhibition of masseter motoneurons following peripheral and cortical stimulation. Following stimulation of the inferior alveolar and masseter nerves an early and late hyperpolarization is observed in masseter motoneurons. The early hyperpolarization is blocked by a systemic application of strychnine suggesting that the hyperpolarization is due to glycine (Nakamura, Y. et al., Brain Res., 57:29, 1973). Following repetitive stimulation of the masticatory cortex, rhythmical jaw movements are observed. A large portion of the rhythmical hyperpolarization seen in the masseter motoneurons is blocked by strychnine (Enomoto, S. et al., Neurosci. Res., 4:396, 1987).

We initiated a series of experiments to identify glycinergic inputs to the trigeminal motor nucleus. A retrograde tracer, gold colloidal-bound WGA-HRP (gWGA-HRP) was injected into the trigeminal motor nucleus of male albino guinea pigs. Following a 72 hour survival time, the animals were transcardially perfused with 4% glutaraldehyde and 0.5% paraformaldehyde. Glycine immunohistochemistry was performed using a polyclonal antibody (Chemicon, Inc.). ABC histochemistry with DAB as a chromagen was used to label the immunoreactive complex. Neurons which co-localized the connectivity marker and neurotransmitter marker were identified bilaterally in the parvocellular reticular formation, supratrigeminal nucleus, intertrigeminal nucleus, spinal trigeminal nucleus oralis and lateral paragigantocellularis. Immunoreactive cells, not containing the retrograde marker, were identified in the superior olive, dorsal cochlear nucleus, trapezoid body, vestibular nuclei, nucleus gigantocellularis, perihypoglossal area and spinal trigeminal nucleus interpolaris. Based on these data we conclude that glycinergic inputs to the trigeminal motor nucleus converge from several distinct portions of the brainstem. Funded by NIH-NIDR grant DE06193.

408.3

SPATIAL AND TEMPORAL OPTICAL RECORDING OF THE EXCITATION OF SINGLE NEURONS COMBINING A COOLED CCD AND A PHOTODIODE ARRAY. M.G. Rioult+, P. Gogan#*, H.-R. Lüscher+ & S. Tyc-Dumont#. Dept. of Physiology (+), University of Bern, CH-3012 Bern, Switzerland and Unité de Neurocybernétique cellulaire (#), CNRS UPR 418, F-13009 Marseille, France.

Investigations of synaptic integration in complex neurons necessitates simultaneous recording of the spatial and temporal patterns of excitation and inhibition in the entire cell. Optical recording of the membrane potential by means of voltage-sensitive dyes (VSD) and a photodiode array (PDA) gives sufficient temporal resolution but only a CCD-array (CCDA) can provide the necessary high spatial resolution. However, a CCDA cannot provide the necessary speed. In order to overcome the limitations inherent in the two techniques, a combined approach was developed.

Rat sensory ganglia grown in an organotypic culture for 14 days were stained with the fluorescent VSD RH-237. The neurons were impaled with microelectrodes for intracellular stimulation and recording of electrical activity. The concomitant changes in fluorescence intensity were recorded simultaneously by a square 124 element PDA and a liquid nitrogen cooled CCDA, both mounted in the image plane of an inverted microscope. A 40x/1.3 n.a. objective provided a spatial resolution of 15 μm/PD and 1 μm/pixel for the CCDA. The signal from each PD was amplified and digitized at a rate of 3 Khz. The CCDA integrated the fluorescence signal for a period of 10 msec coinciding with the evoked action potential. A second CCD-image was taken for the same time period without stimulation. The difference and the fractional change of the two fluorescence images were calculated.

The fluorescence signals registered from the excited neuron by the PDs reproduced the intracellularly recorded action potential with high fidelity. The CCDA produced an image which localized the electrical activity to the membrane of the excited neuron. This demonstrates that the two optical recording techniques are complementary. The high spatial resolution achieved with the CCDA localizes neural activity to small membrane areas, while the PDA provides the necessary temporal information.

408.4

ELECTRICAL INDUCTION AND MODULATION OF RESPIRATION IN VITRO. O. Hamada*, T. Iwahara *, E. Garcia-Rill and R.D. Skinner, Department of Anatomy, University of Arkansas for Medical Sciences, Little Rock, Arkansas.

Recently, we described the ability to induce locomotion using electrical stimulation of the brain stem and spinal cord in the neonatal rat in vitro brain stem-spinal cord preparation (J. Neurophysiol. 64, 1990). The purpose of the present study was to determine if electrical stimulation of the spinal cord can be used to induce respiration in vitro. EMGs were recorded from intercostal muscles in the brain stem spinal-cord, rib-attached, 0-3 day rat in vitro maintained in artificial cerebrospinal fluid. All preparations showed spontaneous, rhythmic and synchronized upward movements of the rib cage at a frequency of 0.06±0.01 Hz (range 0.04-0.07 Hz). The threshold for mechanical or twitch contraction-induced respiration was 16.0±13.5 μA, with the frequency of stimulation determining mechanical respiration from 0.1-4 Hz. In most cases, lower current amplitudes (11.0±3.1 μA) increased the frequency of spontaneous respiration to 0.07±0.03 Hz (range 0.06±0.09 Hz). The optimal frequency and duration of stimuli for induced and modulated respiration were 3 Hz (range 0.5-4 Hz) and 1 ms (0.5-2ms), respectively. The optimal stimulation sites were located in the C4 and C5 segments. These preliminary findings suggest that respiration can be induced and/or modulated by cervical spinal cord stimulation in vitro.

Supported by NIH grant NS 20246.

408.5

A SLOW EPSP CONTRIBUTES TO BURST DISCHARGE OF TURTLE RED NUCLEUS NEURONS REVEALED BY INTRACELLULAR RECORDING *IN VITRO*. J. Keifer and J.C. Houk, Dept. of Physiology, Northwestern University Medical School, Chicago, IL 60611.

The *in vitro* turtle brainstem-cerebellum is a useful model in which to study the circuit and cellular mechanisms underlying central motor commands in the cerebellorubral system. Previously we reported that long duration burst discharges were recorded from red nucleus neurons in this *in vitro* preparation. These bursts are postulated to represent motor commands and are generated by excitatory amino acid neurotransmission mediated by both NMDA and non-NMDA receptors (Keifer & Houk, *J. Neurophysiol.* 65: 454, 1991). It was postulated that burst discharges are triggered by a fast EPSP mediated by QUIS/KA receptors and sustained by an NMDA-mediated slow EPSP whose duration is prolonged by excitation from cerebellorubral recurrent pathways. Here we present evidence from intracellular recordings of red nucleus neurons in the *in vitro* brainstem-cerebellum for the presence of a slow EPSP that may contribute to burst discharge.

Intracellular recordings from 14 red nucleus neurons (RP < -55 mV) were obtained using microelectrodes containing 4 M KAc (D.C. resistance, 80-100 MΩ). Intracellular voltage was measured in the bridge balance mode. A stimulating electrode in the contralateral spinal cord was used to identify rubrospinal neurons by antidromic or synaptic activation. Current-voltage relations having slight inward rectification were obtained from red nucleus neurons and all cells showed anode-break excitation to current offset. Many cells (7 of 14) received spontaneous IPSPs and EPSPs. Responses to synaptic input were assessed by single shock contralateral cord stimulation which activates red nucleus neurons through synaptic pathways. In 6 of 14 cells, a slow EPSP having a mean latency of 11 ms (9-12 ms range) and a duration of 200-600 ms was observed following the stimulus. A higher stimulus intensity evoked an action potential at antidromic or synaptic latency (3-7 ms) followed by the slow EPSP. Action potentials were observed riding on the voltage envelope of the slow potential. The results provide support for the hypothesis that both cellular and circuit mechanisms contribute importantly to the generation of burst discharges in the red nucleus.

408.6

DEPRESSION OF TRANSMISSION FROM GROUP II MUSCLE AFFERENTS BY ELECTRICAL STIMULATION OF THE CUNEIFORM NUCLEUS AND SUBCUNEIFORM REGION IN THE CAT. B.R. Noga[1], E. Jankowska[2]* and B. Skoog[2]*Department of Physiology, [1]University of Manitoba, Winnipeg, Canada and [2]University of Göteborg, Göteborg, Sweden.

The effects of electrical stimuli applied within the cuneiform nucleus and the subcuneiform region were examined on transmission from group I and group II muscle afferents to neurones located in mid-lumbar spinal segments. Changes in the effectiveness of transmission from these afferents were assessed from changes in the sizes of the monosynaptic component of extracellular field potentials evoked following stimulation of muscle nerves.

Field potentials evoked from group II muscle afferents in the dorsal horn, intermediate zone and ventral horn were reduced in size when the test stimuli applied to peripheral nerves were preceded by conditioning stimulation (6-8 shocks at 400 Hz, 0.2 ms duration, 1/sec, 50-100 μA) of either the anterior and posterior cuneiform nucleus or the subcuneiform region ipsilaterally or contralaterally to the recording site. The depression of group II field potentials occurred at conditioning-testing intervals of 20-400 ms, being maximal at intervals of 35-70 ms and 40-105 ms for potentials recorded in the dorsal horn and intermediate zone/ventral horn, respectively. At the shortest intervals the depression of these field potentials was combined with a depression of the group I field potentials (maximal at intervals of 20-25 ms) in the intermediate zone. Thoracic spinal cord reversible cooling experiments revealed that the effects of stimulation of the cuneiform nucleus or subcuneiform region at long-conditioning test intervals were mediated via pathways travelling through the ipsilateral ventrolateral quadrant.

Intravenous administration of ketamine was found to antagonize the depression of dorsal group II field potentials evoked following stimulation of the cuneiform nucleus at long conditioning-test intervals. It is suggested that this effect was due to the action of ketamine on NMDA receptors of neurones activated by stimulation of the cuneiform nucleus and was not due to the dissociative anaesthetic properties of this drug since the depression evoked following conditioning stimulation of the raphe obscurus was unaffected and that evoked by Kölliker-Fuse nucleus stimulation was only partially antagonized.

408.7

SELECTIVE MODULATION OF THE PAD OF SINGLE Ia AND Ib AFFERENTS PRODUCED BY SURFACE STIMULATION OF THE MOTOR CORTEX IN THE CAT. J. R. Equibar, J. Quevedo*, I. Jimenez and P. Rudomin. CINVESTAV, Mexico DF 07000 and ICUAP.

The possible specificity of the control exerted by the motor cortex on primary afferent depolarization (PAD) of single muscle spindle and tendon organ afferents was studied in 6 anesthetized, paralyzed and artificially ventilated cats. PAD was inferred from the intraspinal threshold changes produced in pairs of single group I afferents either from the gastrocnemius (GS) and/or semitendinosus (ST) nerves. Surface anodal stimulation of motor cortex with trains of 8 pulses, 700 Hz, 200-1500 μA facilitated the PAD produced by group I flexors in 8 Ib fibers and inhibited the PAD elicited in 14 Ia fibers. The lowest threshold regions for the 2 actions were located mainly in the hindlimb area of the posterior sigmoid gyrus, and were usually separated by about 2 mm. With low strengths of cortical stimulation some sites were found that affected the PAD of Ia fibers without affecting the PAD of Ib fibers, while other sites affected to a similar extent the PAD of Ia and Ib fibers. The sites of overlapping actions increased with higher intensities of cortical stimulation. No obvious differences were found in the distribution of the most effective sites affecting the PAD of Ia and Ib fibers originating from the GS and ST muscles. The results suggest that neurons in the motor cortex are organized in discrete ensembles that are able to control the effectiveness of specific sets of group I muscle afferents. Supported by grants NIH NS 09196 and CONACyT 41739.

408.8

MODULATION OF SYNAPTIC EFFECTIVENESS OF GROUP I MUSCLE FIBERS BY ARTICULAR AFFERENTS. J. Quevedo*, J.R. Equibar, I. Jimenez, R.F. Schmidt and P. Rudomin. CINVESTAV and ICUAP, Mexico and University of Würzburg, Germany.

Schaible et al. (J. Physiol, 372: 575, 1986) suggested that electrical stimulation of the cat's posterior articular nerve (PAN) produces little or no presynaptic inhibition. We have reinvestigated this issue in the anesthetized, paralyzed and artificially ventilated cat. PAN stimulation with single pulses (<1.6 xT) produced a negative wave (onset latency 5.2±.5 ms) in the cord dorsum but no dorsal root potentials (DRPs). With higher strengths (1.7-4 xT) a second negative wave (peak latency 15.7±1.2 ms) followed by a P-wave and DRPs were recorded. In 2/6 experiments the DRPs were further increased by stimulation of the PAN with intensities (>7 xT) producing a third negative wave (latency 31.8±5 ms). Stimulation with trains (7 pulses 400-700 Hz) lowered the threshold for DRP generation (1.1-1.3 xT). Electrical stimulation of the PAN as well as innocuous and noxious stimulation of the knee joint depressed the DRPs produced by group I afferents of the posterior biceps and semitendinosus (PBSt) nerves. Analysis of the intraspinal threshold changes of single Ia and Ib fibers showed that repetitive stimulation of the PAN (8-10 xT) inhibited the PAD of Ia fibers produced by group I PBSt afferents (n=19). It also produced PAD in some Ib fibers (n=3) and inhibited the PAD in other Ib fibers (n=2). It is suggested that PAN afferents act on Ia and Ib fibers in a similar way as intermediate and high threshold cutaneous afferents. Supported by the VW Foundation, NIH (NS 09196) and CONACyT (41739).

408.9

IS THE PAD EVOKED IN Ia FIBERS RELATED TO THEIR RESPONSES TO STRETCH? M. Enriquez*, O. Hernandez*, I. Jimenez and P. Rudomin. CINVESTAV, Mexico D.F. 07000.

Intracellular recordings were made from single gastrocnemius (GS) afferent fibers in the spinal cord of anesthetized and paralyzed cats. In 17 Ia fibers a detailed analysis was made of their responses to segmental and descending inputs and to longitudinal ramp stretches of the GS muscle (8-16 mm/s). Stimulation of the posterior biceps and semitendinosus (PBSt) nerve (3 shocks, 330 Hz, 2xT) produced PAD in these fibers (mean 370±235 μV). Stimulation of the sural nerve (5xT) produced PAD only in 1 fiber (182 μV), and stimulation of the bulbar reticular formation (4-7 pulses, 331 Hz, 2xT) produced PAD in 4 fibers (mean 405±176 μV). These 2 inputs inhibited the PBSt-induced PAD in other Ia fibers (to 83±13%, n=10 and to 73±10%, n=3, respectively). The amplitude variations of the PAD evoked by PBSt stimulation appear not to be related with the conduction velocity of the Ia fibers (r=0.34). In 3 out of 4 experiments the PBSt-induced PAD of Ia afferent fibers with a low (<20 Hz) dynamic index (DI) was larger than the PBSt-PAD evoked in fibers with a higher (30-60 Hz) DI. The low DI/high DI PBSt PAD ratios were of 1.98, 1.59 and 2.97. The coefficients of correlation between the PBSt-induced PAD and DI derived from the 13 mm/s stretches were of -0.84 (n=5), -0.71 (n=5) and -1 (n=2) in these 3 experiments. It is suggested that the strength and/or density of the connections of the segmental interneurons mediating PAD may vary according to the DI of the target Ia fibers. Partly supported by NIH grant NS 09196 and CONACyT grant PCEXCCNA 41739.

408.10

MODULATION OF JAW MUSCLE SPINDLE AFFERENT ACTIVITY BY STIMULATION OF THE VENTRAL DIENCEPHALON. D. Dessem and H. Juang*, Department of Physiology, University of Maryland Dental School, Baltimore, MD 21201

Anatomical studies showing the presence of synaptic contacts on first-order afferent cell bodies in the mesencephalic trigeminal nucleus (MesV) suggest they are subject to central modulation. This study investigated the diencephalic modulation of jaw-muscle spindle afferent activity recorded in MesV. Peri-stimulus time histograms (PSTH) were generated (1000-10,000 stimuli) from the spontaneous activity of single muscle-spindle afferents triggered from bipolar, constant current stimulation (0.1 ms) of the ventral diencephalon. Jaw-muscle spindle afferents were identified by their response during: muscle palpation, ramp and hold jaw displacement, and the infusion of succinylcholine. Twenty afferents were studied in 8 rats anesthetized with pentobarbital and paralyzed. The spontaneous firing of 8 spindle cells was inhibited at latencies of 0.8-1.6 ms ($\bar{x}$=1.26 ms) and persisted for 2.2-8.0 ms ($\bar{x}$=4.7 ms). The inhibition was characterized by a distinct threshold at 50-300 μA and graded with stimulus intensities above threshold. The inhibition gradually decreased as stimulation frequencies were increased above 10-25Hz. PSTH's generated from non-spindle and some spindle cells showed no effect at stimulus intensities up to 1000 μA. Studies are in progress to determine whether this inhibition is mediated via the hypothalamic pathway to MesV reported by Nagy et al., (Neuroscience, 1986).

408.11

PHASIC MODULATION OF TRANSMISSION FROM VESTIBULAR INPUTS TO RETICULOSPINAL NEURONES DURING FICTIVE LOCOMOTION IN LAMPREYS. N.Bussières* and R.Dubuc. Dép. de Kinanthropologie, Univ. du Québec à Montréal, Montréal, H3C 3P8 and Centre de Recherche en Sciences Neurologiques, Univ. de Montréal, Montréal, Québec, Canada H3C 3J7.

Reticulospinal neurones (RS) play a key role in the supraspinal control of locomotion in lampreys. They provide excitation to motoneurones and to spinal interneurones involved in the generation of locomotion. In turn, they receive sensory inputs from many sources. For instance, electrical stimulation of vestibular nerve afferents induces di-synaptic responses in RS neurones on both sides (Rovainen *J Neurophysiol* 1979 *42* 745). The present study was aimed at examining whether the transmission of these synaptic responses is phasically modulated during locomotion. The brainstem-spinal cord of lampreys was isolated *in vitro*. Vestibular nerves on both sides were stimulated during different phases of locomotor activity induced by bath application of NMDA (50 μM). Reticulospinal neurones showed phasic membrane potential oscillations related to the locomotor activity with a depolarization during ipsilateral activity and hyperpolarization during contralateral activity in the rostral spinal cord. On top of this modulation, excitatory synaptic responses evoked in RS neurones by ipsilateral stimulation were phasically modulated, reaching a minimum during depolarization and a maximum during hyperpolarization of the RS neurone. Stimulation on the contralateral side induced opposite effects: responses were maximal during depolarization and minimal during hyperpolarization. No phasic changes of the input resistance were observed in these RS neurones, suggesting that the modulatory effects are pre-reticular. Microstimulation of two relay nuclei (intermediate and posterior octavomotorius) induced similar results, although the phasic modulation was slightly less. Responses to ipsilateral stimulations were minimal during depolarization whilst those to the contralateral side were minimal during hyperpolarization. Taken together, these results suggest that a large part of the modulation of vestibular input transmission to RS neurones takes place either at the level of the soma of relay cells and/or at presynaptic terminals innervating RS neurones. (Supported by: Canadian MRC, FCAR and FRSQ Québec)

408.12

THE EFFECTS OF ACOUSTIC STARTLE RESPONSE ON LUMBAR 1A INHIBITORY INTERNEURONS. L.E. Tremblay, U. of Ottawa, Canada and P.J. Delwaide*, U. of Luik, Belgium.

The acoustic startle response (ASR) to unexpected auditory stimulation was studied in 15 healthy subjects aged 23-40 years. The present study was undertaken to investigate the effects of ASR on lumbar motoneurons (MNS) excitability and lumbar 1a inhibitory interneurons (1a IN). The ASR was evoked by a binaurally conditioning test (1000 Hz 120 db tone). The audiospinal facilitation was measured on the soleus H-reflex using a lower conditioning stimulus (1000 Hz 85 db tone). The effect of ASR on soleus was measured from 50 to 300 ms. The effect of ASR on 1a IN was elicited the same way as the soleus H-reflex protocol but preceded by a subthreshold electrical stimulus on the common peroneal nerve. The maximal effect of audiospinal facilitation was at a 100 ms delay 222.9% ± 67.5 expressed in percentage of H-reflex control ($P<0.001$). The audiospinal facilitation on (1a IN) was significant at delays of 100 and 125 ms. The inhibition was facilitated by 20% ± 10 and 18% ± 6 ($p<0.05$) respectively. These results on the ASR confirm the time course and variability of audiospinal facilitation on lumbar MNS. The ASR facilitates the lumbar 1a IN at delays of 100 and 125 ms post-acoustic stimulation. At this time only the activation of the nucleus of gigantocellularis of the reticular formation can explain the facilitation effect on 1a IN.

408.13

ANTIDROMIC DISCHARGE OF SINGLE UNITS IN DORSAL ROOTS AND HINDLIMB CUTANEOUS NERVES. A. Niechaj, L. LaBella and S. Rossignol. Centre de Recherche en Sciences Neurologiques, Univ. de Montréal, Québec, Canada, H3C 3J7.

Our previous work on presynaptic mechanisms during fictive locomotion has indicated that some cutaneous and muscle primary afferents, recorded intracellularly at the dorsal root entry level, may discharge rhythmically. In the present series of experiments, spike-triggered averaging (STA) was used to show that action potentials in dorsal roots as well as in cutaneous nerves may be propagated antidromically (AD) in such preparations.

Experiments were performed on 21 spinal cats, 15 of which were spinalized 7-22 days prior to surgery. Single spikes recorded from a dorsal root by bipolar hook electrodes were used to trigger an average of the mass activity recorded from the same root with a second electrode and digitized at 20 KHz. Similar methods were used in the periphery to make STA in the medial (MS) or the lateral sural (LS) nerves. Single units were found to discharge tonically in both intact and cut dorsal rootlets. Such antidromic discharges could last for several minutes. In a few cases, these units were rhythmically active during fictive locomotion induced by Nialamide and DOPA. AD discharges were also found in 17 LS and 10 MS recordings and were also shown in 7 semi-chronic spinal preparations in which the spinal canal was not opened in order to avoid spinal trauma or changes in the temperature of the spinal cord. In a few cases, a weak modulation of such peripheral cutaneous units could be shown during fictive locomotion. It is concluded that, in such acute preparations with or without fictive locomotion, single primary afferents, recorded both in cut or intact dorsal roots, as well as in peripheral cutaneous nerves, may discharge antidromically, either tonically or rhythmically. (Supported by the MRC and by the FRSQ).

408.14

POSTURAL AREAS IN THE FROG LUMBAR SPINAL CORD. S. F. Giszter, F. A. Mussa-Ivaldi* and E. Bizzi. Dept. Brain and Cognitive Sciences, M. I. T., Cambridge, MA 02139.

Recently, we have examined the role of the premotoneuronal spinal areas in organizing posture and movement in spinalized frogs. Our investigations revealed that microstimulation of a single site in the upper and middle layers of the frog spinal cord, in conjunction with positioning of the leg in different workspace locations, generated a force field with a single equilibrium point. The equilibrium point is that spatial location at which the leg would be at steady state were it free to move. In the work described here, we have examined the spatial distribution of the convergent force fields (CFFs) in the lumbar cord.

We investigated whether the areas that specify different equilibrium-limb positions are topographically organized. After sampling the premotor area of the lumbar cord, we concluded that there are at least four areas from which four distinct types of CFFs are elicited. These regions form stripes oriented rostro-caudally. Within each region, a qualitatively similar set of x and y forces are produced. We also found that the simultaneous stimulation of two different points in the areas we have outlined results in a force field proportional to the sum of the fields obtained by the stimulation delivered at each point. This result is surprising given the complex nonlinearities that characterize the interactions both among neurons, and between neurons and muscles. We view a superposition mechanism as the simplest way to explain how the spinal cord generates a vast repertoire of force fields from the limited variety of available fields.

This work was supported by NIH grants NS09343 and AR26710, and ONR grant N00014/K/0372.

408.15

DIFFERENTIAL EFFECTS OF SPINAL TRAUMA IN PRIMATES AND CATS. M.Jayachandra* and V.E.Amassian. Dept. of Physiology, SUNY-Health Science Center at Brooklyn,Brooklyn, NY 11208.

We demonstrated changes in direct impulse conduction in the dorsal columns (DC) and corticospinal tracts (CT) following a midthoracic (T6) weight drop in fully anesthetized cats (J.Physiol.(Lond).,426,103P,1990). DC function was assessed by recording the posterior tibial nerve responses monophasically to antidromic epidural stimulation at C1 or T5. A second epidural stimulus was also applied at T10 below the trauma zone and these responses were used to correct (normalize) for any local changes at the recording site over a period of 8-29 hrs. DC population responses showed an immediate conduction loss after submaximal trauma, followed by recovery within 30-60 min and subsequent secondary decay beginning within 2-3 hrs; the normalization process increased the definition of kinetic changes in all the models used. Following trauma, both sensory and motor systems showed latency shifts, decreased amplitudes, and selective conduction loss of the fastest elements of the population responses. In the motor system this sensitivity was seen in population studies of antidromically excited single units recorded from primary motor cortex; the shortest latency units, presumably reflecting the largest diameter fibers, were more affected.

In the cat the DC conducted responses were less vulnerable, recovered faster and to greater extent than the CT. Extending the same paradigm to primates two phases of decay were identified, but the secondary decay took longer to develop. However, unexpectedly, the DC conducted response in primates (n=4) was more sensitive to trauma than the direct CT response (recorded from the pyramid to antidromic stimulation at T10). This finding cautions against extrapolating results derived from models in lower forms to primates.

408.16

FORELIMB MOTOR PERFORMANCE FOLLOWING CERVICAL SPINAL CORD CONTUSION INJURY, DORSAL COLUMN LESION, OR DORSOLATERAL FUNICULUS LESION IN THE RAT. G. W. Schrimsher and P. J. Reier. Dept. of Neuroscience, Univ. of Florida, Col. of Medicine, Gainesville, FL 32610.

The focus of this study is to compare the effects of weight-drop contusion injury with either selective dorsal column (DC) or dorsolateral funiculus (DLF) lesions at the C4 spinal segment on the following behavioral tests: forelimb reaching and pellet retrieval, vibrissae-induced forelimb placing, and forehead adhesive sticker removal. Contusion injuries were produced by dropping a 10 gm weight from a height of 2.5 cm onto a 2.5 mm tip diameter impounder resting on the exposed dura. Of 13 rats receiving this injury, 9 failed to recover reaching ability, 3 recovered reaching ability with a greatly reduced retrieval rate, and one rat approximated normal performance levels. The inability to contact an adhesive sticker placed on the forehead or a greater than 50% drop in forelimb placing at one week post-injury were each predictive of permanent failure to recover reaching ability. The DC corticospinal tract was severely disrupted in all animals, regardless of whether or not reaching ability recovered. Animals that recovered reaching ability were found to have a greater sparing of lateral column white matter as compared to animals which failed to recover. Initial results from the surgical lesion studies revealed that reaching ability was spared and pellet retrieval performance approached normal levels even when the DC lesion disrupted the DC corticospinal tract. Confined bilateral DLF lesions, however, produced a profound grasp deficit involving a prominent loss of digit flexion. Lesions which also extended into the ventrolateral white matter produced a marked hypometria in forelimb extension during reaching attempts. These findings indicate possible roles for the rubrospinal tract and the small DLF component of the corticospinal tract in distal limb motor performance in the rat. This work was supported by PHS Award MH15737-12.

408.17

EFFECTS OF RETICULOSPINAL INPUT ON MONOSYNAPTIC REFLEXES IN PARTIAL SPINAL CORD INJURY. W. H. Lee*, Y.G. Park, A. Chen*, J. H. Kim, The Miami Project To Cure Paralysis, Dept. of Neurol. Surg., Univ. of Miami, Miami, FL 33136

Rats with a spinal cord (SC) lesion sparing only the ventral quadrant at T6 level recovered coordinated fourlimb locomotion within 2-3 weeks. The time course of the functional recovery of the hindlimb movement is temporally correlated with recovery of reticulospinal nuclei (RtN) input to the lumbar SC. However, the physiological role of RtN in motor function is not clear. The objectives of this study was to investigate effects of RtN activation on monosynaptic reflex (MSR) in the normal and chronically injured SC. Sprague-Dawley rats were used. Under sterile conditions, SC lesions were made at the T6 level sparing one ventral quadrant. Rats survived from one day to ten weeks. During terminal experiments, a laminectomy was performed to expose L1-S1 SC. Left and right L4 dorsal roots (DR) were freed and mounted on two pairs of bipolar stimulating electrodes. MSR was produced by stimulation of DR, and was monitored on the sciatic nerve . Chronic changes in the RtN inputs to the SC were estimated by changes in MSR due to RtN conditioning stimulus. Immediately after partial SC lesion, both MSR and the RtN evoked outputs were depressed on the completely lesioned side of the cord. Within 2-3 days dramatic increases in MSR appeared on the left side and peaked within one week after the lesion. Following the lesion, the excitatory effect was completely abolished on the left, whereas it remained intact on the right where the ventral quadrant was spared. **(Support: NIH NS28059, The Miami Project Research Fund)**

408.18

Loss of Hindlimb Stepping after Unilateral Lumbosacral Deafferentation in Cats. M.E. Goldberger E. Coleman-Paige, K.L. Ziegler. Medical College of Pennsylvania, Phila PA, 19129.

We have shown (Goldberger & Ziegler, 1988, Soc Neurosci) that, after unilateral dorsal rhizotomy, L1-S2, the deafferented hindlimb recovers quadrupedal locomotion overground and on a treadmill, but not bipedal treadmill locomotion. In order to test the hypothesis that this failure might be due to the presence of descending inhibition, the spinal cord was transected at L1 in 3 cats with chronic hindlimb deafferentation in order to abolish descending inhibition and perhaps bring back bipedal locomotion. The deafferented hindlimb, however, does not recover bipedal locomotion and even the stepping responses of the afferented limb recovered slowly and were permanently weaker than in transected-only cats. Speculating that pattern generating neurons of the afferented side were unable to acivate those of the deafferented side, we examined c-fos expression in one animal after the chronic combined lesion and treadmill exercise. C-fos was expressed in interneurons on both sides of the cord. The results, taken together suggest that deafferentation reduces critically the input to spinal pattern generators and that even though neurons are activated, activation is inadequate to mediate locomotion unless the influence of descending input is permitted (or specific drugs are used). Supported by NS 228081.

408.19

THE EFFECT OF LESIONS TO THE NUCLEUS TRACTUS SOLITARIUS (NTS) ON THE OROPHARYNGEAL SWALLOW IN CATS. M. Kobara-Mates*, J. Logemann, C. Larson, and P. Kahrilas. Dept. of Communication Sciences and Disorders, Northwestern University, Evanston, IL 60208.

Although the NTS area has been shown to be involved in the central control of swallowing, it is unknown whether this area merely initiates the pharyngeal swallow or is involved in the motor programming of the pharyngeal swallow, as well. The purpose of this study was to examine the effect of small lesions in the NTS on the physiology of the oropharyngeal swallow in awake cats through the use of simultaneous videoradiography (VRG) and electromyography (EMG).

Hooked wire EMG electrodes were implanted into six muscles (posterior tongue, mylohyoid, thyrohyoid, thyroarytenoid, inferior constrictor, cricopharyngeus) active during swallowing. Sites in the NTS area near the level of the obex that elicited swallowing upon electrical stimulation were stereotaxically identified and then lesioned by passing anodal DC current. Cresyl violet staining revealed similarly placed lesions in the ventromedial portion of the NTS and surrounding area, near the obex.

Following the lesions, VRG revealed that two of the cats exhibited difficulty initiating pharyngeal swallows of small bolus volumes only. Intra-oral bolus transport was not affected. Post-lesion changes in laryngeal elevation, a pharyngeal stage event, were also found for all three cats. Although amplitude and duration measures of EMG were affected by lesioning, the sequence of EMG bursts was not affected in any of the cats. Thus, the results of this study support the assertion that the ventromedial NTS and immediately surrounding area is not only involved in the initiation of the pharyngeal swallow, but may be involved in programming the duration and amplitude of pharyngeal stage events. Although these results are preliminary, small lesions to the NTS do not appear to affect the sequencing of EMG activity during the pharyngeal stage of the swallow.

CONTROL OF POSTURE AND MOVEMENT I

409.1

STANCE PERTURBATIONS FOLLOWING BILATERAL LABYRINTHECTOMY IN THE CAT. J.M. Macpherson and J.T. Inglis. R.S.Dow Neurol. Sci. Inst., Portland, OR 97209 and Dept. of Anatomy, Queen's Univ., Kingston, Ont., Canada K7L 3N6.

The aim of this study was to determine whether vestibular afferent inputs are critical for triggering the rapid postural responses that are evoked by movements of the support surface under the freely standing cat. Four cats were trained to stand quietly on a moveable force platform. The animal's stance was unexpectedly perturbed by applying a linear ramp-and-hold translation to the support surface in each of 16 different directions in the horizontal plane. The postural responses were quantified in terms of the 3-D ground reaction forces under each paw, and the EMG activity evoked in selected hindlimb muscles. Following the collection of control data, the animals were labyrinthectomized bilaterally in one surgical procedure. The vestibule on each side was opened by drilling into the temporal bone, and the membranous labyrinth was suctioned away, presumably destroying the hair cells.

The cats were able to stand stably on the platform within 2-3 days of the lesion. The responses to platform translation were characterized by normal patterning and latency of EMG activity. Furthermore, all cats continued to use the force constraint strategy that is characteristic of the intact animal (Macpherson, J. Neurophysiol. 60(1988):204-217). The only clear deficit in performance was a transient hypermetria, characterized by an over-response in the forces and often, a subsequent oscillation. The hypermetria disappeared after 8-10 days. Although the cats over-responded, they were still able to maintain their balance successfully. These results suggest that vestibular inputs are not necessary for the triggering or patterning of postural responses evoked by support surface translations.

Supported by the MRC of Canada and Queen's University.

409.2

INTERACTION OF VESTIBULOSPINAL AND SOMATOSENSORY INPUTS IN THE CONTROL OF BALANCE IN MAN. C.W.Y. Hui-Chan, E. Lo Monaco*, N. Paquet*. School of Phys. and Occup. Ther., McGill Univ., Montreal, Canada, H3G 1Y5.

Despite considerable investigations into the control of posture during *slow* perturbations to free standing, there has been few systematic studies directed at the control of balance during *more rapid* whole head-and-body tilts. The objectives of our study were 1) to determine the effects of different magnitude of head acceleration on lower limb muscles responses evoked by sudden whole head-body tilts during stance; and 2) to delineate the modifyability of tilt-evoked responses by foot/ankle somatosensory inputs. Seven young normal subjects were blindfolded and fixed on a tilting apparatus. They underwent sudden forward tilt of 15° from the vertical position at acceleration of 0.5, 0.7, 1.4 and 1.8g without, and at 0.7 and 1.8g with, concommitent passive ankle dorsiflexion. Acceleration was measured with a linear accelerometer mounted on a dental bite, and ankle position with a potentiometer. Surface EMG was recorded from the tibialis anterior (TA), medial gastrocnemius (MG) and biceps femoris (BF) muscles.

Our results showed that the number of respondent to tilt with or without ankle rotation, and the frequency of occurrence of tilt-evoked responses in the respondents, increased progressively in the three lower limb muscles with increasing magnitude of head acceleration. With the addition of ankle rotation, the only change observed was in the relative onset latencies of the two extensor muscles (MG and BF). More specifically, with no ankle rotation, the proximal muscle (BF) was activated before the distal one (MG) in 4 of 5 subjects; while with ankle rotation, this pattern was reversed (MG before BF) in 5 of 6 subjects. These preliminary results suggest that the occurrence of tilt-evoked responses is dependent on the intensity of the vestibular stimulation and that the patterns of muscle activation in the lower limb muscles could be subjected to modification by somatosensory inputs interacting with vestibulospinal influences.

409.3

MECHANISM OF γ_D INDUCED SLOW DECAY OF CAT SPINDLE IA RESPONSE TO STRETCH. M. Hulliger, B. Wang*, M.S. Tabet* and E. Otten[1*]. **Dept. of Clinical Neurosciences, Univ. of Calgary, Calgary, Alta., Canada T2N 4N1, and Dept. of Neurobiology, Univ. of Groningen, NL-9712 KZ Groningen.**

The sensitization of spindle IA response to stretch during dynamic fusimotor activation has been attributed to stretch induced excitation of bag1 fibres, and the slow decay of IA firing following dynamic stretch to a slow decay of stretch activation (Boyd, Q.J. Exp. Physiol. 61: 203, 1976). However, experimental assessment of this hypothesis has been inconclusive (Dickson et al., Prog. Brain Res. 80:9, 1989), leaving open the possibility that IA sensitization is due to other mechanisms, including activation of unusual visco-elastic properties of bag1 fibres.

Acute experiments in cat soleus nerve muscle preparation were combined with mathematical modelling of spindle receptor mechanisms (Schaafsma et al., J. Neurophysiol., in press) to explore the nature of the slow decay. Trapezoidal stretch (8 mm) of the parent muscle was combined with quick release (at the end of the dynamic stretch) and with various patterns of γ_D activation. Modelling predicted that slow decay of firing due to slow deactivation should be immune to rapid release steps, regardless of amplitude. In contrast, slow decay due to a visco-elastic force transient would be abolished by release. This was confirmed experimentally. A slowly decaying transient of IA firing (evoked by exponentially decaying rate-modulated γ_D activation) persisted during release steps (up to 8 mm.) In contrast, with tonic γ_D activation the slow decay of IA response after stretch was completely abolished by release steps of around 4 mm.

These findings suggest that the slow decay of γ_D-sensitized stretch response is **not** due to a decay of **stretch activation.** Moreover, modelling indicates that it is most parsimoniously attributed to - exceptional - force-velocity characteristics of activated bag1 fibres. **Supported by AHFMR (Canada).**

409.4

RESTRUCTURATION OF THE FICTIVE LOCOMOTOR CYCLE BY TONIC PROPRIOCEPTIVE INPUTS. P. Saltiel, T. Reader and S. Rossignol. Ctr. Res. Sci. Neuro., Univ. Montréal, Québec, Canada, H3C 3J7.

In spinal cats, studies on the initiation of the swing phase have suggested that interactive signals responsible for interlimb coordination might be maximal in mid-stance and mid-swing. Is there evidence, firstly, that such mid-phase events are present in the fictive, centrally-generated, locomotor pattern and, secondly, that these events are involved when restructuration of the fictive locomotor cycle occurs with changes in limb position?

In high decerebrate, spinalized (T13) and paralyzed cats, fictive locomotion was recorded in both forelimbs from cut nerves to long head of triceps (TriLo, shoulder retractor and elbow extensor), lateral head of triceps (TriLa, elbow extensor) and cleidobrachialis (ClB, shoulder protractor and elbow flexor).

At 55% of the extensor phase, three events occur simultaneously: ipsilateral (ipsi) TriLa abruptly rises in amplitude (inflexion point), ipsi TriLo and contra ClB start to decrease in amplitude. When the ipsi shoulder is tonically protracted or retracted or when the elbow is flexed or extended, cycle duration changes little, but burst durations and amplitudes are markedly altered. However, the simultaneity of the ipsi TriLa inflexion point and contra ClB onset of descent is preserved with each kind of perturbation. Restructuration of the cycle is achieved by lengthening or shortening the duration of the bursts components before or after these points. This pattern of cycle restructuration was seen in five cats.

The observations that the ipsi Trila inflexion point and contra ClB onset of descent remain coupled as the cycle is restructured by a change in ipsi forelimb tonic position and that these points distinguish which burst components are modified in this restructuration suggest that these centrally- generated events may be important for interlimb coordination. (Supported by the MRC of Canada).

409.5

SPINAL MOTOR CENTER EXCITABILITY CHANGES DURING EXERCISE OF DISTANT MUSCLES AS MEASURED BY TENDON TAP RESPONSE.

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Division of Restorative Neurology and Human Neurobiology, Baylor College of Medicine, Houston, Texas 77030.

The Jendrassik's maneuver (strong volitional effort to pull apart clasped hands) will increase stretch reflex responses in muscles not involved in the maneuver. Likewise, maximal volitional contraction (MVC) of a muscle group will increase stretch responses in other ipsilateral and contralateral muscles. Intermittent, graded volitional ankle dorsiflexion at 20, 40,60, 80 and 100% of MVC with visual force feedback on contralateral quadriceps tendon tap responses were studied in six adult subjects. Surface EMG responses to tendon taps delivered with a constant force by an electrodynamic hammer at regular intervals were analyzed. Spinal motor center excitability was increased during the contraction phase at contraction/relaxation cycle rates of 6/4 s and 12/8 s. In contrast, at the 3/2 s cycle rate, increased excitability is present in the relaxation phase as well. This paradigm provides insight into the behavior of sub-components of voluntary motor control.

409.6

DIFFERENCES IN MODULATION OF EXTENSOR AND FLEXOR SPINAL MOTOR CENTERS DURING GAIT.

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Suprasegmental modulation of spinal motor center (SMC) excitability occurs during gait, but little is known about how central mechanisms influence extensor and flexor SMC excitability prior to movement. We studied excitability changes in extensor and flexor SMCs using triceps surae (TS) and tibialis anterior (TA) H-reflexes in 18 healthy subjects during treadmill walking. Stimuli were delivered relative to heel strike at 20 ms intervals across a 500 ms window. We found that facilitation of extensor H-reflex occurred 100 to 300 ms prior to EMG activation of TS, while flexor H-reflex facilitation preceded EMG activity in TA by 0 to 100 ms. These temporal differences indicate that there is a difference in the central mechanisms modulating extensor and flexor SMCs.

409.7

SPONTANEOUS ACTIVITY IN VENTRAL SACRAL ROOTS OF ISOLATED SPINAL CORD OF THE ADULT HAMSTER WITH AND WITHOUT 4-AMINOPYRIDINE.

A. J. Dekhuijzen[1], J. Bagust*[2], and V. R. Edgerton[1],
[1]Brain Research Institute and Dept. Kinesiology, UCLA, Los Angeles, CA 90024, U.S.A. and [2]Dept. Physiology and Pharmacology, Univ. of Southampton SO9 3TU, U.K.

In vitro ventral root activity has been recorded from the lumbar isolated spinal cord of hamsters weighing 15-25 g (Bagust, J, et al, *Brain Res.*, 479: 138, 1989). The feasability of recording from the isolated spinal cords of larger hamsters was explored in the present study in order to expand the utility of this preparation to study long term neuroplasticity. Whole isolated spinal cords, bathed in artificial cerebro-spinal fluid were studied in conditions with and without 4-aminopyridine (4-AP) and with and without Mg^{2+}, at temperatures of 18-21 ^{o}C in hamsters weighing 56-65 g. Electrically induced and spontaneous activity was recorded from ventral roots. Although spontaneous bursting activity occurred in 4-AP/Mg^{2+}-free conditions, 4-AP (10^{-5} M) increased the burst frequency from 0.25-0.50 Hz to 1.0-6.0 Hz. The burst durations found in 4-AP/Mg^{2+}-free conditions decreased from 0.5-1.0 s, to 0.02-0.4 s in the conditions with 4-AP. It is concluded that ventral root activity can be recorded in an isolated spinal cord from a sexually mature animal. [1]SUPPORTED BY NIH GRANT NS 16333 AND [2]NUFFIELD FOUNDATION.

409.8

EFFECTS OF PRACTICE OF FAST VOLUNTARY MOVEMENTS IN DOWN SYNDROME INDIVIDUALS. G.L. Almeida*, D.M. Corcos, M.L. Latash
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The effects of extensive practice of aimed elbow flexions in a standardized experimental situation upon kinematic and electromyographic (EMG) variables in two young adults with Down Syndrome (DS) were studied. The protocol included a pre-test during which DS-subjects performed series of trials including isotonic elbow flexions of varying amplitude "as fast as possible", slower isotonic flexions, maximal isometric flexions and extensions, and different perturbation paradigms. Then, the subjects practiced the 36° elbow flexion movements to a 6° target over 10 sessions (1100 movements). They were encouraged to increase movement speed. The training was followed by a post-test similar to the pre-test.

The following major differences were observed between the pre- and post-tests. In both subjects, there was a nearly three-fold increase in the peak movement speed with a corresponding decrease in movement time. The amount of agonist EMG during the acceleration phase and the amount of antagonist EMG during the movement increased five- to ten-fold. In the pre-test, the standard deviation of the final position (SD_f) did not show any clear relation to movement distance, while during the post-test, in one of the subjects, SD_f increased linearly with distance corresponding to the linear speed-accuracy trade-off. The rates of EMG and acceleration rise were much higher during the post-test than during the pre-test.

We conclude that extensive training of DS-subjects in standardized conditions can lead to a dramatic improvement in their performance which demonstrates the effects of transfer being equally observed during movements over practiced and unpracticed distances. Practice can lead to emergence of previously absent features that were seemingly absent in the DS-individuals, e.g. the linear speed-accuracy trade-off.

The study was supported by a grant from the Down Syndrome Research Fund.

409.9

FREQUENCY DEPENDENT MODULATION OF MOTOR UNIT FIRING DURING QUASI-SINUSOIDAL ISOMETRIC MUSCLE CONTRACTIONS.

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This study utilizes time- and frequency-domain analyses to examine the firing behavior of human motor units (MUs) during time-varying isometric muscle contractions. Subjects produced nearly sinusoidal abduction forces with their index finger. Single-MU discharges and multi-unit EMG were recorded from the antagonistic first dorsal and palmar interosseii for several frequencies (range 0.25 to 5 Hz), amplitudes and mean levels of sinusoidal muscle force. As previously reported (Iyer et al., Soc. Neurosci. Abstr. 16, 1990), MU discharge rates are modulated at the frequency of the force oscillation, as indicated by a distinct component in their auto-spectra. Further, the unitary modulations show widespread correlations, as indicated by prominent coherences between single-unit and population activities. The present results extend these findings to the antagonist muscle. In addition, we find a dependence of the modulations on the frequency of the force oscillation. For both agonist and antagonist, the modulating spectral components of MUs increased in size with frequency; and for some MUs, particularly of the antagonist, such components were present only at high frequencies. The unit-to-population coherences also increased with frequency, indicating augmented synchrony (correlations) between the modulations of the MU populations. The observed increase in depth and synchrony of MU rate modulations as well as the increased modulation of the antagonist may serve to counteract the effects of the low-pass filtering property of muscle.

Supported by NS 2271

409.10

BIMANUAL MOTOR CONTROL IN YOUNG CHILDREN.

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Past research on bimanual motor control in adults indicates that the upper extremities to move as a single unit. Dynamical theory of motor control suggests that the motor system can accommodate systematic departures from such linkage and that such deviations depend on both task and environmental demands. The purpose of this study was to examine the development of bimanual motor control in young children and to describe conditions under which linkage of the two hands is more or less likely to occur.

4 age groups (6, 8, 10, & 18+ yrs) performed 4 single & 4 two-hand movements to targets located at different distances (7.5cm, 15cm) under four visual conditions: full, hands-only, targets-only, & hands-targets illuminated. Movement time, reaction time, error, & accuracy of responses were analyzed using repeated measures MANOVA techniques.

Bimanual movements of both children and adults were more tightly linked when movements were to equidistant targets and in asymmetrical movements where the RH covered a long distance. Movements were also more tightly linked under reduced visual conditions. Errors in performance were greatest under limb-only conditions. These data suggest that both task and environment are significant factors in the degree to which the upper extremities are constrained to act as a single unit.

409.11

INDEPENDENCE OF REFLEX AND VOLITIONAL EMG MODULATION DURING SINUSOIDAL TRACKING IN NORMALS.

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Stretch reflex gain and volitional muscle activity are linked in the "automatic (reflex) gain compensation" and "servo-assistance" hypotheses. Experiments are reported illustrating conditions in which the normal comodulation of reflex and volitional EMG is altered, violating hypotheses rigidly linking reflex and volitional muscle activation.

A sinusoidal visually guided wrist tracking task was perturbed by step torque transients every 45° of the tracking phase. Evoked EMG and wrist deflection were acquired and used to assess modulation of the stretch reflex amplitude and wrist compliance as functions of the tracked position. Behavior at odds with a rigid linkage between the reflex and volitional control systems may be summarized as 1) a reflex gain modulation corresponding to joint kinematics, independent of the volitional EMG modulation and 2) a task and load specific shift of reflex gain modulation, independent of the volitional EMG modulation. Four observations underscore these behaviors. First, servo-driven passive joint movement through the sinusoidal tracking cycle produces a normally placed, statistically significant reflex amplitude peak with undetectable volitional EMG modulation. Second, unloaded active tracking in a subset of subjects results in a normally placed single reflex peak and bimodal peaks of volitional EMG serving functions of propulsion and cocontraction through the tracking cycle. Third, the imposition of an anti-elastic load (additive position feedback) retards the volitional EMG by approximately 180° compared to that resulting from an elastic load, yet the reflex peak remains normally situated at maximal velocity in the direction of muscle shortening. Fourth, associated with the inherently unstable external anti-elastic loading and during employment of a "precision" tracking strategy is an increase of reflex activity in tracking phases of homonymous muscle lengthening. This shift of the reflex activity corresponds to a decrease in joint compliance, and is postulated to counter destabilizing loading or increase the precision of movement. The tracking phase sequencing of the reflex and volitional EMG in these experiments points to a flexible relationship between reflex and volitional control systems, altered by peripheral input, external load, and task intent.

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409.12

ALTERATIONS IN POINTING AND HAND CONTROL PRODUCED BY PRISMS AND BY INTERPOSITUS INACTIVATION IN THE MONKEY.

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Accurate pointing and hand control is an important action of the neuromuscular system. To test control and adaptation we perturbed the system using either prisms or muscimol inactivation of the interpositus nucleus. A monkey was over-trained to make rapid reaches (time restricted reach) to a 6 by 6 array of touch detectors placed vertically in front of him within easy reach. We compared this to a task in which the monkey reached freely (free reach) for food pieces. Both tasks were performed with the head immobilized. When displacing prisms (8 Δ left or right) were added, changed or removed during either task, the monkey required several trials to adapt. When prism adaptation took place during the free reach task and then the monkey was switched immediately to the time restricted task (prism unchanged), there was only partial transfer of adaptation; 50% as many trials were required to complete adaptation as when the prism was changed during the time restricted reach task. Similarly, transfer from time restricted reach to free reach was also only partial.

Inactivation of the interpositus nucleus by injections of muscimol produced errors in both pointing and overall hand control during the performance of both tasks. While the speed of movement was unimpaired, the monkey made grossly inaccurate reaches, with horizontal and vertical pointing errors on the 6x6 array. A large amplitude tremor was evident and especially pronounced as the target was neared. Visually and/or tactilely locating the target using the unaffected hand reduced these errors. Together these results demonstrate that prism adaptation during performance of a task does not generalize completely to other tasks and that the interpositus nucleus plays an important role in the performance of accurate pointing and hand control.

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409.13

VOLTAGE-DEPENDENT EXCITATION OF MOTONEURONES FROM SPINAL CENTRES DURING FICTIVE LOCOMOTION IN THE CAT.

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It is well known that spinal networks can generate a locomotor rhythm in response to tonic input (either by stimulation of brainstem regions or by systemic application of L-DOPA). It has previously been shown that there is a wide convergence of inputs to these spinal centres including those from the mesencephalic locomotor region, FRA, and Ib afferents (in the mesencephalic cat, Shefchyk & Jordan, J Neurophysiol 53, 1985; in the spinalised L-DOPA treated cat, Conway et al, Exp Brain Res 68, 1987). The excitation of motoneurones from these convergent pathways and hence from the output of the spinal locomotor network was examined in the current study.

Using these two preparations, it was found that the excitatory phase of the locomotor drive potentials (LDPs) in motoneurones showed a voltage-dependent increase in amplitude, and that the EPSPs evoked in motoneurones from both Ib and FRA stimulation showed a similar voltage-dependent increase in amplitude.

Furthermore, the question of whether this voltage-dependent increase in the LDP was secondary to intrinsic properties of the motoneurones such as plateau potentials, or rather due to a voltage-dependency of the synaptic excitation per se such as that seen with NMDA receptor activation, was investigated using iontophoretic application of the NMDA antagonist D-APV. Following such an application, the voltage-dependent increase was reversibly eliminated.

It is concluded that the excitatory half-centres which receive convergent inputs from multiple sources project to motoneurones leading to a voltage-dependent excitation of these cells. It is suggested that this is at least in part due to activation of NMDA receptors.

409.14

NEURAL COORDINATION DYNAMICS OF SINGLE, MULTIJOINT LIMB MOVEMENTS: SPATIAL BIFURCATIONS AND HYSTERESIS.

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Recent work (Kelso, Buchanan and Wallace, Exp. Brain Res. 1991) has identified relative phase as an order parameter for the neural organization of single, multijoint limb movements. Phase transitions (or bifurcations) were observed when a temporal control parameter, movement frequency, was systematically varied. In KBW (1991) we hypothesized that bifurcations may also result when a spatial parameter, forearm orientation, is manipulated. To test this hypothesis, six subjects rhythmically coordinated flexion and extension of the elbow and wrist joints of the right arm under the following initial conditions: 1) forearm supine (0° of orientation): wrist flexion with elbow flexion and vice versa; and 2) forearm prone (160° of orientation): wrist flexion with elbow extension and vice versa. Starting in (1) or (2) subjects rotated the forearm in eight 20° steps, each step consisting of 15 cycles of motion at a constant frequency of 1.25 Hz. Switching from pattern (1) to pattern (2) and vice versa was observed at a critical spatial orientation. Loss of stability appears as the chief transition mechanism: a) Fluctuations in the relative phasing between the joints were observed en route to the transition in both directions; and b) The critical point depended on the direction of forearm orientation change, revealing the hysteretic nature of the phase transition.These results show that the coordination dynamics of the nervous system are highly nonlinear, both temporally and spatially, thereby assuring stability and flexibility of function.

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409.15

THE DYNAMICS OF SPONTANEOUS TRANSFER IN LEARNING NOVEL PHASE- AND FREQUENCY-LOCKED COORDINATION PATTERNS. P.G. Zanone and J.A.S. Kelso. Center for Complex Systems, Program in Complex Systems & Brain Sciences, Florida Atlantic University, Boca Raton, Fl. 33913.

The tendency toward phase and frequency synchronization is ubiquitous in neurobiology and behavior, ranging from studies in visual cortex, hippocampal learning, central pattern generation, and human sensorimotor coordination. Previous work in humans [1] has shown that learning a *novel* coordination pattern may involve a nonequilibrium phase transition, or bifurcation. This qualitative change in the coordination dynamics corresponds to the emergence of the to-be-learned pattern (e.g., a frequency-locked bimanual coordination pattern with relative phase, $\phi = \pi/2$ rad., between the oscillating components) as an *attractor* (i.e., an asymptotically stable state) accompanied by destabilization of intrinsically attractive states (e.g., $\phi = \pi$ rad.).

The present study reports *spontaneous* transfer of learning in which the pattern symmetric (e.g., $\phi = 3\pi/2$) to the pattern learned becomes stable and attractive as well. Subjects learned a visually-specified phase relation between the hands of either $\phi = \pi/2$ or $3\pi/2$ rad. Probes of the phase diagram, the set of relative phase patterns in the interval 0 to 2π rad., were conducted during the learning process. When $\pi/2$ rad. was the pattern to be learned, spontaneous generalization to its symmetric partner, $3\pi/2$ rad., was found, and vice-versa. Thus, learning and transfer of relative timing patterns are governed not by specific lead-lag relations between the components, but by intrinsic dynamical constraints founded on symmetry.

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[1] P.G. Zanone, J.A.S. Kelso, *J. Exp. Psychol.: Hum. Perc. Perf.* (in press).

409.16

RELATIONSHIP BETWEEN STIFFNESS AND NET JOINT TORQUE DURING BALLISTIC ELBOW JOINT MOVEMENT. D.J. Bennett. Dept. of Brain and Cognitive Sciences, MIT, Cambridge, MA 02139.

Previously we demonstrated that the stiffness of the elbow joint drops to as low as 5 $N \cdot m/rad$ during slow arm movements. We hypothesized that stiffness changes may be proportional to changes in the net joint torque (muscle torque) used to accelerate the limb inertia, but we were unable to fully test this hypothesis because of the limited range of movement speeds studied, and the confounding influence of co-contraction induced by the perturbations. The objective of the present study was to measure the stiffness changes over a large range of movement speed and load conditions, while minimizing and controlling for the effects of co-contraction. Subjects executed ballistic movements (1 *rad*) with strict velocity constraints (< 10 % error). On occasional movements a torque motor applied a small pseudo random binary (PRBS) *position* perturbation (< 0.1 *rad*), servoed about the mean unperturbed trajectory. In contrast to the random torque perturbations that we used previously, the position perturbations did not obligate the subject to co-contract, or otherwise stiffen the joint, to remain near (< 0.1 *rad*) the nominal trajectory. EMG recordings indicated that during the movement the reflex activity contributed to the joint stiffness, but little co-contraction was induced by the perturbations. As found previously for slow movements, the stiffness dropped during movement, with a minimum (< 6 $N \cdot m/rad$) at the zero crossing in the net joint torque. This phenomena persisted even for very fast movements. After compensation for the passive stiffness, the peak stiffness was found to increase proportionally to the peak joint torque. Unlike the net joint torque, the stiffness changes were often abrupt, dropping steeply at the movement onset and rising gradually as the target was approached.

409.17

A MODEL OF MOTOR UNIT RECRUITMENT WHICH RESULTS IN INDEPENDENT CONTROL OF EQUILIBRIUM POSITION AND STIFFNESS OF THE JOINT. D.J.Reed and D.R.Humphrey.Lab of Neurophysiology,Emory Univ.Sch.of Med.,Atlanta,GA30322.

We have previously proposed separate descending cortical systems for the control of co-contraction and joint position(Humphrey and Reed, Neurosci. Abstr.,7:740,1981). The present paper discusses a hypothetical model which could provide such control at the level of the motor pool. The model separates the motor pool innervations into common mode(co-activation) and reciprocal components. It assumes that the motor units are recruited according to size(Henneman,JN 28,1965) with the increment being proportional to the existing recruitment, resulting in a logarithmic recruitment function. As a direct result joint stiffness and equilibrium position become independent and are functions of the common mode and reciprocal components respectively.

For the direct control of Alpha motor units(open loop) the model was first developed for an antagonist pair acting about a single joint, then the results were shown to be general for any arbitrary muscle-joint arrangement(multi- muscle,multi- articular). For the reflex case it was assumed that that both Gamma and Alpha motor units are recruited alike,innervated by the same reciprocal component, as suggested by Alpha-Gamma coactivation(Valbo,Acta Phys Scand 80,1970). For this situation the model predicts that the reciprocal component of the net stretch reflex (static) output is null at the Alpha(open loop) equilibrium point. Thus the equilibrium points of Bizzi(Polit&Bizzi,JN 42,1979) and of Feldman(J Motor Behav. 18,1986) would occur at the same joint position. (Supported by NIH Grants NS 10183 and NS20146 to DRH.)

409.18

CONTRIBUTIONS OF THE PERONEI, THE TRICEPS SURAE, AND TIBIALIS ANTERIOR TO ANKLE TORQUE IN THE CAT. J. H. Lawrence,III*, T. R. Nichols, and A. W. English. Depts. of Physiology and Anatomy and Cell Biology, Emory University Atlanta, GA 30322.

Although locomotion in quadrupeds and bipeds occurs largely through sequences of flexion and extension, the full repertoire of movement requires torques in three dimensions. Abraham and Loeb (Exp. Brain Res. 85, 580-593, 1985) showed coactivation of peroneus longus (PL) and brevis (PB) with ankle extensors during walking. However the relative contributions of the peronei to eversion and to sagittal plane movements are not known. We stimulated muscles crossing the ankle joints in cats deeply anesthetized with pentobarbital, and used a multiaxis force-moment sensor to measure the resulting isometric torques. The directions of torque and relative magnitudes were as follows:

Soleus	plantarflexion
PL	dorsiflexion and eversion
PB	eversion > toe out > plantarflexion
MG,LG	plantarflexion > toe out > eversion
TA	dorsiflexion > toe in > inversion

(MG,LG: medial & lateral gastrocnemius; TA: tibialis anterior) The eversion torques via LG and MG were each comparable to those from PB and PL combined. MG and LG gave relatively large toe out torques, but less than that of the peronei group. Therefore,with respect to the inversion/eversion axis, MG and LG were both synergistic to the peronei and antagonist to TA.

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409.19

EVIDENCE THAT REFLEXES BETWEEN PLANTARFLEXORS AND EVERTERS DECOUPLE ACTIONS OF THESE MUSCLES AT THE CAT ANKLE JOINT. S.J. Bonasera* and T.R. Nichols. Department of Physiology, Emory University, Atlanta GA 30322.

It is well established that the peroneal muscles of the cat hindlimb are coactivated with the triceps surae during stance and locomotion (Rasmussen et al., *J Morphol* 155:253-270, 1978). Additionally, while the major action of peroneus brevis (PB) is to provide eversion and toe out at the ankle joint, gastrocnemius (G) also provides a major component of toe out and eversion at the ankle (Lawrence et al., *Soc Neurosci Abst* 19, this volume).

Since both PB and G act in ankle eversion, we investigated stretch-evoked reflex interactions between PB and G, as well as soleus (S), tibialis anterior (TA), and other lateral and posterior compartment muscles in decerebrate cats. At low forces, reflex interactions between PB and G were weak, while interactions between PB and S were asymmetric, with PB contributing a stronger inhibition onto S. However, at high forces, mutual inhibition occurred between PB and both G and S. Reflex interactions between PB and TA were weak. The inhibition observed between the plantarflexors G and S, and the everter PB may serve to decouple actions of G and S from actions of PB under high force conditions, and thus partially compensate for the significant eversion torque generated by G and the slight plantarflexion torque generated by PB.

(Supported by NIH grant NS20855)

409.20

GENERATING MOTOR REPERTOIRES BY FORCE FIELD SUMMATION. F.A. Mussa-Ivaldi*, S.F. Giszter and E. Bizzi. Dept. of Brain and Cognitive Sciences, MIT, Cambridge, MA 02139.

The purpose of this work is to investigate how motor outputs might be combined by the central nervous system for generating a wide repertoire of behaviors. Recent experiments in the spinal frog have shown that the focal microstimulation of the premotor layers in the lumbar grey results in a field of forces acting on the frog's ankle. We have observed this field by measuring the isometric forces induced at different ankle locations by the stimulation of the same spinal site. Our experiments have produced three major resuts. (1) The stimulation of the premotor layers induces a field of forces converging toward an equilibrium point at which the ankle force is zero. (2) There are at least four distinct regions in the lumbar grey matter. Microstimulation of these four areas generates four distinct fields with different equilibrium points. (3) We found that simultaneous stimulation of two different spinal sites generates a field that is proportional to the vector sum of the fields elicited by stimulating each site independently. These results suggest that the neural circuits in the spinal cord "store" a few limb postures in the form of fields acting on the limb's end-point. Using computer simulation, we have investigated the competence of vector summation to provide a larger repertoire of behaviors using such fields. Our approach is equivalent to combining a number of predefined functions for approximating the shape of an unknown surface from a set of sampled points. The simulation results indicate that by a simple vector summation of motor outputs the CNS may generate a wide variety of force patterns and postures from the combination of four convergent force fields. Thus, the combination of force fields is an appropriate framework for motor planning, adjustment and control.

This work was supported by NIH grants NS09343 and AR26710, and ONR grant N00014/K/0372.

409.21

CONTROL OF BODY MOMENTUM DURING STANDING LEG FLEXION MOVEMENTS IN MAN. M.W. Rogers and Y.C. Pai.* Physical Therapy, Northwestern University Medical School, Chicago, IL 60611

The organization of postural responses underlying dynamic transitions in stance support during single leg flexion movements was examined in man. Previous observations led us to hypothesize (1) an invariant structure of the impulse-linear momentum (LM) relationship of the body center of mass over a range of flexion speeds, and (2) an intralimb and interlimb coupling of hip abductor (AB) and adductor (AD) muscle activations for the control of lateral body momentum. Eight adults stood on 2 separate force platforms and performed rapid and slower leg flexion movements. Surface EMG was recorded from the knee flexor biceps femoris and AB and AD muscles. Kinematic data was obtained via motion analysis.

The findings generally supported both hypotheses. First, the normalized time when the peak LM occurred and the absolute magnitudes of the force-time integrals for propulsion (P), braking (B) and P/B components remained constant across speeds. Second, EMG responses revealed an apparent reciprocal organization of bilateral hip AB and AD muscle pairs linking anatomically antagonistic but functionally synergistic interlimb postural muscle elements related to the control of body momentum in the frontal plane.

409.22

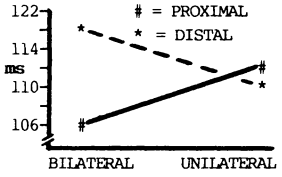
HEAD AND NECK KINEMATICS DURING VERTICAL PLANE ROTATIONS. E.A. Keshner, R. Cromwell*, G. Rovai*, and B.W. Peterson. Dept. of Physical Therapy, Univ. of IL at Chicago and Dept. of Physiology, Northwestern Univ. Medical School, Chicago, IL 60611.

In our previous study of rotations about the vertical axis, longer latency, voluntary mechanisms dominated stabilization at frequencies < 1 Hz while reflexes and biomechanics contributed at higher frequencies (Keshner & Peterson, Soc. Neurosci. Abstr. 14:1235). In this study, we recorded chair and head velocities, and surface EMGs from bilateral semispinalis (SEMI) and sternocleidomastoid (SCM) muscles. Seated subjects underwent pitch rotations in the dark about a horizontal, bitemporal axis. A triaxial angular rate sensor and a laser pointer, affixed to a headband worn by the subjects, were positioned at the vertex of the head. Stimuli were predictable sine waves and a random sum-of-sines stimulus with 5 frequencies ranging from 0.35 to 3.05 Hz which should be high enough to elicit vestibulocollic (VCR) and cervicocollic responses. One test condition required voluntary attempts at head stabilization (VS), and the second used mental arithmetic to minimize voluntary intervention (MA). Gain and phase of head velocity and EMG responses were calculated using a best fit sinusoid and analyzed with respect to chair velocity. **At 0.3-1 Hz** the head was well stabilized in VS and had stabilizing responses with gain ~ 0.3 in MA. Both SEMI and SCM EMG responded in phase with chair position. **At 1-2 Hz** good head stability was seen in both conditions. SEMI was modulated in phase with chair velocity indicative of a VCR response but SCM continued to have a position-related response. **Above 2 Hz** mechanical responses dominated head motion in both conditions and large EMG responses related to this motion were recorded in SEMI and SCM. Thus, compensatory responses appeared at lower frequencies and were sustained over a greater frequency range than during horizontal rotations. EMG data were consistent with the idea that SEMI is dominated by vestibular, SCM by neck receptor input. *Supported by grant NS22490.*

409.23

FRACTIONATED REACTION TIME IN SIMPLE, BILATERAL, UNILATERAL, PROXIMAL, AND DISTAL MOVEMENTS. J.G. Anson and Y. Bird*. School of Physical Education and Neuroscience Research Centre, U. of Otago, Dunedin, New Zealand.

Effects on simple reaction time of initiating unilateral vs bilateral and proximal vs distal responses were investigated in 12 college-aged females. Distal responses were unilateral or bilateral index finger extension; proximal responses were unilateral or bilateral elbow flexion. Two blocks of 20 trials were obtained for each condition. EMG from the agonist muscles was demeaned, rectified and smoothed before fractionation into premotor- (PMT) and motortime components. Early results show an interaction between proximal-distal and bilateral-unilateral conditions for PMT. An explanation based on differences between the neuromuscular structure and motor program demands for each condition is proposed.



409.24

KINESTHETIC INPUTS AND MOVEMENT SEQUENCING. L. Bevan, L. G. Carlton*, M. J. Carlton* and P. J. Cordo. R. S. Dow Neurological Sciences Institute, Portland, OR 97209.

The present experiment examined how kinesthetic inputs from one joint are used to trigger movement at other joints in activities requiring movement sequences. Human subjects were required to open their right hand as their right elbow was passively rotated. The criterion elbow angle for hand opening was fixed at 145 deg (180 deg equals full elbow extension). The elbow was rotated at one of seven constant velocities (18-85 deg/s) and from one of seven different starting angles (111-129 deg). Both velocity and starting angle were presented in random order. The results were compared to control trials where the elbow starting angle was held constant, and velocity was randomly varied. The arm and hand were screened from view, but information about hand opening errors was presented after each trial.

The results indicated that there was a large and systematic bias in opening angle when starting angle was randomly varied. Hand opening occurred before reaching the target with longer movement distances (111-117 deg starting angle), and after the target for shorter distances (123-129 deg starting angle). This finding was independent of the velocity manipulation. These results suggest that kinesthetic inputs representing absolute elbow angles are not used to trigger hand opening, rather, the movement is triggered by components of velocity and time, or the angular distance from the starting angle.

409.25

RECRUITMENT ORDER OF MOTOR UNITS IN WRIST EXTENSOR MUSCLES. K.E. Jones*, S. Riek* and P. Bawa. Kines., S.F.U., Burnaby, B.C., V5A 1S6.

Extensor carpi radialis (ECR) contributes to wrist extension and radial deviation, while its neighbour, extensor digitorum communis (EDC) contributes to extension of the wrist and extension of various fingers. The following study examined recruitment order of motor units within various task groups of ECR and EDC in human subjects. ECR units tested for wrist extension and radial deviation did not show separate subpopulations contributing to the two tasks. Motor units were recruited in an orderly fashion both for wrist extension and radial deviation. EDC motor units were seen to fall into 3 subpopulations, one contributed to middle finger (MF) extension, the second contributed to ring finger (RF) extension, while the third group (common) contributed to both MF and RF extension. When the fingers were disengaged, EDC motor units were recruited in an orderly fashion for wrist extension. All subpopulations of EDC contributed together towards wrist extension. Similarly, for MF extension, 'MF' and 'common' subpopulations pooled together to show an orderly recruitment.
Supported by BCHCRF and NSERC grants.

409.26

THE EFFECT OF VARYING PURE INERTIAL LOADS ON THE MECHANICAL PERFORMANCE OF CAT SOLEUS MUSCLE. D.C. Lin and W.Z. Rymer. Depts. of Biomedical Eng. and Physiology, Northwestern Univ., Chicago, IL 60611.

The objective of this project was to evaluate the mechanical performance of eccentrically contracting soleus muscle under different sizes of pure inertial loads and in reflexive and areflexive conditions. Specifically, our aim was to examine the dissipative properties of muscle, assessing its performance by using kinematic variables such as energy and momentum. Pure inertial loads were simulated by a motor and analog circuit using force feedback. The force was integrated twice and referred to the motor which acted as a length servo. The resulting gain and phase of the simulated load plotted as a function of frequency corresponded exactly to a mass.

In the first class of experiments the soleus muscle with the reflex intact from a decerebrate cat was attached to the motor and the crossed-extension reflex was used to elicit a background force of 5N. An initial stretch velocity was set and then the muscle was free to move under the simulated mass, which is equivalent to giving a force impulse to the mass. Both the initial velocity and magnitude of the mass could be independently specified. In the second class of experiments the protocol was repeated except the muscle was originally inactive and then the nerve was electrically stimulated for two pulses, providing a transient force in an open loop condition.

The first experiment shows that the damping coefficient increased with increasing mass. (Usually in a linear spring-mass system with damping, the damping coefficient decreases with increasing mass). In the second set of experiments, both the energy and momentum transferred to the mass were measured. It was found that the momentum transferred always increased with inertial size and the energy transferred also increased with inertial size if the initial velocity was above a certain value.

The end result of the project is to measure the contribution of intrinsic muscle properties in compensating for changes in inertial loads, which has been proposed by Partridge (1965). These inertial-dependent dissipative properties of eccentrically contracting muscle promote smoothness in movements and can simplify the control mechanism needed for the damping of voluntary movements, thus helping to solve the inverse dynamics problem associated with changes in inertial loads.

This work was supported by NIH grants NS-19331 and NS-28076.

409.27

FUNCTIONAL CLASSIFICATION OF NERVE FIBERS VIA SCALE-INVARIANT CLUSTER ANALYSIS ALGORITHM. M. Maltenfort*, T.S. Buchanan, and W.Z. Rymer, Sensory Motor Performance Program, Rehabilitation Institute of Chicago, and Departments of Rehabilitation Medicine & Biomedical Engineering, Northwestern University, Chicago, IL 60611.

This study tested a new method of identifying active fibers in an intact peripheral nerve bundle by performing cluster analysis to group spikes in the multi-unit record. The analysis was similar to that of Camp and Pinsker [1]: two electrodes are placed along the nerve, and the clustered data vectors consist of the amplitude of the spike on the proximal channel; the amplitude of the spike on the distal channel; and the conduction delay between them. In the new method, modified mode analysis is used to identify initial cluster centers and the determinant of the covariance matrix is a scale-invariant measure of distance. The spikes were pre-processed by low-pass differentiating (LPD) digital filters to resolve overlapping spikes.

Analyses were performed on real data sets from a medial gastrocnemius nerve bundle in a barbiturate anesthetized cat, and on simulated data in which the clusters were known *a priori*. To test the effects of the LPD filter, parallel analyses were performed using a low-pass Butterworth filter instead of the LPD. The sorting of the real data was compared to physiologic expectations, while the analyses of the simulated data was compared to the known input. For both types of data, an FFT-based template matching algorithm was used to find within-cluster scatter and between-cluster scatter for the spike waveforms.

It was observed that although mathematical criteria for a successive clustering of the real data were met (amplitude and conduction velocity correlated, amplitudes on both channels correlated, within-cluster scatter less than between-cluster scatter) it was difficult to justify a one-to-one correspondence between fibers and individual clusters. The LPD filter did aid in spike detection, but it somehow reduced the number of Type II fibers clustered and also reduced the ratio of between-cluster scatter to within-cluster scatter.

The new algorithm is recommended as a way of estimating relative changes in total fiber activity at different time periods, and in applications where correlations between spike amplitude and velocity are not necessarily present.

This work was supported by NIH grant NS-19331.

[1] C. Camp and H. Pinsker, "Computer Separation of Unitary Spikes from Whole-Nerve Recordings," *Brain Res.*, vol. 169, pp. 455-479. 1979

409.28

MODIFICATION OF RESPIRATION AND MASTICATION DURING DEGLUTITION IN THE ADULT HUMAN. D. McFarland, C. Valiquette*, and J.P. Lund. Centre de recherche en sciences neurologiques, Université de Montréal, Montréal, Canada, H3C 3J7.

Previously, we have described the interactions that occur between respiration, mastication, and deglutition in the awake, unanesthetized rabbit (Neurosci. Abstr. 16:50.5, 1990). We concluded that changes in respiration and mastication to accommodate swallowing are accomplished through independent commands from the swallowing central pattern generator. We extend these findings by describing the interrelationships between swallowing, respiration, and mastication in the adult human.

Movements of the jaw, rib cage, and larynx were recorded during spontaneous breathing and the mastication of apple, sausage, and candy. The laryngeal signal was used as a marker for swallowing, and three characteristic patterns were observed. Interposed (I) swallows occurred during the sequence, Terminal (T) swallows ended the sequence, and Spontaneous (S) swallows occurred sporadically between masticatory sequences. The present report focuses on the first two swallow types.

Respiratory pauses usually occurred during the expiratory phase (versus inspiration in the rabbit) of the breathing cycle, before or during elevation of the larynx for swallowing. Jaw movements typically paused during the opening phase and after changes in respiration and movements of the larynx. There was no direct linkage between the onset of pauses in respiration and mastication. Unlike the rabbit, the durations of perturbed respiratory and masticatory cycles in man were related to the phase in which swallowing occurred.

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409.29

PERTURBATION PRIOR TO TWO-JOINT POINTING MOVEMENTS. G.F. Koshland & Z. Hasan Dept. Of Physiology, Univ. of Arizona, Tucson, AZ 85724.

The aim of this study was to measure reflex responsiveness to a perturbation, delivered just prior to the onset of voluntary pointing movements in different directions. We expected that the EMG response to the perturbation would be systematically modulated with direction, and in particular, would be least when the intended direction coincided with the perturbation direction.

Motion of the arm was restricted to rotations about the shoulder and elbow joints in the horizontal plane, and in some trials, a force perturbation (30 ms) was unexpectedly applied to the forearm, extending the elbow joint and to a lesser extent the shoulder joint. A clear response to stretch in elbow flexors was observed over the period 30-60 ms from the start of the perturbation, and response amplitude was relatively constant for all intended directions. Soon after this short-latency response, the perturbation consistently elicited an earlier and faster target-reaching movement, also evidenced by earlier and larger EMG activity in the agonist and antagonist muscles appropriate for moving in the target direction. Indeed, in the case where the directions of the perturbation and intended movement coincided, a switch occurred from short-latency activity in flexor muscles to activity in shoulder and elbow extensor muscles, initiated as early as 60 ms after the perturbation. This early onset was always observed despite variations in the experimental paradigm (amplitude and timing of perturbation, type of Go signal, presence of warning tone), suggesting that subjects were not jumping the gun before the Go signal.

The findings suggest that apart from the short-latency response in a stretched muscle, the choice of muscles activated in response to a perturbation reflects the pattern that has already been selected for the upcoming volitional movement. This is consistent with other studies in which perturbations by themselves elicited non-monosynaptic EMG responses that mimicked multijoint voluntary patterns. (Supported by NIH grants NS19407 & NS07309.)

409.30

THE PREDICTED DIRECTIONAL TUNING OF CELLS WHICH ARE CORRELATED WITH MUSCLE KINEMATICS. S. A. Ellias. Parkinson's Disease and Movement Disorder Unit, Roger Williams Med. Center, Providence RI. 02908

The activity of many movement related cells in primate motor cortex are characterized by a 'tuning' curve (TC) that varies as the cosine of the difference between the direction of hand movement and a preferred direction (Georgopoulos et. al. 1982;1983; Kalaska et. al. 1989). However, some cells exhibit a TC that is too skewed to show a good fit to a sinusoid (Kalaska et. al. 1989). Here, I will show that broadly sinusoidal and sharply tuned and skewed TC can be created by cells which either command or are correlated with muscle velocities. The TC of these cells are calculated by computer simulation for 1 cm to 8 cm primate arm movements with a bell shaped hand velocity profile in a horizontal plane. The instantaneous firing rate is assumed proportional to instantaneous muscle velocity. Muscle moment arms are kept constant to isolate the effects of arm geometry. Average firing rates are computed for different portions of the movement for different muscles and for different starting locations. The TC show the following properties: 1) The TC for the instantaneous firing rates are sinusoidal for movements of less than 4 cm regardless of starting location. This is consistent with results derived analytically by linearizing the arm kinematics (Mussa-Ivaldi, 1988). 2) The TC for the instantaneous firing rates are more skewed and sharply tuned for movements larger than 5 cm. This skewing is enhanced for movements starting with the elbow more flexed for both single-joint and double-joint related cells. 3) The TC for the average firing rate reflects these same properties: for the first half of the movement it is sinusoidal, but it can be very skewed if the average includes the last half of the movement. 4) The amplitude of the TC for shoulder related cells increases with elbow flexion, and decreases for elbow related cells and some double-joint cells. These TC characteristics may help to subclassify movement related cells, and should be applicable to some sensory cortical cells. Since force commands may be realized by changes in rest length, a distortion of some of these TC properties may also be found in cells which influence muscle force.

CONTROL OF POSTURE AND MOVEMENT IN HUMANS

410.1

RESTORATION OF PRECISION GRIP USING TACTILE FEEDBACK AND FUNCTIONAL ELECTRICAL STIMULATION. J.A. Hoffer and M. Haugland. Dept. Clin. Neurosci., Univ. Calgary, Calgary, Alberta T2N 4N1, Canada; and Dept. Med. Informatics and Image Analysis, Aalborg Univ., Aalborg, Denmark.

In the intact human, cutaneous mechanoreceptors are essential for the control of precise manual tasks. Of particular importance is the high sensitivity of glabrous skin mechanoreceptors to small slips of a gripped object that can occur if the grip force declines, or if a load is added to the object. By 80 ms after the start of a slip, a spinal reflex of cutaneous origin can recruit sufficient additional force to hold the object securely again (Johansson & Westling, Exp Br Res 71:72, 1988).

In persons paralyzed by stroke or spinal cord injury it has been possible to restore some motor function with Functional Electrical Stimulation (FES) of limb muscles. However, fine motor control with FES is difficult because the forces produced with electrical stimulation depend markedly on muscle length, external load, and prior history of activation. We have investigated whether skin mechanoreceptor signals, recorded chronically from sensory nerves, can provide feedback information suitable for *closed-loop* control of FES, an approach that is likely to approximate more closely the performance of the intact motor system.

The cat footpad served as a model of human glabrous skin. Cats were implanted with tripolar nerve cuff electrodes on the tibial nerve and four bipolar intramuscular stimulating electrodes in the heads of triceps and plantaris. In experiments under anesthesia, the leg was secured with atraumatic clamps. When the plantarflexor muscles were stimulated, the footpad pressed against an object that would fall if not held by the paw. In runs simulating fatigue, the force produced with FES was made to decline gradually until the object slipped. In other runs a weight was dropped, causing the object to slip. In both cases, the ENG signal showed a large burst as the object started to slip, that was invariably detected by a computer within 10 ms. The controller responded with an immediate increase in the stimulation of the muscles that allowed the paw to reliably and reproducibly stop the object after only a 1-2 mm fall.

We suggest that feedback signals generated by skin mechanosensors could be similarly recorded from human nerves, and used for automatic regulation of the activation of paralyzed muscles with FES in quadriplegia, paraplegia or hemiplegia.

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410.2

OPTIMIZED STIMULATION OF MUSCLE AFFERENTS IN HUMANS WITH A SERVOCONTROLLED TENDON VIBRATOR S.C. Gandevia, P.J. Cordo, D. Burke*, J.P. Hales*, The Prince Henry Hospital and University of New South Wales, Sydney, Australia

Tendon vibration is known to be an effective stimulus for muscle receptors. In reduced preparations, vibration can be delivered directly to and in the same axis as the excised tendon. In humans, however, vibration must be delivered through the skin, perpendicular to the tendon, making it more difficult to control precisely the mechanical characteristics of the stimulus. We have constructed a tendon vibrator for humans in which the force and displacement of vibration can be controlled, even when the muscle and tendon move over short distances during active contractions and joint rotations.

We recorded the activity of single muscle afferents in the nerves supplying the tibialis anterior (TA) in humans using microneurography. We classified afferents as muscle spindles or tendon organs using muscle twitches produced by intrafascicular stimulation and as group Ia or II spindle afferents with ramp stretches delivered by the vibrator. We defined the optimal force and displacement of vibration to entrain each afferent at vibration frequencies of 20, 40, 80 and 120 Hz in the passive muscle, during isometric contractions, and during passive and active changes in the length of the TA.

We were able to entrain these receptors (especially Ia afferents) in the passive limb as well as during active contractions. Our determination of optimal vibration parameters will allow this vibrator to be used to investigate quantitatively how proprioceptive input is used to control human movement.

410.3

TRANSIENT DYNAMICS IN MOTOR CONTROL OF PATIENTS WITH BASAL GANGLIA OR CEREBELLAR DISEASES. A. Beuter, C. Labrie*, K. Vasilakos*. Université du Québec à Montréal, CP 8888, Station A, Montréal, Qc, H3C 3P8 and Centre for Nonlinear Dynamics in Physiology and Medicine, McGill University.

Experimental observations of movement disorders including tremor and voluntary microdisplacements recorded during a simple visuomotor tracking task in patients with Parkinson's disease and in patients with lesions of the cerebellum are analyzed. The task required the patient to maintain a constant index position relative to a stationary baseline displayed on an oscilloscope. The displacement signal of the finger was delayed up to 1400 ms, recorded for 80 s and digitized at 102 Hz. Typically in control subjects, insertion of a time delay produces large amplitude low frequency oscillations (with a period two to four times the delay) superimposed over physiological tremor. The performance of patients with Parkinson's disease (whose tremor amplitude is large) and of patients with cerebellar disease (with more severe deficits) is characterized by the intermittent appearance of transient dynamics or by the presence of sudden transitions in the amplitude and/or frequency of the signal. The need to (1) develop new tools to characterize changes in dynamics (i.e., transitions) and (2) redefine movement pathologies following neurological degeneration, in terms of qualitative changes in oscillatory behaviors is emphasized.

This work is supported by NSERC (Canada) and FCAR (Québec).

410.4

ANALYSIS OF VARIABLITY IN KINEMATIC CONTROL IN ASSESSMENT OF LANDING STRATEGIES IN CHILDREN. L.Pelland and P.McKinley. Physical, Occupational Therapy, McGill, Montreal, Que., H3G 1Y5

Landing from a knee-height jump down was studied in 10 children 7-13 yrs of age under normal vision. Subjects (Ss) led with the left leg and landed with both feet simultaneously onto a rigid surface. The jumps were videotaped (60Hz) and analyzed off-line (Peak Performance). Hip, knee, and ankle angles for each subject were normalized to flight time duration, and a 95% confidence interval was obtained at 1% bin widths. For each subject, the bins were normalized to maximum variability (100%) and classified into epochs of ***Low*** (<30%), ***Average*** (30-70%) or ***High*** (>70%). From previously defined categories (Pelland et al., *Xth Intl. Symp. Posture and Gait,* pp 549-552,1990) Ss were placed into one of three developmental categories: ***Adult***, ***Transitional***, or ***Proximal to distal***. In Ss classified as *adult*, 2 points of modulation were identified for all three joints prior to landing: **Low** variability when the limb was in maximum extension, and **high** variability for the 30-42ms (90% of flight time) prior to landing. For the *transitional* group, modulation was less specific although all subjects showed some modulation during 1 of these epochs. For the *Proximal to Distal* group, there were no specific adjustments except at the ankle in the prelanding epoch for 2/5Ss. It is concluded that ability to modulate variability in limb position during landing approach is another important descriptor in categorization of development. Supported by NSERC and FRSQ

410.5

POSTURAL CONTROL IN ALZHEIMER'S DISEASE

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While a motor degenerative syndrome is observed in endstage dementia of the Alzheimer type (DAT), clinical motor abnormalities are uncommon in patients with mild to moderate dementia. However accidental falls are commonly observed in this population. We used a mechanical force plate to measure the maximal fore-aft excursion of the center of pressure (CP) in response to a self-paced forward lifting task in 28 patients with mild to moderate DAT (Mini-Mental State Exam >10) as compared to 34 elderly (EC) and 30 young controls (YC). Appropriate anticipatory postural adjustments would minimize CP excursion. Mean maximal fore-aft CP excursion was greater in patients with DAT (3.07 ± 1.59 cm.) as compared to EC (2.41 ± 0.84) and YC (2.13 ± 0.77) ($p < .05$). As these patients do not have neuromuscular deficits, we suggest that disordered postural control is reflective of defective anticipatory postural responses to minimize the postural perturbations associated with voluntary movements of the extremities. The anticipatory response requires an estimate of this perturbation. We suggest frequent falling in DAT is reflective of this deficit in postural control.

410.6

MEDIUM LATENCY STRETCH REFLEXES ARE NOT ENLARGED IN NEUROLOGICALLY NORMAL HIV-POSITIVE PATIENTS.

D.J. Beckley, B.R. Bloem*, V.P. Panzer, and M.P. Remler. Depts. of Neurology, UC Davis, Davis, CA, University Hospital Leiden, The Netherlands, and NeuroCom, Clackamas, OR.

Patients with the AIDS dementia complex frequently manifest features of parkinsonism which may be related to lesions in the basal ganglia. Damage to these brain areas is also associated with enlargement of medium latency (ML) stretch reflexes. To determine whether ML enhancement may serve as an early electrophysiological marker for CNS involvement by HIV-1, we studied amplitudes of these reflexes in legs of neurologically asymptomatic HIV-positive patients (ADC Stage 0) (8 males, 1 female; age range 26-59, mean 38.7) and 10 controls (5 males, 5 females; age range 25-41, mean 34.3). Free-standing subjects received ten toe-up perturbations of 4^{o} amplitude at 50^{o}/sec of a movable forceplate. ML reflexes were recorded from the left medial gastrocnemius with surface EMG electrodes and corrected for background activity. There was no significant difference in ML amplitudes between the two groups ($p > 0.22$, t test). These results show that enlargement of the ML stretch reflex is not present in neurologically normal HIV-positive individuals. While assessment of ML reflexes is unlikely to be useful as a preclinical electrophysiological marker of HIV-induced CNS involvement, we cannot exclude the possibility that ML reflex abnormalities may parallel clinical manifestations in more advanced disease stages.

410.7

RELATIONSHIP BETWEEN TREMOR AND THE ONSET OF RAPID VOLUNTARY CONTRACTION IN PARKINSON'S DISEASE

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Hallett et al. hypothesized that "some of the delay in initiating movement in patients with tremor-at-rest might come from 'waiting to get into correct time of the cycle'" (J Neurol Neurosurg Psychiatry 1977 40: 1133). We tested this hypothesis by examining the onset of the voluntary rapid response in relation to tremor in 5 patients with Parkinson's disease (PD). Isometric index finger abductions were performed to two targets (20% and 60% of subject's maximum voluntary force) in selfpaced (SP) and reaction time (RT) trials. Each subject produced 200 contractions acquired in blocks of 50 trials per condition. The time intervals between the last two consecutive tremor peaks prior to the voluntary response and also from the last tremor peak to response onset were evaluated. Corresponding intervals between tremor EMG bursts and the phasic EMG burst were calculated from surface EMG. The last-peak-to-response-onset interval was 35% ± 18% (mean ± SD) of the length of the peak-to-peak interval (all subjects and experimental conditions). Therefore subjects most often (69% ± 3.5% of trials) initiated their voluntary contraction during the descending phase of tremor cycle, both in SP and RT trials. The phasic EMG burst usually occurred in synchrony with the tremor burst. This systematic phase relationship between resting tremor and the onset of voluntary motor response might indeed contribute to the prolongation of RT in patients with tremor at rest in PD. (Supported by DFG grant)

410.8

SENSORY-MOTOR PERFORMANCE CHANGES IN HEALTHY AGING SUBJECTS.

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Disorders of balance and stability are among the most significant health problems of the aging population; falls are a major cause of mortality, morbidity, immobility, and premature nursing home placement). The annual incidence of falling for persons living in the community is 30% of those over the age of 65. The "elderly" (65 years and older) face increased risk of death from a fall; they are also more likely to suffer severe nonfatal injuries from falling than younger persons. Falls account for 87% of all fractures in the elderly. Among people age 65 years or older, approximately 9500 deaths per year are attributable to falls; the incidents in which a fall initiates or contributes to a chain of events that culminates in death is probably greater.

The objective of this comprehensive, multi-dimensional study is to identify the neurophysiologic and biomechanical determinants of functional changes associated with aging. The true effects of growing old are often difficult to distinguish from disease processes that may appear or become more pronounced with time but are not necessarily related to the underlying processes of aging. Because many changes associated with aging reflect a reduction in adaptability and performance that may also characterize a specific disease, the effects of aging and disease are often difficult to separate. Consequently, conflicting reports are often found in data on "normal aging" individuals.

Research on 62 healthy elderly subjects in our laboratory has identified 46 subjects whose measurements are different from normal young adult subjects, using standard tests of functional performance (gait and balance), motor control (ankle joint compliance and reflexes), sensory processing (somatosensory evoked potentials), and physical and neurological examinations. However, none of our subjects had deficits in all measures. Twenty-three subjects had deficits in at least one motor control test, without abnormalities in sensory processing or functional measures. Six subjects who reported a history of unsteadiness also had deficits in motor control tests, the neurological examination, and in gait or standing balance.

Acknowledgment: This work has been supported by funds from VA Rehabilitation R&D.

410.9

COORDINATE TRANSFORMATION BETWEEN VISUAL AND MOTOR SYSTEMS: COMPATIBILITY EFFECTS BASED ON VISUAL FIELD, NOT MUSCLE SYNERGY DIRECTIONS. C.J. Worringham and D.B. Beringer.* Department of Movement Science, The University of Michigan, Ann Arbor, MI 48109; Department of Psychology, New Mexico State University, Las Cruces, NM 88003.

Most natural reaching and manipulation take place with gaze directed at the limbs, but many artificial tasks require humans to look at a display while the limbs are neither within view nor aligned with the display. What is the underlying principle spontaneously used by the nervous system to select the limb movement direction necessary to move an object (e.g. a cursor) in the display? Young adult male and female subjects (n=128) performed a target acquisition task with a joystick in one of eight conditions, involving combinations of "Control-Display", "Visual Field", and "Muscle Synergy" compatibility. Results show significant advantages in reaction and execution times and in error minimization when the required movement was in the same direction in the subject's "virtual" visual field as was the required display motion in his or her actual visual field. This effect was independent of limb positions, muscle synergy, and control-display relationships. The results suggest "visual field compatibility" is a universal principle of directional compatibility.

410.10

MOTOR LEARNING IN OLIVO-PONTO-CEREBELLAR ATROPHY: SCHEMA FORMATION, ADAPTATION TO ALTERED GAIN, AND USE OF ERROR INFORMATION. A.L. Smiley-Oyen, C.L. Cross* and C.J. Worringham. Department of Movement Science, The University of Michigan, Ann Arbor, MI 48109.

Animal work shows a crucial olivo-cerebellar system role in motor learning, but there is little equivalent human data. In this study, 7 Olivo-ponto-cerebellar atrophy (OPCA) patients and age-matched controls attempted to learn a task requiring linear arm movements proportional in length to bars presented on a screen. Error feedback was given initially on alternate trials. Tests of retention (no feedback), extrapolation (responses to longer bars than practiced), and gain change (same targets requiring longer movements than previously learned) were given. Measured by absolute constant error, OPCA group improvement during acquisition, retention scores, ability to extrapolate, and adaptation to altered gain did not differ significantly from controls. All OPCA patients could use error information from one trial to improve on the next, and could detect and correct errors even without error feedback. Despite motor control deficits, OPCA patients showed little sign of impaired motor learning in this task.

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410.11

ALTERED MOTOR UNIT DISCHARGE PATTERNS IN SPASTIC PARETIC MUSCLES. J.J. Gemperline, D. Walk*, and W.Z. Rymer. Department of Biomedical Engineering, Northwestern University, Sensory Motor Performance Program, Rehabilitation Institute of Chicago, and Department of Neurology, Michael Reese Hospital. Chicago, IL 60611.

The paresis associated with cerebral and spinal lesions is usually attributed to a loss of descending excitation of the affected muscles. However, previous studies from our laboratory on patients with spastic hemiparesis show an increased amount of EMG in the spastic limb for a given level of motor output, suggesting that other mechanisms may be responsible as well (Tang and Rymer, 1983). In particular, abnormally low motor unit rates in the spastic limb may contribute to the decreased ability of the muscle to produce force.

Patients with spastic hemiparesis were asked to perform a series of contractions at different force levels using both the affected and unaffected limbs. Elbow torque, surface EMG from several major muscles acting at the elbow, and intramuscular EMG from either the biceps or brachioradialis muscle were recorded. Off-line analysis of the intramuscular signals was performed in order to determine the firing patterns of several motor units which contributed to the EMG signal.

As in previous studies, the spastic limb was seen to generate more EMG in order to obtain a given torque output. However, motor unit rates were approximately 30% lower in the spastic limb for matched torques. For matched EMG, motor unit rates were lower by approximately the same amount. Recruitment rates of some units in the paretic muscle were over 50% lower than recruitment rates on the unaffected side. Units on the affected side were regularly observed to fire at rates below the recruitment rates of the motor units of the unaffected side.

The lowering of motor unit rates in the paretic muscle suggests that the motor units in that muscle are being driven inefficiently, producing reduced amounts of force. This necessitates the recruitment of additional motor units in order to produce a given level of force, which is reflected in the elevated surface EMG levels. The recruitment of previously high-threshold units at even very low force levels may have implications regarding the fatiguability of spastic muscle.

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410.12

CHANGES IN THE STRETCH REFLEX THRESHOLD IN SPASTIC MUSCLE AS A RESULT OF ELECTRICAL STIMULATION. J.D. Given*, J.P.A. Dewald, C.J. Heckman and W.Z. Rymer. Sensory Motor Performance Program, Rehabilitation Institute of Chicago, Chicago, IL 60611.

Prolonged electrical stimulation of spastic muscle (for example as utilized for FES treatment of spinal paraplegia) has been reported to reduce the severity of spastic hypertonia. In an effort to study the effects of electrical stimulation on spastic muscle we compared pre- and post- electrical stimulation elastic properties of a spastic muscle.

A hemiparetic stroke subject was seated next to a servo controlled DC motor. Elbow joint extensions of 1 radian at velocities of 0.25 or 0.5 rad/s were used to determine the passive and reflexive stiffness of the spastic elbow flexors. The stretch reflex threshold was defined as an increase in the torque-angle slope (i.e. joint stiffness) in combination with an increase of rectified EMGs from the Biceps and Brachioradialis muscles. Subsequent to the determination of the stretch reflex threshold, the subject's Biceps muscle was stimulated for 10 minutes (20 Hz, duty cycle of 2.5 s on, 2.5 s off). Within 10 s after stimulus termination, 3 elbow extensions were imposed to determine changes in the threshold of the stretch reflex and/or slope of torque-angle relations. The elbow extensions were repeated every 3 min for up to 20 min after cessation of the electrical stimulation.

Compared to the pre-stimulation case, in 2 subjects tested, peak torques were 50% higher at the end of the ramp-stretch immediately after electrical stimulation of the spastic biceps muscle. However, no slope changes of either the passive or reflexive stiffness were observed, indicating that the increase in peak torque was due to a marked reduction of the stretch reflex threshold. This threshold shift persisted for at least 20 min.

The decrease in stretch reflex threshold could be related to the stimulation of cutaneous and Ia afferents, resulting in an increase in the excitability of the flexor motor nuclei. The inhibitory Ib force feedback on these same nuclei appears to be less effective under these stimulatory conditions. Our results contradict several clinical reports claiming a reduction in spasticity after stimulation of the spastic muscle.

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410.13

INHIBITORY EFFECTS OF MAGNETIC CORTICAL STIMULATION IN MAN I: NORMAL SUBJECTS. DP Cros*, WJ Triggs*, RAL Macdonell*, KH Chiappa*, BJ Day*, JJ Fang*, BT Shahani.
Clin. Neurophysiology Lab., MA Gen. Hosp., Boston, MA 02114

We studied inhibitory effects of magnetic cortical stimulation (MCS) in 6 healthy subjects using a magnetic stimulator (Magstim 200) with 9cm coil centered and fixed over the vertex. Electromyography (EMG) was recorded with disk electrodes during monitored 10% contraction of abductor pollicis brevis (APB) or flexor carpi radialis (FCR) muscles with subjects immobilized in bivalve fiberglass casts attached to a force gauge. MCS produced motor evoked potentials (MEPs) followed by a period of EMG silence. The duration of this silent period (SP) increased with MCS intensity, but abbreviated with effort. An SP never occurred without preceding MEP, however SP duration was not a direct function of twitch amplitude. The amplitude of the H-reflex (APB 1 subject; FCR 3 subjects) was facilitated at the beginning of the SP relative to the response at rest, showed progressive inhibition as the SP evolved, and recovered with facilitation after the SP ended. In some subjects, F-wave amplitudes were similarly altered; however inhibition of the F-wave tended to begin after inhibition of the H-reflex. Neither response was ever completely abolished during the SP. Analysis of post-stimulus time histograms of single motor units activated voluntarily at 10Hz revealed that those units that fired in direct response to a threshold magnetic stimulus showed an interval to resumption of firing in excess of the inter-unit interval preceding MCS. Together, these observations suggest MCS has both excitatory and inhibitory effects, which may manifest at both cortical and spinal levels.

410.14

INHIBITORY EFFECTS OF MAGNETIC CORTICAL STIMULATION IN MAN II: LESSONS FROM PATHOPHYSIOLOGY. WJ Triggs*, DP Cros*, RAL Macdonell*, BJ Day*, JJ Fang*, M Hayes*, KH Chiappa*, BT Shahani, A Beric. Clin. Neurophysiology Lab., MA Gen. Hosp., Boston, MA 02114

We studied responses to magnetic cortical stimulation (MCS) in 3 patients with spasticity secondary to motor neuron disease (MND), and in 1 patient with a deafferentation syndrome secondary to sensory neuropathy. We used a magnetic stimulator (Magstim 200) with 9cm coil centered over the vertex. Electromyography (EMG) was recorded with disk electrodes during isometric contraction of abductor pollicis brevis (APB) and abductor digiti minimi (ADM) muscles. In these MND patients, MCS failed to elicit motor evoked potentials (MEPs), but produced a period of silence in the EMG after a latency of 45-50 ms. The duration of this silent period (SP) increased with intensity of MCS. In 1 of these spastic patients, the amplitude of an ADM H-reflex was inhibited during the SP relative to the H-reflex evoked with voluntary facilitation, but not relative to the H-reflex produced at rest. In the deafferented patient, MCS produced normal MEPs with accompanying SPs indistinguishable from responses obtained in normal subjects. This patient showed no SP with supramaximal electrical stimulation of mixed and cutaneous peripheral nerves. These observations indicate that the inhibitory effects of MCS are not due to preceding MEP or changes in muscle length, and indeed are not mediated by Ia peripheral afferents.

410.15

THE USE OF ADVANCED INFORMATION IN MOTOR RESPONSES BY HUNTINGTON'S DISEASE PATIENTS. D. B. Willingham[1], W. J. Koroshetz[2], & J. Treadwell*[1]. [1]Dept. of Psych., Williams College, Williamstown, MA 01267.[2] Huntington's Disease Clinic, Mass. Gen. Hosp., Cambridge, MA

Previous studies have shown that patients with striatal abnormalities do not benefit from a warning signal prior to a GO signal in a two-button choice response time (CRT) task, or that patients are not faster in a simple RT task than a CRT task. We compared the abilities of Huntington's disease (HD) patients and normal controls (NC) on three CRT tasks offering different types of advance information: *Watch*, in which a warning appeared; *None* in which the word "Where?" appeared instead of the warning; and *Push*, in which a warning appeared, subjects pushed a button in response to it, and then pushed again when the GO signal appeared. Subjects also performed a simple RT task, *Single*, in which there was one stimulus and one response button. Time between the warning and GO signal was varied from trial to trial, but trials were blocked by type of warning.

The benefit of the warning in the *Watch* and *Push* conditions was the same for HD patients and NC. Both groups responded more quickly in the *Push* than the *Watch* condition. Patients did not show a normal benefit in the *Single* condition, while NC responded as quickly in this condition as they did in the *Push* condition. The results are discussed in terms of simple model of a motor output buffer.

410.16

AGING AND EXTRAPYRAMIDAL MOTOR FUNCTION. MP Caligiuri Department of Psychiatry, University of California and the VA Medical Center, San Diego, CA 92161.

Mild extrapyramidal motor signs such as rigidity, hypokinesia, tremor and postural disturbances are often associated with "normal" aging leading some to suggest that Parkinson's disease (PD) may serve as a model for the understanding of the aging motor system. Objective data supporting this contention are not available to compare with patients who exhibit clinically overt PD. The present study was designed to test the hypothesis that age-related changes in extrapyramidal motor function do not parallel the cardinal motor signs seen in PD. Sensitive electro-mechanical devices were used to examine the motor systems of over 100 non-parkinsonian individuals. Subjects ranged in age from 21 to 90 years. Correlational analyses between motor function and age failed to reveal a relationship for tremor amplitude (r=0.21), movement time (r=0.04) and rigidity (r=0.18). However, the age-motor function relationship was stronger for females than males suggesting an age-gender interaction. These findings suggest that aging-related motor declines play an inconsequential role in the pathogenesis of idiopathic Parkinson's disease.

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410.17

LOWER ARM ELECTROMYOGRAPHY IN SCHIZOPHRENICS WITH TARDIVE DYSKINESIA. P. B. Vrtunski, K. Kwon* and H. Y. Meltzer. VA Medical Center and Dept. of Psychiatry Case Western Reserve University, Cleveland, OH 44141.

There are numerous descriptions of motor manifestations in schizophrenics with tardive dyskinesia (TD). The neuromuscular substrate of this impairment, however, is not well known. The aim of this study was to examine the EMG activity in agonist and antagonist muscles of the lower arm during a button-press response in a force control task. Seven schizophrenics with TD and 8 without dyskinesia participated in study. The task consisted of matching a target by generating the appropriate force with the index finger of the dominant hand, and maintaining this force for 6 seconds. There were 10 targets ranging from 5 to 560 cN in 90 quasi-randomly ordered trials of the test. The EMG was recorded from m. extensor dig. and m. flexor dig. superficialis. Results indicated that in most subjects a predominant EMG pattern reflected a reciprocal amount of activity along the force continuum, i.e., the antagonist activity was highest at low force targets and lowest at high force targets, while the reverse held true for agonist activity. In four TD patients, the antagonist activity was proportional to that in agonist, i.e., a substantial amount of cocontraction was observed. The increase in cocontraction may represent one of the markers of motor impairment in tardive dyskinesia. (Supp. by VA and MH-46630).

410.18

REACTIVE AND PREDICTIVE SCALING OF POSTURAL RESPONSES IN PARKINSONIAN PATIENTS F. Horak, J. Frank, J. Nutt*, C. Shupert R.S.Dow Neurological Sciences Inst., Good Samaritan Hospital, Portland, OR 97209

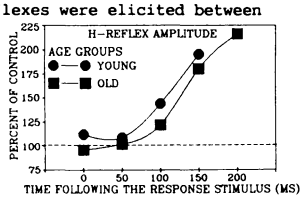
We investigated whether parkinsonian patients' postural deficits could be related to an inability to scale the magnitude of responses to center of mass (CM) displacements. Fifteen parkinsonian and elderly control subjects were exposed to backward surface displacements to determine whether they could use sensory feedback and prior experience to scale postural responses to displacement velocities or amplitudes.

Although parkinsonian patients scale postural responses, their torque responses were less sensitive to fast and large displacements and more sensitive to slow and small displacements than controls. Parkinsonian EMG responses were normal latency but EMG magnitudes saturated at 50-80% of control values for the fastest and largest displacements. Agonist burst durations were shorter with multiple bursts and antagonists were larger and earlier than controls. Although peak center of pressure (CP) initially moved beyond the peak CM, CP was not sustained to maintain equilibrium for large perturbations. Thus, the primary postural deficit was in sustaining tonic postural force and not in reactive or predictive scaling of the initial triggered postural patterns. (Supported by NIH AG0647 and DC00205.)

410.19

MOTOR PREPARATION IN YOUNG AND OLD ADULTS. J.R. Burke[1] and G. Kamen[2]. (Motor Control Laboratory, Indiana University, Bloomington, IN). [1]Department of Exercise Science, University of South Carolina, Columbia, SC and [2]Department of Physical Therapy, Boston University, Boston, MA.

Motor preparation was assessed in young (n = 20) and old (n = 20) adults by eliciting a right leg tibial nerve H-reflex (50% of the maximum H-reflex) prior to the onset of a voluntary response. The motor task was a right plantar flexion in response to a visual stimulus. A visual warning stimulus preceded the response stimulus by 1 second. The motor preparation test intervals were incremented by 50 ms between the presentation of the response stimulus and the onset of the voluntary response. On each trial, a H-reflex was elicited at one of the motor preparation test intervals. Control H-reflexes were elicited between randomly selected trials. The results supported a delayed onset of alpha motoneuron facilitation prior to a voluntary movement with age, as shown for H-reflex amplitude.



410.20

CHANGES IN MOTOR STRATEGIES WITH NORMAL HUMAN AGING. A.S.Buchman,*G.L.Gottlieb,D.M.Corcos,C.H.Chen,G.L.Almeida, Rush-Presbyterian St.Lukes Medical Center,Chicago,IL 60612, University of Illinois, Chicago, IL 60612, Universidade Estadual de Campinas, Ircamp/Conselho Nacional de Pesquisa, Campinas, SP 13100, Brazil

Recent studies of single-joint movements in healthy young normal subjects have led to the development of a Dual Strategy hypothesis for voluntary control of single joint movement. This hypothesis postulates that tasks can be performed by one of two strategies: speed-insensitive (SI) or speed-sensitive (SS). It is proposed that the SI strategy is generated by modulating the duration of the excitation pulse to the motor neuron pool while the SS strategy modulates the intensity of the excitation pulse. In light of ongoing changes in the motor unit with normal aging we have compared 8 normal subjects older than 50 years with 8 sex-matched subjects less than 35 years old.

To elicit the SI strategy, subjects performed discrete elbow flexions in the horizontal plane from a stationary initial position to visually defined targets at different distances with a constant inertial load. To elicit the SS strategy, subjects made discrete elbow flexion movements over a fixed distance with changes in movement speed elicited by explicit instructions to the subject and also knowledge of results concerning their movement time. Joint angle, acceleration, and electromyograms from two agonist and antagonist muscles were measured.

Younger subjects all demonstrated both SI and SS strategies. In contrast, only three of eight older subjects exhibited both strategies. The other five exhibited SS appearing behavior for both paradigms.

These findings are consistent with the loss of neuromotor control with normal aging that has been suggested by studies of motor unit firing changes in normal aged. The loss of motor units and changes in motor unit firing with normal aging may limit excitation pulse duration modulation necessary for generating the SI strategy.

This study was supported by NIH grants AR33189, NS28176 and NS23593

410.21

RELATIONSHIP BETWEEN FUNCTIONAL DEFICITS IN SENSORY AND MOTOR SYSTEMS AND POSTURAL DYSCONTROL IN MULTIPLE SCLEROSIS (MS) PATIENTS. C. A. Pratt, F. B. Horak, and R. M. Herndon. Dept. of Neurology and R.S. Dow Neurological Sciences Institute of Good Samaritan Hospital, Portland, OR 97209

Because the distribution of demyelinating lesions varies across MS patients, we hypothesized that *different forms* of postural dyscontrol exist among MS patients depending on the relative impairment of functional systems involved in postural control. In this study, we have used a battery of clinical and electrophysiolgical tests to assess function of three sensory systems (somatosensory, visual, and vestibular) and the corticospinal tract in 10 mild-moderately disabled, ambulatory MS subjects aged 39-52 yrs. Functional deficits were correlated with postural control abnormalities revealed by horizontal translations of a posture platform.

One subgroup (N=6) consisted of MS subjects with significant (> 2 S.D.) bilateral delays in the onset of postural responses, recorded electromyographically (EMG) in the leg muscles. In these subjects, there was a high correspondence between bilateral delays in the onsets of postural responses and bilateral delays in central afferent conduction time (ACT), as revealed by somatosensory evoked potentials, but not with delays in central motor conduction times (MCT), evoked by magnetic stimulation of the cortex. EMG latencies were normal in MS subjects with normal ACT or impaired ACT on just *one* side, indicating that normal somatosensory inputs from just one leg are sufficient to trigger postural responses of normal latency bilaterally. A second subgroup (N=3) consisted of MS subjects who had normal VORs but had abnormally low gains in tests of fixation suppression, thought to involve the cerebellum. This subgroup may have impaired feedforward control of posture, similar to previously tested cerebellar patients.

410.22

AUTOMATIC & VOLUNTARY POSTURAL RESPONSES IN PARKINSON'S DISEASE. V.P. Panzer*, L.M. Nashner and T.N. Chase, NINDS, NIH, Bethesda, MD 20892 and NeuroCom International, Clackamas, OR 97015.

Limited success has been derived from the study of automatic postural responses in Parkinson's disease (PD); whereas more revealing, though contradictory results are reported in studies of voluntary limb movement. We therefore used a battery of tests of automatic and voluntary postural movements to evaluate 10 PD patients and 10 age-matched controls. Automatic response tests included 6 conditions of posturography and 3 sizes of platform translation. To examine voluntary postural control, patients made single weight shifts to random targets and continuous weight shifting movements. Measures of COG displacement, response latency, movement speed and amplitude were evaluated. Automatic response latencies to translations and COG displacement were normal. In contrast, measures of voluntary movement amplitude were significantly abnormal ($p<.005$). Postural reaction time in single weight shifts was improved by optimal dose levodopa therapy. While automatic postural responses are in the normal range in PD, voluntary movements are abnormal and may be responsive to drug therapy.

LIMBIC SYSTEM III

411.1

OPTICAL REFLECTANCE IN THE CAT DORSAL HIPPOCAMPUS CORRELATES WITH SLOW ELECTRICAL ACTIVITY G. R. Poe*, D. M. Rector*, S. S. Chirwa, and R. M. Harper, Department of Anatomy and Cell Biology and the Interdepartmental Neuroscience Program, UCLA School of Medicine, Los Angeles, CA, 90024-1763.

We monitored optical reflectance at 700 nm from the dorsal hippocampus using a previously described fiber optic probe. Under surgical anesthesia, electrodes were placed to record cortical EEG, and the image probe was lowered to the surface of the dorsal hippocampus. Fine wires were attached to the side of the optic probe to record hippocampal slow electrical activity. Diaphragmatic leads were placed to record respiratory and ECG activity.

To assess optical activity, images from the dorsal hippocampus were gathered at 2 second intervals in synchrony with the ECG R-wave for 2 hour periods which incorporated both sleep and waking states. Recordings of hippocampal and cortical EEG, EOG, diaphragm EMG, and ECG were digitized simultaneously with the images and scored for sleep state. Epochs of particular EEG spectral composition (slow waves, desynchronized) were selected within each state. Images within similar epochs were averaged, then compared by subtraction and ANOVA analysis with an alpha of .05. The dorsal hippocampus exhibited spontaneous fluctuations in optical activity coincident with slow electrical waveforms. Maximal reflected activity occurred during desynchronized EEG activity from phasic REM sleep and during spindling discharge of quiet sleep. The least reflected activity was observed during desynchronized EEG associated with particular waking epochs.

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411.2

CHOLINERGIC GATING OF AUDITORY RESPONSE IN THE HIPPOCAMPUS. R. Freedman, V. Luntz-Leybman, P. Bickford-Wimer, and L. Olson, Center for Neuroscience and Schizophrenia, Denver VAMC and Univ. of Colorado Health Sciences Center, Denver, CO 80262 and Karolinska Institute, Stockholm, Sweden

Decreased response to repeated sensory stimuli is characteristic of the hippocampus. Innervation from the medial septal nucleus has been shown to be necessary for such gating of auditory response, which is observed in the evoked activity of pyramidal neurons in the CA3 region. The purpose of this study was to determine if cholinergic neurotransmission is critical to this effect and to identify which of the several cholinergic receptor types are involved. Sprague-Dawley rats were anesthetized with chloral hydrate, and recordings of auditory-evoked N40 responses were made from microelectrodes in the CA3 pyramidal layer. Auditory stimuli were presented in a paired-pulse or conditioning-testing paradigm, with 0.5s intrapair interval. In control recordings, the test N40 response was less than 40% of the amplitude of the conditioning N40 response. Cholinergic antagonists were subsequently infused into the lateral ventricle. The muscarinic antagonist scopolamine and the nicotinic ganglionic antagonist mecamylamine were without effect. However, the neuromuscular antagonist curare blocked gating of the test response in a dose-dependent fashion, as did α-bungarotoxin (α-BT). κ-BT had no effect. α-BT infused into the lateral ventricle binds to large neurons outside the CA3 pyramidal layer. There is also binding in the stratum oriens of CA1, but little drug reaches the dentate. Some of the α-BT-labelled neurons in CA3 also stain for GABA by immunocytochemistry. The results suggest that gating of response to auditory stimuli in the hippocampus is mediated by cholinergic activation of GABA-ergic interneurons in CA3 through an α-BT-sensitive receptor.

411.3

FINE STRUCTURE AND CELLULAR CORRELATES OF HIPPOCAMPAL SHARP-WAVE BURSTS. Z. Horváth, R. Urioste*, J. Hetke*, K. D. Wise* and G. Buzsáki, Center for Neuroscience, Rutgers University, Newark, NJ 07102 and Center for Integrated Sensors and Circuits, University of Michigan, Ann Arbor, MI 48109[1]

Neuronal correlates and bilateral synchrony of hippocampal sharp-waves were investigated with single and integrated multisite microelectrodes in the awake rat. The probability of single pyramidal cell firing in CA1 and CA3 during the sharp-wave burst varied from 0 to 55 percent. The probability that 2 or 3 pyramidal cells fired within a time window of 100 msec was several-fold higher during sharp-waves than during sharp-wave-off periods. Interneurons in the stratum oriens selectively increased their discharges during the population bursts, whereas some feed-forward interneurons in CA3 were not affected at all. This finding suggests that sharp-wave bursts are not triggered by subcortical inputs. A spindle-like oscillation (ripple) with 150-250 Hz and 1 mV amplitude was riding on the peak of each sharp-wave. Action potentials were associated with the negative phase of the ripple. The amplitude of ripples was maximal in the pyramidal layer. Ripples were synchronous within 2 msec in the two hippocampi. Ripples may represent a series of population spikes and/or fast repetitive IPSP events. These findings suggest that "epileptiform" fast oscillation is a normal feature of the intact hippocampus.

411.4

SPONTANEOUS AND EVOKED THETA FIELD AND THETA-RELATED CELL ACTIVITY OF THE ENTORHINAL CORTEX IN THE URETHANE-ANESTHETIZED RAT. C.T. Dickson and B.H. Bland. Beh. Neurosci. Res. Grp., Dept. Psyc., Univ. of Calgary, Calgary, AB, Canada T2N 1N4.

Theta (θ) is a near-sinusoidal waveform generated by the hippocampal formation and is associated with a well documented nosology of θ-related hippocampal cellular activity. Retrohippocampal areas, such as the entorhinal cortex, also generate θ but the theta-related cell activity is less well known. This study extends the classification system for hippocampal θ-related cell activity to the entorhinal cortex and to examine the control of cell and field activity by cholinergic agents. Both cellular and field activity were sampled from this region using an approach normal to its laminar structure and the effects of various cholinergic agents on both field and cell activity were monitored. Thus far, θ-on cells (i.e., those which fired more during θ) and one θ-off cell (i.e., one which fired less during θ) have been found. θ-on cells were found to have either rhythmic or non-rhythmic firing properties and to be either linearly or nonlinearly related to the frequency of θ in hippocampus. Systemic and intrahippocampal administration of cholinergic agonists activated entorhinal θ and at a lag, θ-on cell discharge. Thus, the nosology of θ-related units in the entorhinal cortex and their control via cholinergic agents seems to be similar to that seen in the hippocampus.

411.5

EFFECTS OF A REVERSIBLE BLOCKADE OF THE DORSO-MEDIAL POSTERIOR HYPOTHALAMUS ON HIPPOCAMPAL FIELD ACTIVITY. S.D. Oddie, L.V. Colom, and B.H. Bland. Beh. Neurosci. Res. Grp., Dept. of Psyc., Univ. of Calgary, Calgary, AB, Canada T2N 1N4.

The effects of dorsomedial-posterior hypothalamic (DMPH) stimulation and inactivation on hippocampal field activity was investigated in urethane-anesthetized rats. Hippocampal theta (θ) could be elicited with either tail pinch or electrical stimulation of DMPH in the range of 0.1-1.0 mA. Reversible blockade of DMPH by micro injection of procaine hydrochloride abolished spontaneous, tail pinch, and stimulation elicited θ and resulted in a reduction in the power of hippocampal field activity. Frequency, amplitude, and power measures of hippocampal field activity showed progressive recovery during the post-procaine period as tested by tail pinch and DMPH stimulation. A second series of experiments investigated the effects of DMPH stimulation and blockade on hippocampal θ elicited by intra-hippocampal microinjection of carbachol. Contrary to Colom, et.al. (1991) where blockade of medial septum abolished carbachol elicited θ, blockade of DMPH did not abolish θ elicited by carbachol in the hippocampal formation or lower the pre-procaine power level of the field activity. After recovery, electrical stimulation of DMPH at appropriate levels resulted in an upward modulation of the carbachol-induced hippocampal θ frequency.

411.6

DORSOMEDIAL-POSTERIOR HYPOTHALAMIC INFLUENCES ON THE SEPTO-HIPPOCAMPAL PATHWAY IN THE GENERATION OF HIPPOCAMPAL THETA. B. H. Bland, L. V. Colom and S. D. Oddie. Beh. Neurosci. Res. Grp., Dept. Psyc., Univ. of Calgary, Calgary, AB, Canada T2N 1N4.

We have hypothesized that the dorsomedial-posterior hypothalamic (DMPH) region may form part of the ascending synchronizing brainstem pathway originating in the pons region and projecting to the medial (MS)/vertical limb of the diagonal band of Broca (vDBB). The influence of the DMPH on the generation of hippocampal formation theta (θ) field activity was thus investigated in urethane-anesthetized rats. Microinfusion of procaine (PRO)-hydrochloride (HCl) into the DMPH blocked tail pinch elicited θ, spontaneously occurring θ, and θ produced by electrical stimulation of the nucleus pontis oralis. Systemic administration of eserine was able to generate hippocampal formation θ field activity during reversible PRO-HCl blockade of the DMPH. Microinfusion of carbachol into the DMPH resulted in intense activation of hippocampal θ. The results suggest that the DMPH forms part of the ascending brainstem synchronizing system. Furthermore, at least part of the influence of DMPH in the generation of hippocampal θ is mediated by cholinergic receptors.

411.7

PONTINE AND DORSOMEDIAL-POSTERIOR HYPOTHALAMIC INFLUENCES ON THETA-RELATED CELLS IN THE MEDIAL SEPTUM. L. V. Colom and B. H. Bland. Beh. Neurosci. Res. Grp., Dept. of Psyc., Univ. of Calgary, Calgary, AB, Canada T2N 1N4

The effects of electrical stimulation of the nucleus pontis oralis (PO) and dorsomedial-posterior hypothalamic (DMPH) region, along with reversible inactivation of the DMPH with procaine hydrochloride (PRO-HCl), on the discharge rates of theta (θ)-related cells in the medial septum (MS)/vertical limb of the diagonal band of Broca (vDBB), were investigated in urethane-anesthetized rats. Electrical stimulation of either the PO or DMPH resulted in intense activation of θ-on cells in the MS/vDBB. The microinfusion of PRO-HCl into the DMPH abolished the activation of MS/vDBB θ-on cells produced by electrical stimulation of the PO. The spontaneous discharge rates of θ cells in the MS/vDBB showed two types of responses to reversible inactivation of the DMPH. Some θ-on cells reduced their discharge rates significantly while others increased their discharge rates significantly, compared to the rates accompanying hippocampal θ conditions. Microinfusion of carbachol into the DMPH also produced intense activation of θ-on cells in the MS/vDBB which was abolished by subsequent microinfusion of atropine sulfate into the DMPH.

411.8

ATROPINE-RESISTANT AND ATROPINE-SENSITIVE HIPPOCAMPAL THETA IN THE FREELY MOVING RAT DEPENDS ON SEPTAL AFFERENTS. V.H. Lawson and B.H. Bland, Beh. Neurosci. Res. Grp., Dept. Psych., Univ. Calgary, Calgary, AB, T2N1N4

The role of the medial septum (MS)/vertical limb of the diagonal band of Broca (vDBB) in hippocampal theta (θ) generation and behavior was assessed using microinfusions of drugs into the MS/vDBB. Rats were chronically implanted with electrodes in the stratum moleculare of the dentate gyrus and a microinfusion guide cannula above the MS/vDBB. Microinfusion of procaine-HCl (PRO) produced a bilateral reversible suppression of θ. The beginning of the PRO infusion was characterized by increased motor activity followed abruptly by an immobility state resembling catatonia, associated with the suppression of θ. The duration of suppression was dose-dependent and recovery was associated with intense feeding. Microinfusion of carbachol also initially produced motor activity followed by long periods of immobility which were associated with θ activity at a constant dose dependent frequency. Any voluntary movements resulted in upward θ frequency shifts. Subsequent microinfusions of either PRO or atropine sulfate abolished immobility related θ and in the case of atropine, movement correlated θ was re-instated. Thus, the septo-hippocampal pathway is critical for the generation of both atropine-resistant and atropine-sensitive θ and their behavioral correlates.

411.9

FIRING RELATIONS OF ENTORHINAL NEURONS TO THE HIPPOCAMPAL THETA RHYTHM IN WALKING AND URETHANE ANESTHETIZED RATS. M.Stewart, M.Barry*, G.J.Quirk and S.E.Fox. Dept. Physiol., SUNY Health Sci. Ctr., Brooklyn, NY 11208.

Recordings from afferent neurons, together with data on the anatomical targets and the excitatory/inhibitory nature of the input, aids the interpretation of field potentials underlying the hippocampal theta rhythm. Extracellular recordings were taken from single entorhinal neurons in rats during the two most widely studied theta mode conditions: walking and urethane anesthesia. Cell firing in relation to the simultaneously recorded theta rhythm was examined. In walking rats, 22/23 histologically identified cells from layer II/III of medial entorhinal cortex (MEC), were significantly phase-locked to the hippocampal theta rhythm with a mean phase of 5° (0°=dentate theta pos. peak). In contrast, only 8/23 layer II/III MEC cells in urethanized rats were phase-locked with a mean phase of 90°. The firing of 4/8 layer II/III cells from lateral entorhinal cortex (LEC) in urethanized rats was significantly phase-locked to the theta rhythm with a mean phase of 134°. The rhythmic firing of one MEC cell (layer II), driven at constant latency from the hippocampal fissure, was reversibly abolished by cooling the supracallosal striae at the genu of the corpus callosum, suggesting that septo-entorhinal projections rhythmically excite these cells.

MEC cells tend to fire near the positive peak of the dentate theta rhythm in both walking and urethanized rats. Hippocampal pyramidal cells also fire on this phase. Distinguishing the two preparations, a larger proportion of MEC cells are phase related to the theta rhythm during walking. Although the MEC phase data are consistent across preparations, they cannot completely account for hippocampal field potentials at the depth of the MEC afferents. The firing of LEC neurons near the negative peak of the dentate theta rhythm may account for the large current sink near the hippocampal fissure. (Supported by NIH NS17095.)

411.10

HOMO- AND HETEROSYNAPTIC PAIRED-PULSE MODULATION OF PERFORANT PATH TRANSMISSION: ROLE OF CONDITIONING PULSE INTENSITY.

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To study short-term synaptic modulation in the lateral (LPP) and medial (MPP) perforant path we have adopted a paired-pulse (P-P) procedure of varying the level of C pulse and keeping the intensity of T pulse constant. The effect of C pulse intensity and interpulse interval was studied in urethane anesthetized rats. Stimulation electrodes were placed in the LPP and MPP and the recording electrode at the granule cell layer. Analysis of the population spike revealed homosynaptic facilitation in the MPP at levels of C pulse below threshold for firing granule cells (100-200% interpulse intervals 15-100ms). At higher intensities of C pulse there was inhibition at the shortest (15-30ms) and the longest (above 300ms, "late inhibition") intervals. In the LPP, homosynaptic facilitation was seen but there was no evidence of inhibition at any intensity of C pulse. Heterosynaptic facilitation was present at all levels of C pulse in both pathways except for the highest intensity of MPP C pulse, which induced heterosynaptic inhibition at shortest intervals. These results reveal a wide dynamic range of the short-term modulation in both the LPP and MPP which has not been reported using conventional P-P procedures.

411.11

INTRACELLULAR RECORDINGS OF RAT SUBICULAR NEURONS. J.S. Taube and C.W. Cotman. Department of Psychology, Dartmouth College, Hanover, NH 03755 and Department of Psychobiology, University of California, Irvine, CA 92717.

The electrophysiological properties of CA1 pyramidal neurons have been well-characterized. These cells send a major projection to the subiculum, which in turn projects to the presubiculum. Although recent studies have determined the behavioral/spatial correlates of neurons in the subiculum and presubiculum, little is known about the electrophysiological properties of subicular neurons. Using the in vitro slice preparation and intracellular recording techniques, the present study was conducted to assess the basic properties of subicular neurons.

Potentials recorded from subicular neurons in response to intracellular depolarizing current pulses differed from CA1 pyramidal neurons. Following a 100 msec, 0.5 nA depolarizing current pulse, subicular neurons responded with an initial burst of 3-4 spikes contained in a 40-50 msec depolarizing envelope, followed by the discharge of 1-2 single spikes not contained in a depolarizing envelope. This initial burst of spikes appeared to be mediated by calcium. Subicular cells showed an EPSP/IPSP sequence in response to electrical stimulation of CA1 radiatum, oriens, or alveus. The EPSP was blocked with addition of CNQX to the bathing medium. In a Mg^{++} free, CNQX bathing solution, a longer lasting depolarization was recorded, which was blocked with application of AP5. Iontophoretic application of glutamate or quisqualate into the apical or basal dendritic layers of subiculum lead to a short-latency depolarization. Application of GABA produced a short-latency hyperpolarization when it was applied near the recording site, but a depolarization when it was applied in the apical dendritic region. These results illustrate common properties of CA1 and subicular neurons, with the exception of their response to a depolarizing current pulse.

411.12

SHORT TERM ALTERATIONS IN RESPONSES OF ENTORHINAL CORTICAL NEURONES DURING ACTIVATION OF SYNAPTIC INPUTS *IN VITRO*. R.S.G. Jones. Department of Pharmacology, University of Oxford, Oxford OX1 3QT.

The perforant path, which arises from neurones in layers II-V of the entorhinal cortex (EC) is pivotal in controlling hippocampal functions. To understand these functions it is important to know how the EC processes information which is destined for the hippocampus. The present experiments have examined responses of neurones in layers IV/V and II of the medial EC (MEC) to repetitive activation of afferent inputs at different frequencies. Slices of EC were prepared from Wistar rats and maintained in an interface chamber. Synaptic responses were evoked in layer IV/V neurones by stimulating in the lateral EC and recorded with conventional intracellular electrodes. Responses were evoked in layer II neurones by stimulating in layer V/VI of the MEC or the parasubiculum. Stimulation at relatively low frequencies (<1.0 Hz) elicited a fast, AMPA/kainate mediated EPSP in layer IV/V neurones which was often followed by a slow NMDA mediated EPSP. In some cells (20 %) the NMDA EPSP showed dramatic enhancement during low frequency stimulation and elicited bursts of action potentials. Cessation of stimulation resulted in a rapid (1 min) decline of the slow EPSP to control levels. Higher stimulation frequencies usually resulted in failure of the slow EPSP. The fast EPSP was little altered by changes in frequency. Responses of layer II neurones were dominated by fast ($GABA_A$) and slow ($GABA_B$) IPSPs although an NMDA mediated depolarization often intervened. Both IPSPs showed a frequency dependant decrement and could practically disappeared at higher frequencies (> 2.5 hz). Simultaneously, the NMDA EPSP was enhanced and could elicit action potentials. These effects reversed rapidly (1-2 min). 2-AP5 blocked the enhanced slow EPSP but not the fading of the IPSPs. The results indicate that the routes taken by synaptic information through the EC and therefore its destination in the hippocampus are probably dependant on the frequency of the inputs.

411.13

A MODEL OF THE EXTRACELLULAR FIELD POTENTIALS GENERATED BY POPULATIONS OF HIPPOCAMPAL PYRAMIDAL CELLS. R. Costalat*, S. Genet*, E. Thiels, T.W. Berger and G. Chauvet. *Inst. de Biologie Théorique de l'Ouest-Atlantique, 49100 Angers, France, and Depts. of Behavioral Neuroscience and Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA 15260.*

In order to analyze some of the determinants of extracellular field potentials generated by activation of hippocampal CA1 neurons upon stimulation of commissural afferents, a two-neuron model of variation of field potential in space and time was expanded to a population of neurons. The model uses the Laplace equation to describe intra- and extracellular potentials, and Hodgkin-Huxley and linear cable equations to describe active and passive membrane currents, respectively. Computer simulations of the two-neuron system served to derive ionic current lines between sources and sinks and determine from them dynamics of extracellular field potentials. Because the field potential of a population of neurons at a given point in the extracellular space depends on the summation of the extracellular field potentials of all neurons of that population, the ionic current lines for individual neurons were used to deduce population field potentials.

Comparison of the thus derived waveforms with ones obtained from extracellular recordings of CA1 pyramidal cells activated by stimulation of commissural fibers in intact rat hippocampus indicate that the model can predict qualitatively the changes in waveform observed as a function of: (1) location along the dendrite-cell body extent, (2) magnitude of commissural fiber stimulation, (3) efficacy of commissural-CA1 synapses, varied experimentally by induction of LTP, and (4) blockade of commissural-CA1 synapses located deep vs superficial to pyramidal cell bodies, varied experimentally by microinjection of the quisqualate receptor antagonist CNQX in the respective dendritic zones. We conclude that the two-neuron model is sufficient to describe extracellular field potentials generated by populations of neurons.

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411.14

OPEN-LOOP NONLINEAR RESPONSE PROPERTIES OF RABBIT HIPPOCAMPAL DENTATE GRANULE CELLS RECORDED FROM THIN AND "MINI" *in vitro* SLICES. T. Patrick Harty, German Barrionuevo, Robert J. Sclabassi and Theodore W. Berger. *Depts. of Behavioral Neuroscience, Neurosurgery and Psychiatry, University of Pittsburgh, Pittsburgh, PA, 15260.*

The objective of these experiments was to use nonlinear systems analytic techniques to characterize the input/output properties of dentate granule cells in an open-loop condition, i.e., when feedback from other hippocampal subfields and feedforward or feedback modulation from dentate interneurons is eliminated. Although this condition is at least partly met by the *in vitro* hippocampal slice preparation, populations of interneurons exist in a slice that may contribute to granule cell response properties. Two additional experimental manipulations were used in an attempt to isolate granule cells from all but perforant path input. First, slice thickness was reduced from 600 µm to the minimum thickness that reliably produced detectable population spikes (300 µm). In 600 µm slices, second order nonlinearities were characterized by facilitation of population spike amplitude when a preceding impulse occurred with an interval of 10-100 ms, and suppression when the interval was 150-800 ms. The $GABA_A$ antagonist bicuculline (BMI) increased facilitation, demonstrating an influence of GABAergic interneurons. Although 300 µm slices exhibited similar second order nonlinearities, they were insensitive to BMI. The second manipulation involved physically separating approximately 40% of the dentate gyrus from the rest of a 600 µm slice. Compared to intact slices, granule cell nonlinearities in these "mini" slices were unchanged, and were sensitive to BMI. These results strongly suggest that feedback from interneurons in the deep hilus and from areas CA3 and CA1 do not contribute to nonlinear response properties of granule cells in the *in vitro* slice preparation. In addition, the contribution of GABAergic interneurons is eliminated in a 300 µm slice, suggesting that open-loop conditions for granule cells can be achieved. Supported by MH09857, ONR, AFOSR, MH00343.

411.15

COMPARISON OF THE FUNCTIONAL PROPERTIES OF DORSAL VERSUS VENTRAL DENTATE GYRUS OF THE *in vitro* HIPPOCAMPAL SLICE USING NONLINEAR SYSTEMS ANALYSIS. Theodore W. Berger, Choi Choi*, T. Patrick Harty, Robert J. Sclabassi. *Departments of Behavioral Neuroscience and Neurological Surgery, University of Pittsburgh, Pittsburgh, PA 15260.*

Neuroanatomical studies of the hippocampal formation have shown that circuitry as well as cell density vary along the dorso-ventral axis. To what extent anatomical differences maintained within the reduced circuitry of the transverse hippocampal slice result in functional differences is the focus of this study. Nonlinear systems analytic techniques were used to compare the input-output functions of the dorsal versus the ventral dentate gyrus in the rabbit hippocampal slice.

Slices of 600 µm thickness were cut transverse to the longitudinal axis of the hippocampus from the dorsal third and the ventral third of the left hippocampus. The perforant path projection to dentate granule cells was stimulated with a train of 4064 electrical impulses with randomly distributed interimpulse intervals. The resulting extracellular potential was recorded from the granule cell layer. The relationship between the interimpulse intervals and the amplitudes of the resulting population spikes were expressed as the kernels of an orthogonalized functional power series. The second order kernel represents the changes to the population spike amplitude due to any preceeding impulse with interval Δ, and the third order kernel represents the changes to the population spike amplitude due to any two preceeding impulses of intervals Δ_1 and Δ_2.

Second and third order kernels were computed for responses from dorsal/ventral slice pairs from the same hippocampi. Preliminary results show no significant differences between the dorsal and ventral slices with respect to either second or third order kernels. These results suggest that the functional dynamics of the transverse dentate gyrus slice, in response to perforant path input, remain constant along the dorso-ventral axis. Supported by ONR, AFOSR, and MH00343.

411.16

CLOSED LOOP COMPUTATIONAL EVALUATION OF HIPPOCAMPAL MODEL CONTAINING UNOBSERVABLE ELEMENTS

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Nonlinear systems analytic techniques are being utilized to experimentally characterize the transformational properties of the hippocampus by recording electrophysiological responses to random impulse train stimulation and by expressing the relationship between the input and output activity as the kernels of a functional power series, which are interpreted as nth order impulse responses. In-vivo and in-vitro experiments have been performed to characterize both the closed- and open-loop properties of this system. However, some elements cannot be isolated experimentally and their input/output properties must be inferred from their effect on the other directly observable groups of neurons. To accomplish this the nth order Laplace transforms of the impulse responses are computed and algebraically manipulated. Models of the observable and unobservable subsystems, as well as the entire system, may then be computed in terms of nth order convolution operators.

This paper presents an example utilizing data from three sets of experiments: the intact hippocampal formation, with the dentate granule cell layer considered as a feedforward element, and the remainder of the ipsilateral hippocampus as a feedback element; a partially closed-loop system, where the contralateral hippocampus has been removed, the ipsilateral trisynaptic pathway is open, and the local GABAergic pathways have been blocked, in which: (1) the properties of unobservable elements are estimated, and (2) the response properties of the entire system are computed based on combining the observable and unobservable elements. Supported by NIMH, the Office of Naval Research and the Air Force Office of Scientific Research.

411.17

THREE-DIMENSIONAL SPATIAL DISTRIBUTION OF PERFORANT PATH-EVOKED FIELD POTENTIALS IN HIPPOCAMPUS RECORDED SIMULTANEOUSLY *in vivo* BY MULTIPLE ELECTRODES IN CA1 AND DENTATE. Andrew J. Nowak and Theodore W. Berger, *Depts. of Behavioral Neuroscience and Psychiatry, University of Pittsburgh, Pgh, PA 15260.*

Excitatory perforant path fibers arising from the entorhinal cortex innervate the distal dendrites of dentate granule cells, as well as the apical dendrites of CA1 and CA3 pyramidal cells. The present study examined the three-dimensional spatio-temporal distribution of CA1 pyramidal and dentate granule activated by perforant path stimulation in the *in vivo* rabbit hippocampus. The laminar profiles of extracellular field potentials evoked by perforant path stimulation were recorded simultaneously by an array of electrodes advanced in 100 μm steps through the CA1 and dentate regions of the dorsal hippocampus of halothane-anesthetized rabbits. Electrode arrays consisted of 3-5 insulated, finely-etched insect pins cemented together with tips separated by 500 or 1000 μm. Perforant path axons were stimulated at the level of the angular bundle with two intensities, one which was subthreshold for eliciting a population spike and the second which evoked a 50% maximum amplitude population spike in the dentate. During withdrawal of the array, current was passed through each electrode at the reversal points of the field potentials for histological reconstruction of the electrode tracks.

Results showed, as described previously (Yeckel and Berger, PNAS, 1990), simultaneous monosynaptic excitation of CA1 pyramidal and dentate granule cells with subsequent polysynaptic excitation of CA1 via the trisynaptic pathway. In addition, a medio-lateral gradient of excitation across the CA1 and dentate regions was indicated by: 1) a longer peak latency and decreased slope of population EPSPs for more lateral recording sites in CA1, and 2) longer latency population spikes for more lateral recording sites in dentate. Finally, a significant disparity was found between the locations of orthodromically and antidromically driven CA1 pyramidal cells. Supported by ONR, AFOSR, MH00343.

411.18

EFFECTS OF YOHIMBINE ON THE NONLINEAR RESPONSE CHARACTERISTICS OF HIPPOCAMPAL DENTATE GRANULE CELLS IN THE ANESTHETIZED RAT. Michael J. Mana, Robert J. Sclabassi, and Theodore W. Berger. *Departments of Behavioral Neuroscience, Neurological Surgery, and Psychiatry, University of Pittsburgh, Pgh, PA 15260.*

Nonlinear systems analytic techniques were used to study the effects of the α-2 antagonist yohimbine on the input/output dynamics of hippocampal dentate granule cells to perforant path stimulation involving a random interval train of 2026 electrical impulses; interimpulse intervals (Δ) were determined by a Poisson process with a mean frequency of 2 Hz. Yohimbine (4 mg/kg, i.p.) was administered after the first train; 30 min later, a second train was delivered. First, second, and third-order kernels describing the relationship between Δ and the amplitude of the evoked granule cell population spikes were computed for each train.

Yohimbine produced a 40-50% increase in the magnitude of first order kernels, (h_1), which reflect the average population spike for all impulses in a train. Second order kernels reflect the influence of any preceding impulse on granule cell response to a later impulse in the train, as a function of Δ. The increase in second order facilitation produced by yohimbine when Δ = 60-200 ms was proportionally greater than the drug-induced increase in h_1; the magnitude of suppression when Δ < 50 ms was maximum both pre- and post-drug (i.e., postdrug suppression increased in absolute terms to match the increase in h_1). Yohimbine did not change the Δ associated either with peak facilitation or peak suppression. Third order kernels reflect the influence of any pair of preceding impulses occurring Δ_1 and Δ_2 ms earlier. A marked suppression was observed both pre- and post-drug when Δ_1 and Δ_2 < 200 ms. Yohimbine also enhanced the magnitude of third order nonlinearities, with the increase again being proportionally greater than the increase in h_1. These results suggest that yohimbine increases both the number of granule cells excited by a perforant path volley, as well as the relative contribution -- though not the time course -- of the majority of mechanisms providing feedforward and feedback modulation of granule cell activity. Supported by MH00343, MH43947, NS19608, and an MRC of Canada Postdoctoral Fellowship to MJM.

411.19

AUTONOMOUS AND RHYTHMICALLY DRIVEN THETA-LIKE OSCILLATIONS IN A COMPUTER MODEL OF THE *IN VITRO* RODENT HIPPOCAMPAL CA3 REGION. R.D. Traub, G. Buzsáki and R. Miles. IBM T.J. Watson Research Ctr., Yorktown Heights, NY 10598; Center for Neuroscience, Rutgers Univ., Newark, NJ 07102; Institut Pasteur, Paris, France.

MacVicar and Tse (*J. Physiol.* 417: 197, 1989) have reported a 4-10 Hz *C*arbachol-*D*riven *P*opulation *O*scillation ("CDPO") in the isolated CA3 region *in vitro*. CDPO is characterized by 1) large synchronized EPSPs in pyramidal cells with superimposed bursts in many cells; 2) decrease in amplitude but unchanged frequency after low dose kynurenic acid; 3) insensitivity to bicuculline and phaclofen. We built a network of 1000 pyramidal cells (19 soma-dendritic compartments, 6 voltage-dependent currents - see Traub, Wong and Miles, *Soc. Neurosci. Abstr* 16: 1297, 1990), 100 inhibitory cells (i-cells) producing $GABA_A$ IPSPs and 100 producing $GABA_B$ IPSPs. This network generates population waves similar to CDPO, provided 1) AHP conductance is reduced; 2) EPSP conductances are reduced; 3) IPSPs are blocked; 4) pyramidal cells are depolarized. These requirements are consistent with known actions of carbachol and with MacVicar and Tse's data. In contrast to CDPO, *in vivo* walking theta is characterized by 1) infrequent firing of pyramidal cells with rare bursting; 2) pyramidal firing leads i-cell firing; 3) short bursts in many i-cells during each theta wave. Our network produces this pattern when, at 5 Hz, pulses of excitation are delivered to a small subset of pyramidal cells, followed by (external) phasic depolarizing-hyperpolarizing stimuli to most or all of the i-cells. The i-cell input is consistent with anatomical evidence for cholinergic and GABAergic synapses on these cells. The physiological timing and actions of these i-cell inputs remains to be confirmed.

411.20

AMPA RECEPTORS ARE INVOLVED IN EXCITATORY RESPONSES OF PREFRONTAL CORTICAL CELLS INDUCED BY STIMULATION OF THE HIPPOCAMPAL FORMATION. T.M. Jay, A.M. Thierry* and J. Glowinski*. Lab. de Neuropharmacologie, INSERM U114, Collège de France, 11, Place Marcelin Berthelot, 75231 Paris Cedex 05, France.

In previous studies we have demonstrated that field CA1 and subiculum project to the prelimbic portion of the rat prefrontal cortex (PFC) and that this direct unilateral pathway is excitatory and shows long-term potentiation (Jay et al., Br. Res. 505: 337, 1989; Laroche et al., Neurosci. Lett., 114: 184, 1990).

It was therefore of interest to determine whether the transmitter of the hippocampo-prefrontal cortex pathway is an excitatory amino acid and whether excitatory responses of PFC neurones induced by the hippocampal (HIP) stimulations are mediated by NMDA or non-NMDA receptors. Extracellular single unit recordings were obtained from neurones in the prelimbic area of the PFC in urethane anesthetized rats and low frequency stimulation (1Hz) was applied in the ipsilateral field CA1 or subiculum. The effects of iontophoretic application of agonists and antagonists of NMDA and non-NMDA receptors on PFC cells showing an excitatory response to the HIP stimulation (n=16; latency=18 ms) were analyzed. In 15 of these cells, the application of either CNQX or DNQX (10-80 nA) which antagonized the AMPA evoked excitation, completely and reversibly blocked the HIP-induced excitatory responses. In contrast, the application of APV (6-50 nA), which antagonized the NMDA but not the AMPA evoked responses, failed to reduce the HIP-induced excitatory responses in the same cells.

These results demonstrate that the excitatory responses of PFC neurones to stimulation of the hippocampus are mediated by AMPA receptors and suggest that the transmitter of the hippocampo-prefrontal cortex pathway is an excitatory amino acid.

HYPOTHALAMUS I

412.1

IMMUNOGOLD CYTOCHEMISTRY FOR OXYTOCIN AT THE ULTRASTRUCTURAL LEVEL: REEVALUATION OF THE PLASTICITY IN THE RAT SUPRAOPTIC NUCLEUS (SON) AFTER CHRONIC DEHYDRATION: F. Marzban, C.D. Tweedle and G.I. Hatton. Dept. of Anatomy, Neuroscience Program and Michigan State University, East Lansing, MI 48824.

Vasopressin (VP) and oxytocin (OX) hormones are synthesized in separate magnocellular neurons (MN) in the supraoptic nucleus. These cells show profound plasticity when hormone secretion is elevated by stimuli such as lactation and dehydration. Plasticity of MN was previously seen using EM-immunogold cytochemistry for VP. Both VP(+) and VP(-) neurons underwent ultrastructural changes after chronic dehydration. Here we studied the changes in the cells of the same animals using OX antibody to confirm our findings. Middle portions of SON of chronically dehydrated (10 days 2% saline treated) and control (normally hydrated with tap water) rats were studied. Glial coverage of either type of MN cell body did not change. Percentage of soma-somatic/dendritic membrane contact significantly increased by 11-fold and 3-fold in both (+)-labelled (OX) and (-)-labelled (most probably VP) cells respectively. Percentage of axo-somatic contact significantly decreased by (36%) in OX(-) cells, possibly due to an increase in cell size rather than to a decrease in synaptic contact, with no change in OX(+) cells. Percentage of soma/soma-dendritic multiple synapses per cell significantly increased by (70%) only with OX(+) cells. Multiple synapses only occurred between the (+)- cells or (-)-cells, but not between (+) and (-) cells. Such changes may help synchronization of OX and VP hormonal secretion under the physiological stimulation. The similarity of our findings compared to VP immunolabelling indicates that our technique is adequate and both types of the cells do show plasticity with chronic dehydration. Supported by NS 09140.

412.2

OBSERVATIONS ON A NEW VESICULAR-LIKE STRUCTURE IN THE AXON TERMINALS OF POSTERIOR PITUITARY OF THE FEMALE RAT. J.E. Smith, P.J. Rusch, L.E. Koran, G.I. Hatton, Neuroscience Prog., Michigan State University, E. Lansing, MI 48824.

Large vesicle-like structures were observed ultrastructurally in the axon terminals of posterior pituitaries of female rats. These vesicle-like structures are either round or ovoid, range in size from 0.5-1.9μm diameter, with an average diameter of approximately 1.3μm (typical peptidergic granules in the posterior pituitary axon terminals of these animals ranged in diameter from 0.1-0.25μm), and there are an average of 2-3 per terminal. They are usually not bounded by membrane, although occasionally small segments of membrane form their borders. These structures appear to contain particulate material of the same density as the small peptidergic vesicles in the terminals. Approximately 50% of terminals containing these large vesicle-like structures abut against pituicytes, while approximately 20% are adjacent to basal lamina and approximately 30% are intermingled among other axon terminals (p<.005, Chi Square). 75% of terminals containing these structures are located in the center of the posterior pituitary and 25% are located around the periphery of the pituitaries (p<.005, Chi Square). Alonso and Assenmacher (Cell Tissue Res, 1981, 219:525) have shown that supraoptic nucleus sends terminals primarily to the center of the posterior pituitary, with scattered projections to the periphery, while paraventricular nucleus projects terminals only to the periphery. To the best of our knowledge this is the first report of these large vesicle-like structures in the posterior pituitary of the female rat. Supported by NS 09140 and Trgr NS 07279.

412.3

RAPID SYNAPSE FORMATION AND NEURONAL/GLIAL PLASTICITY IN RAT SUPRAOPTIC NUCLEUS IN RESPONSE TO A SINGLE HYPERTONIC SALINE INJECTION. G.H. Beagley & G.J. Hatton. Psych. Dept. & Neurosci. Program, Michigan State Univ., E. Lansing, MI 48824.
Neurosecretory neurons of the supraoptic nucleus (SON) manufacture, transport and secrete the neuropeptide hormones oxytocin and vasopressin. These neurons have been shown to be osmotically activated by administration of hypertonic NaCl. We examined, ultrastructurally, changes in several morphological parameters in response to an acutely induced rise in plasma osmotic pressure. Male rats were given intraperitoneal injections of isotonic (0.15M) or hypertonic (1.5M) NaCl solution and sacrificed 5 h after injection. Electron micrographs of the SON of 14 NaCl injected rats (6, 0.15M; 8, 1.5M) were compared to determine morphological differences in the magnocellular neuroendocrine cell (MNC) somata in the two conditions. In the SON cell body zone significant decreases were found in the extent of glial contact with the MNC membrane (0.15M=73%, 1.5M=57%, p<.001); amount of nerve terminal contact with MNC membrane increased (0.15M=10%, 1.5M=13%, p <.01); and amount of apposition of MNC membrane with other MNC cell bodies or dendrites increased (0.15=7%, 1.5=19%,p<.001). Number of multiple synapses (one terminal contacting 2 or more cells) per 100 um of somatic membrane in the 1.5M NaCl condition was double that found in the 0.15M NaCl condition. Both size of nucleoli and overall cell body size were significantly larger in the animals injected with 1.5M NaCl. The 1.5M injection increased the amount of Golgi apparatus from 16% to 28% of the cross sectional profile of MNCs (p<.001). These changes accompany the known increased activation of the MNCs of the SON by intraperitoneal injection of 1.5M NaCl. That these morphological changes all occurred within five hours of the injection indicates a degree of plasticity heretofore unappreciated. Supported by NS 09140 and NS07279.

412.4

OSMOTICALLY INDUCED CHANGES IN NEURONAL/GLIAL RELATIONSHIPS IN RAT NEURAL LOBE ARE DEPENDENT ON SYSTEMIC EPINEPHRINE. G.J. Hatton & G.H. Beagley. Neurosci. Program & Dept. of Psychology, Michigan State Univ., E. Lansing, MI 48824.
Posterior pituitary glial occupation of the basal lamina (BL) decreases and neural occupation increases with stimuli that elevate neuropeptide release into the blood. Since the origin(s) of the catecholamines thought to be involved in the glial movement are unknown, we investigated the possibility that blood borne epinephrine might provide a signal. Three groups of male rats were given intraperitoneal injections of either hypertonic (1.5 M) or normal (0.15 M) NaCl and were sacrificed five hours after the injection. One group was given 1.5 M or 0.15 M NaCl with no additional treatment, one group was anesthetized from the time prior to the injection until perfusion, and one group was bilaterally adrenal-medullectomized (MDX) 7 days prior to the NaCl injections. Morphometry on electron micrographs revealed that 1.5 M NaCl alone produced a significant increase in neural occupation of the BL compared to 0.15 M NaCl (63% and 36%; p<.001). Rats given 1.5 M NaCl under anesthesia showed no change in neural occupation when compared to anesthetized rats given 0.15 M NaCl (38% and 34%). Neural occupation of the BL in MDX animals given 1.5 M NaCl injection was the same as in MDX rats receiving 0.15 M NaCl: 33% and 36%, which was no different from neural occupation shown by either anesthetized group or untreated rats receiving 0.15 M NaCl. We conclude that both anesthesia and adrenal-medullectomy prevented glial retraction under a condition that normally changes its occupation of the BL and that systemic epinephrine is involved. Supported by NS 09140.

412.5

RAPIDLY INDUCED SUPRAOPTIC DENDRITIC SYNAPSES: THE SAME AS THOSE FORMED IN PARTURITION? C. D. Tweedle, K. G. Smithson and G. I. Hatton, Neurosci. Prog., Michigan State University, East Lansing, MI 48824.
The magnocellular neurosecretory cells of the adult rat supraoptic nucleus show dramatic ultrastructural synaptic and astroglial changes under several conditions of increased hormone demand. Parturition (mainly involving oxytocin release) is accompanied by increase both in contact of dendrites by multiple synapses (DMS, one pre-synaptic terminal contacting more than one post-synaptic dendrite) and in direct dendro-dendritic contact ("bundling") in the mother rat. We also found evidence for similar changes in the same parameters with 25 min of transcardial perfusion of etherized male rats with high (325 mOsm) vs. lower (302 mOsm) warmed, oxygenated ACSF (Neurosci. Abstr. 15:1224). Further detailed observations and point intersection analysis of electron micrographs now demonstrate that, with parturition or high osmolality perfusion, the percentage of DMS to total number of dendritic synapses significantly increased with increased bundling. Interestingly, the novel synapses are identical with the two stimuli (symmetrical with clear and dense core granules). The time course of the rapidly formed DMS following perfusion indicate they must form by astroglial withdrawal from between dendrites allowing previously single terminals access to new post-terminal dendritic membrane. (Supported by NS 09140)

412.6

LONG-TERM INHIBITION OF VASOPRESSIN (VP) EXCRETION BY USING CENTRAL INJECTION OF AN IMMUNOCONJUGATE (ANTIBODY TO VP LINKED TO RICIN A CHAIN. A.Burlet, M. Chapleur*, B. Haumont-Pellegri*, F. Jansen**, B. Fernette*, J.P. Nicolas* and C. Burlet*. INSERM U 308, Nancy, **Clin-Midy, Montpellier, France.
A monoclonal antibody (MAb) to vasopressin (VP) was specifically taken up and transported in VP producing neurons, when it was injected near the supraoptic nucleus (SON) of the rat brain. At the same time, the diuresis was increased, urine osmolality and VP excretion decreased whereas the ^{35}S-cysteine incorporation was prevented in the target neurons. The duration and the amplitude of these effects depended on the injection site, VP-MAb isotype and activation of the complement cascade, but did not last more than 24hrs. In order to amplify these effects, an immunoconjugate (IT) constructed with purified VP-MAb (IgG1k isotype) and ricin A chain has bilaterally been injected near SON and different parameters measured in freely moving rats. One injection of IT was less efficient than one injection of an ascites fluid containing IgG2a isotype of VP-MAB to increase diuresis and decrease VP excretion. A long-term inhibition of VP excretion was induced by the addition of monensin and plasma complement to IT. Ten days after the last injection of this mixture, the diuresis was high, urine osmolality reduced and urine content of VP lowered (177±35pg/ml vs 648±108pg/ml). Immunocytochemical observations confirmed dramatic disturbances of VP producing neurons at the level of injected nuclei whereas VP producing neurons of other hypothalamic regions were activated.

412.7

DEVELOPMENT OF THE HYPOTHALAMIC CATECHOLAMINERGIC SYSTEM AND ITS PHARMACOLOGICAL DEPLETION DURING ONTOGENESIS. A. Trembleau*, M. Ugrumov*, J. Bernabé*, A. Calas. Lab. Cytol. IDN-CNRS, Lab. Physiol. Reprod., Univ. P. & M. Curie, Paris, France ; Inst. Develop. Biol., Acad. Sci. Moscow, USSR.
The development of the hypothalamic catecholaminergic (CA) system has been studied in rats from the 13th fetal till the 11th postnatal day with the combination of ^{3}H-thymidine radioautography and TH-immunocytochemistry as well as with HPLC of CA. Moreover, HPLC has been used to evaluate the reaction of the hypothalamic CA system in fetuses and in neonates following the treatment of pregnant rats (from the 13th day of gestation) and of neonates (from the 2nd day of life) with the inhibitors of catecholamine synthesis, either α-methyl-m-tyrosine or α-methyl-p-tyrosine. It has been demonstrated that the hypothalamic CA neurons were mainly originated between the 14th and 16th fetal day, that was followed by the synthesis and accumulation of dopamine. The parallel accumulation of noradrenaline (NA) and in a lesser extent of adrenaline (Ad) apparently showed the ingrowth of the NA and Ad fibers to the hypothalmamus via the medial forebrain bundle. By the end of fetal life, the content of dopamine reached the level observed at the 11th postnatal day. On the contrary, NA increased four folds over the same period. Ad remained at the lowest level in each age group. The long-term treatment of pregnant rats and neonates with the inhibitors of CA synthesis did not change the hypothalamic level of dopamine both in fetuses and neonates. On the contrary, the level of NA decreased more than 50% in fetuses and 70% in neonates. It means that this pharmacological model could be valuable to study the role of noradrenaline in the brain development and particularly in the differentiation of the target neurons. This model is currently used to study the possible role of NA in the differentiation of vasopressin and oxytocin neurons using *in situ* hybridization and northern blot methods.

412.8

A T-TYPE CALCIUM CURRENT IN VASOPRESSIN (AVP) AND OXYTOCIN (OT) NEURONS IN GUINEA PIG SUPRAOPTIC NUCLEUS (SON). K.R. Erickson, O.K. Ronnekleiv and M.J. Kelly. Dept. of Physiology, Oregon Health Sciences University, Portland, OR 97201-3098.
Single-electrode voltage clamp studies were performed in SON neurons with biocytin-filled electrodes. A T-type calcium current was characterized in AVP and OT neurons, which were identified with a double labeling procedure (Ronnekleiv et al., BioTechniques 9:432-438, 1990). The T-type calcium current was TTX-insensitive, and was blocked by 100-500 μM NiCl in a dose-dependent manner. The activation threshold was -64 ± 1 mV (N=9), and peak current occurred within 30-50 ms. The T-type current appeared to be a requisite feature for a depolarizing potential (DP) to occur, which in AVP cells was critical for phasic firing. Hyperpolarizing pre-steps elicited a damped oscillating potential (DOP) following the offset of the hyperpolarizing step in cells exhibiting a T-type calcium current (N=10). Cells without a T-type calcium current exhibited no such oscillations (N=12; χ^2: p=.0001, df=2). Since later components of the DOP resembled the DPs observed in phasically-firing neurons in time course and amplitude, it appeared that the T-type calcium current underlies the DP measured in current clamp, and therefore is necessary for phasic firing to occur in AVP neurons of guinea pig SON. (Supported by PHS grants DA05158 and HD00718).

412.9

CONVERGENCE OF μ-OPIOID AND $GABA_B$ RECEPTORS AT AN INWARDLY RECTIFYING K^+ CONDUCTANCE IN HYPOTHALAMIC NEURONS: MODULATION BY ESTROGEN. M.J. Kelly, M.D. Loose and O.K. Ronnekleiv. Dept. of Physiology, Oregon Health Sciences U., Portland, OR 97201-3098.

The effects on neurons of the arcuate nucleus, including β-endorphin neurons, of the $GABA_B$ agonist baclofen and the μ-opioid agonist Tyr-D-Ala-Gly-MePhe-Gly-ol (DAMGO) were compared in hypothalamic slices from guinea pigs (GP) and rats. Intracellular recordings (3% biocytin) were made in hypothalamic slices prepared from proestrous rats or ovariectomized GP which were treated with estradiol benzoate (EB) or oil for 24 hr. Baclofen hyperpolarized all of the cells (GP:12$\pm$2 mV, N=12; rat:8$\pm$3 mV, N=11). DAMGO hyperpolarized all of the GP (N=6) and 4 of 7 rat neurons. Both baclofen and DAMGO caused a decrease in R_{in}. The baclofen-induced current reversed at $\approx E_{K+}$ and exhibited inward rectification as did the DAMGO-induced current. The response to either baclofen or DAMGO was occluded by a maximal dose of the other agonist. EB treatment caused a rightward shift in the dose-response curve for baclofen which was similar to the 3.4 fold shift in the DAMGO dose-response curve. It appears that μ-opioid and $GABA_B$ receptors open the same inwardly rectifying K^+ channels and that estrogen alters a component (G-protein, channel) that is activated by and common to both of these receptors. (PHS grant DA05158).

412.10

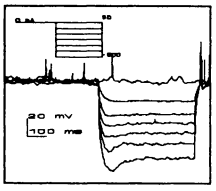
INTRINSIC MEMBRANE CURRENTS IN GUINEA PIG VASOPRESSIN (AVP) NEURONS DICTATING PHASIC FIRING. O.K. Ronnekleiv, K.R. Erickson and M.J. Kelly. Dept. of Physiology, Oregon Health Sciences U., Portland, OR 97201-3098.

Single-electrode voltage clamp studies in AVP-immunoreactive magnocellular neurosecretory cells in the supraoptic nucleus (SON) of guinea pigs revealed that these cells displayed two membrane currents not reported in rat SON, which were associated with phasic firing. The I_h was expressed in a greater percentage of AVP neurons (83%) than OT neurons (40%). The I_h was blocked by 2 mM CsCl (N=24) but not by 100-500 μM $BaCl_2$ (N=8). The time constant of activation followed a single exponential and decreased at more hyperpolarized membrane potentials (700-200 ms). The I_h was modulated by forskolin, an adenylate cyclase activator, which shifted its activation range to more depolarized levels (4 of 6 AVP cells). The I_h appeared to interact with a T-type calcium current by depolarizing the cell into the range of activation of the T-current, which produced a regenerative, depolarizing potential (DP). The DP was associated with phasic firing in guinea pig SON AVP neurons (N=34; χ^2: p=.0004, df=2), and was present only in neurons possessing a T-type calcium current. While both currents were present in oxytocin neurons, these cells did not fire phasically, suggesting that the presence of the I_h and T-type calcium current are necessary but not sufficient for phasic firing to occur. (Supported by PHS DA 05158).

412.11

WHOLE CELL RECORDING OF NEURONS OF THE SUPRA-CHIASMATIC NUCLEI (SCN) STUDIED IN RAT BRAIN SLICE. E.A. Gallman, P.C. Nolan*, T.G. Waldrop and M.U. Gillette. Depts of Cell & Structural Biology and Physiology & Biophysics, University of Illinois, Urbana, IL, 61801.

The SCN in rat are the neuroanatomic substrate for the biological pacemaker underlying circadian rhythmicity. This rhythmicity is expressed *in vitro* in the hypothalamic brain slice preparation as a daily oscillation in overall firing frequency of SCN neurons, with peak activity occurring near the middle of subjective day. The intracellular mechanisms responsible for changes in firing frequency are unknown. As these cells are small (6-15 μm), they have proved hard to study with conventional intracellular techniques, and few such studies exist. We have begun examining these cells using whole cell patch recording in slice. Typically, these cells had resting potentials -40 to -60 mV, after-hyperpolarization (AHP) -5 to -15 mV, and overshooting action potentials of 55 to 85 mV amplitude. Our results also indicate that at least some SCN neurons have an inward rectifier current and many exhibit post-inhibitory rebound (PIR). Support: AFOSR-90-0205 & PHS NS 22155.



BRAINSTEM SYSTEMS

413.1

PATTERNS OF INNERVATION OF LOWER BRAINSTEM AND NODOSE GANGLION CELLS TO THE LIVER AND THE PANCREAS IN NORMAL AND DIABETIC RATS STUDIED BY A DOUBLE LABELING TECHNIQUE. T. Kono, M. Takada, and S. T. Kitai, Dept. of Anatomy and Neurobiology, College of Medicine, University of Tennessee, Memphis, Memphis, TN 38163.

Innervation patterns of the dorsal motor nucleus of the vagus (DMV) and the nucleus ambiguus (NA) to and the nodose ganglion (NG) from the liver and the pancreas were studied by a fluorescent double labeling technique in normal (N=5) and spontaneously diabetic Wistar BB rats (N=10). Under pentobarbital-chloral-hydrate anaesthesia, the cut end of each hepatic and pancreatic nerve was inserted into polyethylene tubings containing either 2μl of 5% True blue (TB) or 3% Diamidino yellow (DY) solution. After four days, animals were anaesthetized (pentobarbital 60 mg/kg) and perfused. The brainstem and NG were sectioned in coronal plane with 40μ thick and labeled neurons were examined under a fluorescent microscope.

In the normal rat, 3-5% of cells were double labeled and 92-95% single labeled in DMV and 40% of cells double labeled and 60% single labeled in NA. In diabetic rats, the number of double labeled neurons remained almost the same in DMV while in NA, they were reduced to only about 6%. In NG, around 40% of the neurons were double labeled in normal while only less than 10% double labeled in diabetic rats. Reduction in double labeled neurons in NA was due to a lack of labeling by pancreatic nerve and in NG due to hepatic nerve. These data indicate that in diabetic rats a strong central control of pancreatic action and sensory inputs on blood sugar from the liver are much reduced. Supported by USPHS grant NS20702.

413.2

COLLATERAL ORGANIZATION OF PARATRIGEMINAL AND SPINAL TRIGEMINAL NUCLEUS PROJECTIONS: A FLUORESCENT RETROGRADE TRACER STUDY IN THE RAT. D.W. Saxon and and D.A. Hopkins. Department of Anatomy, Dalhousie University, Halifax, NS B3H 4H7.

The paratrigeminal islands (PTI) and the marginal layer of the spinal trigeminal nucleus pars caudalis (SpV I) have complex efferent projections to the ipsilateral nucleus of the tractus solitarius (NTS), ipsilateral parabrachial nucleus (PBN) and contralateral ventrobasal complex (VBC) of the thalamus. In order to elucidate the collateral organization of the PTI and SpV I in the rat, fluorescent retrograde tracers (Fluoro-gold, True Blue, Diamidino Yellow and fluorescent latex beads) were injected in combinations into the NTS, PBN and VBC. Injections into the rostral NTS produced labelled neurons throughout the ipsilateral PTI and SpV I. After injections in the VBC labelled neurons were located predominantly in the caudal part of the PTI while after injections in the PBN they were dispersed throughout the PTI and were more numerous . SpV I neurons projecting to the VBC were abundant in the dorsal part of lamina I and tended to be located superficial to those projecting to the PBN. Small numbers of double labelled neurons were found in the PTI and dorsal SpV I following combined injections into the ipsilateral PBN/NTS and VBC/PBN. The topography of afferent projections to and the organization of projections from the PTI suggest that the PTI is distinct from SpV I. The collateralization of the PTI and the known visceral and somatic afferent input to the nucleus support the idea that the PTI plays a role in somatovisceral reflexes. Supported by MRC of Canada, Grant MT-7369.

413.3

PROJECTIONS FROM THE NUCLEUS PARAGIGANTOCELLULARIS IN THE VENTROLATERAL MEDULLA TO THE NEOCORTEX: A DOUBLE-LABELING STUDY IN THE RAT

R. Jaslow*, M. A. L. Nicolelis, R. C.S. Lin, J. K. Chapin and B. D. Waterhouse. Department of Physiology and Biophysics. Hahnemann University, Philadelphia, PA 19102-1192.

The rat paragigantocellularis (PGI) nucleus located in the ventrolateral medulla is known to be a site of convergence for projections arising from nuclei related to the control of autonomic and sensory functions. A broad range of anatomical and physiological studies had suggested that this structure may play a significant role in controlling cardiovascular function, specifically sympathetic tone. Recently, special attention has been drawn to the efferent projections of the PGI since this structure was shown to be one important source of afferents to the locus coeruleus (LC). Here, we provide evidence that the PGI is also a source of direct projections to multiple cortical areas. In this study, adult rats received small injections (0.2-0.4 µl) of two different retrograde fluorescent tracers, fluoro-gold (FG) and rodamine coated microspheres (RCMs), into the frontal and visual cortices respectively. Following a short survival period we observed that in all animals FG injections in the frontal cortex produced bilateral (with ipsilateral predominance) labeling in different nuclei of the ventrolateral medulla, including the PGI. RCMs injections centered in the visual cortex produced less dense labeling in the PGI. Moreover, double-labeled cells were also observed in the PGI, indicating that a small percentage of PGI neurons may give rise to projections to both frontal and visual cortices. These results suggest that PGI neurons have direct access to cortical circuits, providing a possible pathway through which autonomic and sensory information conveyed through this nucleus may influence cortical activity. *Sponsored by grants NS 29161 NS 26722 and FAPESP 88/4044-9.*

413.4

TOPOGRAPHIC ORGANIZATION OF PROJECTIONS FROM THE RAT DORSAL RAPHE TO THE PRINCIPAL NUCLEUS OF V, VPM THALAMUS, AND BARRELFIELD CORTEX. M.L. Kirifides, M.A.L. Nicolelis, R.C.S. Lin and B.D. Waterhouse. Department of Physiology and Biophysics. Hahnemann University, Philadelphia, PA 19102-1192.

Previous studies have demonstrated the existence of a crude topographic organization of the projections from the dorsal raphe nucleus (DRN) to selected target nuclei in the mammalian forebrain. The goal of the present study was to examine the distribution of DRN cells that project to sensory specific structures. In addition, the degree of collateralization of individual DRN cells to multiple sensory modality specific targets was assessed. Adult Long Evans hooded rats (250-350gms) received unilateral injections (.3-.5ul) of fluorescent retrograde tracers (Fluoro gold, rhodamine or fluorescein coated microspheres) into the principal nucleus of V (Vp), ventral posterior medial thalamus (VPM), and the "barrelfield" of the primary somatosensory cortex (BC). Following these injections, coronal sections (80um) through the DRN were examined using fluorescence microscopy. Following injections in VPM and Vp, retrogradely labeled cells were consistently observed in the ventromedial zone of the DRN and to a lesser extent in the dorsomedial region of DRN. Cells projecting to BC were concentrated in the dorsomedial zone of DRN but were also scattered in ventromedial and lateral wing regions of the nucleus. In the sagittal plane, cells labeled from each of the 3 injection sites were found throughout the rostrocaudal extent of the nucleus, with those projecting to VPM more concentrated rostrally and those projecting to Vp more concentrated caudally. Double labeled cells from a paired injection in the VPM and contralateral Vp were observed in the dorsomedial zone of the caudal DRN. In summary, DRN cells that project to different levels of the ascending somatosensory path are distributed within partially overlapping zones of the nucleus. The demonstration of double labeled cells in the DRN suggests that activation of individual dorsal raphe neurons may simultaneously influence efferent targets that are processing the same sensory information. These data are consistent with the notion that DR efferents are organized with respect to the sensory function of target structures. Supported by No. AFOSR-87-0138.

413.5

PROJECTIONS FROM THE DORSAL RAPHE NUCLEUS TO THE PEDUNCULOPONTINE AND LATERODORSAL TEGMENTAL NUCLEI. T.L. Steininger and B.H. Wainer. Dept. of Pharm and Phys. and Comm. on Neurobiology, University of Chicago, Chicago IL 60637.

The pedunculopontine (PPT) and laterodorsal (LDT) tegmental nuclei provide widespread cholinergic innervation of the thalamus, as well as other forebrain and brainstem regions, and are believed to participate in behavioral state control. Previous retrograde tracing studies have demonstrated putative projections to the PPT from the dorsal raphe nucleus (DR) (Steininger and Wainer, '89, Soc. Neurosci. Abstr. 15:452). To confirm DR innervation of the cholinergic PPT and LDT neurons, we have utilized the anterograde transport of *phaseolus vulgaris* leuko-agglutinin from the DR, in combination with choline acetyltransferase immunohistochemistry. Light microscopic examination revealed anterogradely-labeled fibers in the tegmentum. In the PPT and LDT, thin varicose fibers formed numerous pericellular contacts on soma and proximal dendrites of the cholinergic neurons. Innervation of the PPT was particularly denser in the pars compacta than in medial diffuse regions. Similarly, labeling in the LDT was denser in lateral regions. In future studies, putative contacts will be examined at the ultrastructural level to ultimately confirm synaptic innervation. Since the serotonergic DR has been implicated in sleep/wake control as well, the demonstrated projection from the DR to the PPT and LDT may serve as the anatomical substrate for behavioral state changes. (Supported by NS 17661 and MH 09919)

413.6

Origin and function of spiral fiber input to the axon cap of the goldfish Mauthner cell. J.W. Scott[1], N.P. Beatty[2], S.J. Zottoli[2], and H. Korn[3]. Dept. Anatomy, Emory Univ., Atlanta, GA[1], Dept. Biology, Williams College, Williamstown, MA[2], Institut Pasteur, Paris, France[3].

The axon cap is a specialized region enclosing the Mauthner cell (M-cell) axon initial segment. Interneurons entering its periphery inhibit the M-cell by a "field effect" followed by conventional synaptic transmission. Other axons enter the central portion of the cap and spiral around the M-cell initial segment. Some of these axons make a morphologically mixed synapse, but their function is unknown.

HRP placed in the axon cap filled spiral fibers whose somata (about 15µm in diameter) lay contralaterally at the level of the Vth motor nucleus, 300-780µm rostral to the M-cell and 300-540µm lateral to the midline, their dendrites extending ventrally. Electrical stimulation near these somata produces a short latency intracellular M-cell depolarization, which can bring the cell to threshold, with an associated extracellular negativity that is maximal in the axon cap.

Supported by NSF grant BNS 8809445 (S.J.Z.) and an NIH-CNRS Fogerty fellowship (J.W.S.).

413.7

MORPHOLOGICAL PROPERTIES OF NUCLEUS TRACTUS SOLITARIUS NEURONS RECEIVING SENSORY INPUTS FROM UPPER AIRWAY RECEPTORS. R.D. Sweazey. School of Dentistry, University of Michigan, Ann Arbor, MI 48109.

Morphological characteristics of lamb nucleus tractus solitarius (NTS) neurons receiving taste and tactile information from the epiglottis and aryepiglottal folds were investigated using electrophysiological mapping, Golgi impregnations and immunohistochemical techniques. Areas of the NTS responsive to chemical or mechanical stimulation of upper airway receptors occupied about one-third of the total superior laryngeal nerve termination zone in the NTS. Within this NTS area was a region of chemical and tactile sensitivity surrounded by a region of tactile sensitivity. Large multipolar neurons with 3-10 primary dendrites predominate in areas of tactile sensitivity. Smaller multipolar neurons with 3-5 primary dendrites and elongate neurons having 2-3 primary dendrites predominate in NTS areas which responded to both chemical and mechanical stimulation of upper airway receptors. Within this latter region preliminary results suggest that GABA is primarily associated with elongate neuron while aspartate is most often localized in multipolar neurons. These results suggest that NTS neurons processing upper airway sensory information have morphological properties similar to neurons located in rostral NTS areas which process information from oral receptors. Supported by N.I.H. Grant DC00735.

413.8

PEPTIDE CONTAINING NEURONS WITHIN THE NUCLEUS OF THE SOLITARY TRACT OF THE PIGEON. M.L. Berk and L.A. Mullins*. Dept. of Anatomy, Marshall Univ. Sch. of Med., Huntington, WV 25755.

The distribution of neurotensin (NT), leu-enkephalin (LENK), cholecystokinin (CCK), and substance P (SP) containing neurons within the nucleus of the solitary tract (NTS) was determined immunocytochemically in colchicine treated pigeons. Each of these peptides has a unique distribution within the various NTS subnuclei. Many cell bodies containing LENK and NT were observed within the anterior parts of subnuclei medialis dorsalis and medialis intermedius. Fewer cell bodies containing LENK were also found within the posterior parts of subnuclei medialis dorsalis, medialis intermedius, medialis ventralis, lateralis dorsalis, and the caudal most NTS (posterior to the obex). Most of the CCK containing cell bodies were found at the level of the obex in subnucleus medialis superficialis and the adjacent caudal subdivision of NTS. Most of the SP containing cell bodies were located within all anterior-posterior levels of NTS subnucleus medialis ventralis. The peptide containing neurons in these NTS subnuclei may function as interneurons or as long ascending projection neurons in the transmission of visceral sensory information. The anterior divisions of subnuclei medialis dorsalis and medialis intermedius, which contain NT and LENK neurons, are the primary recipients of esophageal afferent fibers (Katz and Karten, '83). These LENK and NT cells probably have local connections based on previous tracing studies. The CCK containing cells in the caudal NTS and subnucleus medialis superficialis pars posterior may have ascending projections to the forebrain and parabrachium (Berk, '87; Arends et al., '88). The SP neurons in the posterior part of subnucleus medialis ventralis may also have ascending projections. Double-labelling experiments are in progress. Supported in part by grants NSF R11-8922106 and NIH RR05870.

413.9

DISCHARGE PROPERTIES OF MEDIAN RAPHE NEURONS IN FREELY-MOVING RATS DURING SLEEP-WAKING STATES. R.P. Vertes and B. Kocsis. Center for Complex Systems, Florida Atlantic Univ., Boca Raton, FL 33431

We previously demonstrated that median raphe (MR) stimulation desynchronizes the hippocampal EEG, and MR lesions have been shown to produce continuously-running theta independent of behavior. We propose that the MR may control the hippocampal desynchronization of quiet waking and slow-wave sleep (SWS). Previous reports in cats have shown that serotonergic MR neurons fire at low rates in all behavioral states (i.e., < 2-3 spikes/sec) and at progressively lower rates from waking (W) to SWS to REM sleep, while nonserotonergic MR neurons discharge at high rates in waking (W) (19-20 spikes/sec) lower rates in SWS (4-5 spikes/sec) and relatively high rates in REM sleep (9-10 spikes/sec).

Adult male rats were permanently implanted with electrodes for recording the cortical and hippocampal EEG, the neck EMG and single unit activity in the MR during sleep-waking states. To date we have sampled the activity of 18 MR neurons; 17 of the 18 cells exhibited discharge characteristics of nonserotonergic MR neurons. The majority of non-5-HT cells fired at very high spontaneous rates during the waking state (i.e., > 30 spikes/sec). Of the non-5-HT cells analyzed across all behavioral states, three populations emerged: (1) cells that fired at approximately equivalent rates in W and REM (21.7) and low rates in SWS (11.3); (2) cells that maintained high rates in W (18.9), very low rates in REM (1.05) and low rates also in SWS (2.4); and (3) cells that discharged at high rates in SWS (29) and low rates in both W and REM (5.1). All of the above values in spikes/sec. The third type of cell may be involved in controlling states of hippocampal desynchronization.

Research supported by NIMH Grant 45075.

413.10

RESPIRATORY NEURONS IN THE CAUDAL MEDULLA OF THE FROG, *RANA CATESBEIANA*. K. Kogo, S.F. Perry* and J.E. Remmers, Dept. of Medical Physiology, Univ. of Calgary, AB, Canada T2N 4N1.

To better understand the neural mechanisms underlying the coordinated cyclic movements of buccal and lung ventilation in the frog, we have begun classifying in the caudal medulla "respiratory neurons", the activity of which correlated with this rhythmic activity. Decerebrated, unanesthetized, paralyzed bullfrogs were used. We recorded the motor output from cranial nerves (CN) V, X and XII as well as the intra- or extracellular activity of respiratory neurons. In addition, the response of these neurons to stimulation of CNIX and X was investigated.

We found respiratory neurons, including motoneurons and non-antidromically activated neurons, from the area in and around the motor nuclei of CNIX, X and XII. Neurons which were active during both buccal elevation (Be) and lung ventilation (L), and others active during both buccal depression (Bd) and during the phase immediately preceding lung ventilation (preL) were designated Be&L and Bd&preL neurons, respectively. Motoneurons which projected to the main branch of CNXII were of the Be&L type, whereas, those projecting to the sternohyoid branch of CNXII were Bd&preL neurons. Some neurons were active (L+), or inactive (L-) only during the L phase. Motoneurons projecting to the laryngeal branch of CNX were either L+ or L-. In addition, we demonstrated Bd-Be neurons which were active during the transition between phases, and a special type of L+ neuron with cardiac modulation. Some of the above neurons could be excited by stimulation of CNIX and X. (Supported by PBF Foundation)

413.11

SYNAPTIC POTENTIALS IN MESOPONTINE CHOLINERGIC NEURONS EVOKED BY LOCAL STIMULATION *IN VITRO*. C.S. Leonard and R. Sanchez, Center for Neural Science, NYU 6 Wash. Pl. NY, NY 10003.

The synaptic activation of pedunculopontine (PPT) and laterodorsal tegmental (LDT) neurons was studied with intracellular recording methods in a guinea pig brain slice preparation. Brain slices were cut in the coronal plane and local stimulation was delivered through an electrode placed at the midline or in the central grey ipsilateral to the recording side. Cholinergic neurons were identified by combined intracellular injection of biocytin and histochemical staining for NADPH-diaphorase. Constant current pulses evoked a sequence of graded psps consisting of a fast epsp followed by a fast ipsp and slow ipsp. The epsp could be large enough to evoke an orthodromic spike and displayed both facilitation and post-tetanic potentiation with stimulus trains (100Hz for 0.3-1S). In normal Ringer (3mM Mg) the epsp was completely abolished by 10uM DNQX suggesting that a major component is mediated by non-NMDA receptors. Blocking the epsp uncovered the fast ipsp which reversed near -75mV and was blocked by picrotoxin suggesting activation of $GABA_a$ receptors. Trains of stimuli also produced facilitation and potentiation of the fast ipsp. The slow ipsp fluctuated considerably following single stimuli. Trains of stimuli enhanced the slow ipsp which could be evoked by stimulus trains at strengths below those necessary for fast psps. The slow ipsp was insensitive to picrotoxin and in the presence of DNQX reversed at more hyperpolarized potentials than the fast ipsp suggesting the slow ipsp was mediated by activation of a K conductance. Since conditions were found to evoke each of these potentials independently, it is likely that mesopontine cholinergic neurons receive projections from both rapid signalling and modulatory systems.

413.12

VOLTAGE-DEPENDENT MEMBRANE CURRENTS IN ACUTELY DISSOCIATED BRAIN STEM NEURONS OF RATS, C.Jiang, T.R. Cummins and G.G. Haddad, Section of Respiratory Medicine, Dept. of Pediatrics, Yale Univ. Sch. of Med., New Haven, CT 06510

In order to elucidate fundamental mechanisms underlying the neural control of cardio-respiratory function, it is essential to identify the inherent membrane properties of brain stem neurons. To study these properties in isolation, neurons from the hypoglossal (XII) nucleus (n=45) and the dorsal motor nucleus of vagus (DMX, n=105) were freshly dissociated separately and studied under whole-cell voltage clamp. Using specific blockers, reversal potentials and ion substitutions, several membrane currents were characterized. XII neurons showed only one TTX-sensitive Na^+ current and two outward currents: one sensitive to TEA (40 mM) and the other to Cs^+ (1 mM), suggesting that there are a delayed rectifier (K_V) and an inward rectifier (K_H) in XII neurons. In contrast to XII neurons, DMX neurons had two inward currents. TTX (3 μM) and Na^+ removal decreased but did not abolish the inward current. Addition of Co^{++} (1-2 mM), however, eliminated all the remaining inward current, indicating that both Na^+ and Ca^{++} (high threshold) currents are present in DMX cells. Two transient outward currents were observed in DMX neurons. One was 4AP-sensitive and the other was Co^{++} but not apamin sensitive. The remaining outward current was blocked by extracellular TEA (40 mM) and Cs^+ (1 mM) suggesting that K_A, K_{Ca}, K_V and K_H exist in DMX cells. We conclude that 1) XII and DMX neurons have different membrane currents and these currents underlie their different repetitive firing properties and 2) these results substantiate our previous data in slices since the same voltage-dependent conductances were also observed in isolated neurons.

413.13

RESPONSES OF RAPHESPINAL NEURONS TO VESTIBULAR NERVE STIMULATION IN THE CAT. B.J. Yates, T. Goto* and P. Bolton. Lab. Neurophysiology, The Rockefeller Univ., New York, NY 10021

Although vestibulosympathetic reflexes have long been known to exist, and to be potentially important in compensating for orthostatic hypotension [Doba and Reis, Circ. Res., 1974], little is known about the neural pathways which relay vestibular signals to sympathetic preganglionic neurons. The caudal medullary raphe nuclei are possible mediators of these reflexes, as considerable evidence suggests they contain many neurons which project to the thoracic intermediolateral cell column [Morrison and Gebber, J. Neurophysiol., 1985]. We tested whether neurons in raphe pallidus and raphe magnus of the decerebrate cat that projected to the thoracic spinal cord responded to electric stimulation of the vestibular nerve. Twenty of 28 raphespinal neurons with conduction velocities between 1 and 4 m/s received vestibular inputs; 13 of the 20 were inhibited, and 7 were excited. Raphe neurons which make synapses upon sympathetic preganglionic neurons have similar slow conduction velocities [Morrison and Gebber, 1985]. In addition, 34 of 42 raphespinal neurons with more rapid conduction velocities (6-78 m/s) also received vestibular signals; 26 were excited, and 8 were inhibited. Raphespinal neurons with conduction velocities > 5 m/s are known to terminate in both the dorsal (laminae I, II, V and X) and ventral horn (lamina VII) of the spinal cord [Light, J. Comp. Neurol., 1985] and therefore are potentially involved in modulating excitability in both sensory and motor pathways.

Slowly-conducting raphespinal neurons thus appear to be a possible route through which vestibular signals can reach sympathetic preganglionic neurons. In addition, rapidly-conducting raphespinal neurons may be in part responsible for vestibular-elicited effects on sensory and motor pathways in the spinal cord.

Supported by NIH grants DC-00693 and NS-02619.

413.14

FOS-LIKE IMMUNOREACTIVITY (FLI) IS INDUCED IN NEURONS OF THE MEDULLA OBLONGATA AFTER STIMULATION OF THE CAROTID SINUS NERVE (CSN) IN AWAKE OR ANESTHETIZED RATS. J.T. Erickson and D.E. Millhorn (*University of North Carolina, Chapel Hill, NC*)

Expression of the protooncogene c-fos has been used as a metabolic marker for tracing polysynaptic neuronal pathways after discrete central or peripheral stimulation (Science 237:192.1987). In this study, CSN afferent fibers were stimulated either electrically or via hypoxia in anesthetized or awake rats. After a suitable recovery period, the medulla was perfused, sectioned, and processed immunohistochemically to detect FLI. A discrete distribution of Fos-labeled cells was observed within nucleus tractus solitarius, area postrema, ventrolateral medulla, nucleus raphe pallidus, and superficially on the ventral medullary surface. We believe these cells represent higher order neurons within the baro- and chemoreceptor reflex pathways. FLI in surgically prepared but unstimulated animals and in animals breathing a normoxic gas mixture was substantially reduced compared to stimulated animals. Studies using immunohistochemical double labeling techniques suggest that some of these functionally activated cells colocalize serotonin or tyrosine hydroxylase, the rate-limiting enzyme for catecholamine biosynthesis. (HL 33831 and AHA 88108).

413.15

BEHAVIORAL AND BIOCHEMICAL STUDIES ON THE ROLE OF GABA-B RECEPTORS IN THE MEDIAN RAPHE NUCLEUS. D. Wirtshafter, T.R. Stratford, M. Pitzer & J.C. Krebs. Dept. Psychology, Univ. Ill. at Chicago, Box 4348, Chicago, Il 60680.

We have shown that injections of the GABA-A agonist muscimol into the median raphe nucleus (MR) produce hyperactivity, a reduction of hippocampal serotonin metabolism and an acceleration of dopamine metabolism in the nucleus accumbens. We examined here whether similar effects could be produced by stimulation of GABA-B receptors. Microinjections of 62.5-500 ng of the GABA-B agonist baclofen into the MR resulted in dose dependent hyperactivity which was immediate in onset. Injections into the the adjacent dorsal raphe nucleus or the ventral tegmental area resulted in smaller, delayed, increases. The baclofen effect was stereospecific and could be eliminated by coinjection of the GABA-B antagonist 2-hydroxy-saclofen. These findings support the anatomical and pharmacological specificity of the baclofen effect. Baclofen produced only small effects on hippocampal serotonin metabolism, but resulted in a large increase in accumbens dopamine metabolism.

The current findings suggest that GABA-B receptors play a functional role within the MR.

413.16

EFFECT OF GABA ON NEURON TYPES IN GUSTATORY ZONE OF RAT NUCLEUS TRACTUS SOLITARIUS USING WHOLE CELL RECORDINGS IN BRAIN SLICES. R. M. Bradley, S. Boucher and Wang Limei*. School of Dentistry, University of Michigan, Ann Arbor, MI 48109-1078.

We have used whole cell recordings in brain slices to characterize the biophysical and pharmacological properties of neurons in the gustatory zone of the nucleus tractus solitarius (NTS). Three neurons types have been characterized using a current injection paradigm which hyperpolarized and then immediately depolarized the neuron. The hyperpolarizing pulse resulted in an irregular pattern of action potentials initiated by depolarization in Type I neurons. In Type II neurons the hyperpolarizing pulse either delayed the initiation of the first action potential or resulted in a long first interspike interval in the spike train initiated by the depolarizing pulse. The hyperpolarizing pulse had no influence on the spike train initiated by depolarization of Type III neurons. When GABA (1 mM) was superfused over the slices, the resting membrane potential of all three types of neuron was hyperpolarized. This was accompanied by a fall in membrane resistance and marked reduction in excitability. These results demonstrate that GABA alters membrane properties of all three types of neurons in gustatory NTS. Supported by N.I.H. Grant DC000288.

413.17

NEURONS RECORDED EXTRACELLULARLY FROM KOELLIKER-FUSE AND PARABRACHIAL REGIONS OF A RAT IN VITRO BRAINSTEM PREPARATION. V.M. Tronnier*, P.C. Rinaldi and R.F. Young. Div. of Neurological Surgery, University of California, Irvine, CA 92717.

As part of ongoing work to study the role of the Koelliker-Fuse (KF) nucleus in pain modulation a slice preparation was developed to define cellular characteristics of KF and parabrachial (PB) neurons and to confirm anatomically described projections to the trigeminal system. Extracellular recordings of the activity of PB and KF neurons were carried out in rat brainstem coronal sections (400 uM). To aid in visualization of anatomic landmarks fast green was initially added to the recording chamber with the slice in static bath conditions. Once appropriate locations for recordings were determined, the slice was maintained and recordings obtained in submerged conditions. Standard extracellular techniques with both pipettes and tungsten microelectrodes were used and signals were recorded digitally for later off-line computer analysis. Stimulation was accomplished with a 50 uM twisted Nichrome wire bipolar electrode. Both spontaneous and evoked unit activity was monitored.

To date, 20 cells have been examined. 15 of these were localized in KF, 3 in the external medial parabrachial and 2 in the external lateral parabrachial subnuclei. In 8 of the cells amplitudes ranged between 20 and 35 uV and in 12 cells amplitudes ranged from 10 to 15 uV. According to frequency pattern, 4 cell types could be distinguished: 4 showed a constant discharge frequency of 10/sec; 6 of 1-3/sec; 6 of 6-8/sec; and 3 cells showed rhythmic patterns of firing. Interestingly, evoked activity could be found in only 2 of 20 cells after stimulation of the motor trigeminal nucleus. Stimulation of principal sensory trigeminal neurons had no influence on the spontaneous activity of KF or PB cells although connections between these structures have been described.

413.18

NICOTINIC PROLONGATION OF HALOTHANE ANESTHESIA BY THE INTERPEDUNCULAR NUCLEUS. R. Wojcik, K. Abate, M. Andresen* and I. D. Hentall. Univ. of Ill. Coll. of Med., Rockford, Illinois, USA 61107.

The metabolic rate of the interpeduncular nucleus (IPN) has been shown to be higher in anesthetized than awake animals. This phenomenon is associated with an increased release of acetylcholine (ACh) (see accompanying abstract). The effect of increased cholinergic activity on the state of anesthesia is thus an important question.

Through a chronically implanted guide cannula, nicotine (2 µl 10^{-5} to 10^{-1} M) or saline was microinjected near the IPN of 300 g female rats (n=20) immediately after discontinuation of 10 min of 3% halothane anesthesia. Fast green dye marks allowed histological verification of injection sites. A dose-dependent prolongation of the time taken to recover righting reflexes was seen; mean prolongation for 10^{-2} M nicotine was 65%. The number of breaths taken by the rat before recovery was similarly increased. The duration of anesthesia was shortened 17% by microinjection of the nicotinic antagonist hexamethonium (2 µl 10^{-1} M, n=10). The nicotinic antagonist mecamylamine (2mg/kg) given intramuscularly prior to anesthesia blocked the effect of 10^{-2} M nicotine (n=10).

These data suggest that ACh release from the IPN contributes to the maintenance of anesthesia.

(Supported by NINDS grant 26116.)

413.19

ACETYLCHOLINE RELEASE IN THE INTERPEDUNCULAR NUCLEUS IS MODULATED BY SUBSTANCE P, PENTOBARBITAL, KETAMINE, AND HALOTHANE. K. Abate, R. Wojcik, K. Taguchi, M. Andresen*, T. Shibuya, Y. Suzuki* and I. D. Hentall. Univ. of Ill. Coll. of Med., Rockford, Illinois, USA 61107.

Many afferents to the interpeduncular nucleus (IPN) contain acetylcholine or substance P. Since the IPN has an unusually high metabolic rate during anesthesia, we tested how the release of ACh is affected by diverse anesthetic agents and by substance P.

Microdialysis tubes were stereotaxically implanted in the rat's IPN, and the extracted ACh was measured by HPLC. In pentobarbital-anesthetized acute preparations, mean [ACh] was 1.32 ± 0.73 pmol (15 min samples of 25 µl). This level was not affected significantly by electrical stimulation (3 Hz, 0.2 ms, 100 µA for 15 min) of the fasciculus retroflexus, which is a major input pathway to the IPN. In chronic awake rats, mean [ACh] was 0.62 ± 0.27 pmol. This value subsequently increased 298% by 100 mg/kg i.m. ketamine (n=3), 212% by 50 mg/kg i.m. pentobarbital (n=2), and 230% by inhaled 3% halothane (n=2). The presence of substance P in the dialysis tube's inflow reduced ACh release in awake animals (20 ± 5% for 10^{-5}M).

We conclude that more ACh is released from the IPN in anesthetized than awake rats, and that substance P directly or indirectly inhibits this release.

(Supported by NINDS grant 26116.)

414.1

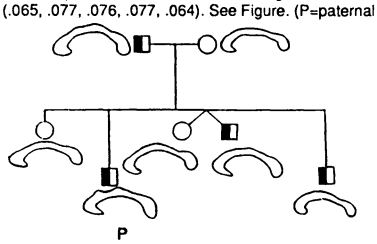
PRENATAL RISK AND CORPUS CALLOSUM DEVELOPMENT IN LANGUAGE AND LEARNING IMPAIRED CHILDREN. P.E. Cowell, T.L. Jernigan, P.A. Tallal and V.H. Denenberg. Biobehavioral Sciences Graduate Program, Univ. of Connecticut, Storrs, CT 06269, Dept. Psychiatry, UCSD, La Jolla, CA 92093 and Center for Molecular and Behavioral Neurosciences, Rutgers Univ., Newark, NJ 07102.

Jernigan et al. (Arch. Neurol., 1991) have shown that regional cortical volume asymmetry patterns differ between language and learning impaired (LLI) children and age matched controls. Since LLI children had substantial aberrations in cortical anatomy, regional differences in callosal (CC) size were investigated. Subjects were 20 LLI males and females (ages 8-10 years). Prenatal risk scores were also available. Callosal drawings were rendered from midsagittal MRIs and digitized using the computer-aided program Stereology. Parameters obtained included callosal area, axis length, perimeter, and 99 widths. The widths were divided into seven clusters defined by previous factor analysis and consisted of averaged widths 3 through 18 (W3-18), W22-39, W49-62, W65-74, W77-85, W89-94, W95-99. Analyses compared LLI subjects on the basis of high vs. low Prenatal risk defined by a median split of scores. In an ANOVA including all Widths, Prenatal risk was highly significant. Overall, high risk children had smaller CCs than low risk children. There was also a marginally significant Sex x Prenatal x Width interaction indicating that the pattern of affected CC regions was not identical for males and females. In addition, there were significant correlations between Prenatal risk score and CC parameters. Sensitivity of this fiber tract to Prenatal risk factors appears to be an important characteristic of LLI children.

414.2

INHERITANCE OF CORPUS CALLOSUM SIZE AND SHAPE IN FAMILIES WITH DEVELOPMENTAL DYSLEXIA.. A.Kushch*, R.Duara, B.Jallad*, H.Lubs*, M.Rabin*,B.Levin*. Univ. of Miami Sch. of Med., Miami, FL 33101.

As part of a three generation familial dyslexia study, 63 members from 8 different families were scanned using magnetic resonance imaging. The corpus callosum and mid-sagittal brain areas were traced in T-1 weighted mid-sagittal slices. Planimetric measures were taken of the genu, splenium, total corpus callosum, and mid-sagittal brain areas. One family showed an inheritance pattern in the total normalized corpus callosal area that was suggestive of autosomal dominant inheritance: with the father and one son having a larger corpus callosal size (.096, .086) and the mother and remaining 4 children having average sizes (.065, .077, .076, .077, .064). See Figure. (P=paternal size)



The corpus callosal size did not distinguish between dyslexics and non-dyslexics in this family. Currently, sib-pair analysis on both corpus callosal angulation and area measurements are in progress on the overall sample.

414.3

ANATOMY OF THE PLANUM TEMPORALE IN RELATION TO SIDE, HANDEDNESS AND SEX S.F. Witelson and D.L. Kigar*, Dept. of Psychiatry, McMaster Univ., Hamilton, Ont., Canada, L8N 3Z5

To assess the relationship of neuroanatomical and functional asymmetries, the planum temporale (PT), part of the posterior language region, was measured in 67 postmortem brains from 24 ♂ and 43 ♀ whose hand preference [consistent-right-handed (CRH) and nonCRH] was tested prior to death. PT was defined as the extent of the dorsal surface of the superior temporal gyrus from Heschl's main transverse sulcus to the end of the Sylvian fissure (SF), and a vertical (V) segment was measured in addition to a horizontal (H) segment when the SF bifurcated in the posterior region. (1) Area of H was greater in the left (L) hemisphere; area of V was greater, to the same extent, in the right (R), making overall size of PT equal in the two hemispheres, although morphologically asymmetrical. (2) Area of H varied with handedness and sex; in contrast, area of V did not differ among the 4 hand-sex subgroups. H was larger in CRH than nonCRH and tended to be larger in ♂. (3) A laterality score for H (L-R/L+R) showed that among ♂, nonCRH had a greater anatomical asymmetry than CRH; among ♀ there was no difference between hand groups. (4) The greater asymmetry in nonCRH men was due to a smaller H in the R hemisphere compared to CRH men; there was no difference between the L hemispheres. To sum, these results indicate that there is marked L-R asymmetry in PT; that PT anatomy is related to hand preference and, by inference, to the pattern of functional asymmetry; but that this relation of structure to function holds only for ♂, not ♀ (as found for the anatomy of the corpus callosum in relation to handedness, Witelson & Goldsmith, 1991). Supported by U.S. NINDS.

414.4

RADIAL ARM MAZE MEASURES DEVELOPMENT OF SPATIAL MEMORY IN YOUNG CHILDREN. A. Peuster*, W.H. Overman, S. Caulfield*, R. Hakan. Psychology Dept., UNC-Wilmington, Wilmington, N.C. 28403.

The radial arm maze (RAM) has been used to measure spatial memory and hippocampal function in animals; however, it has rarely been used with humans. In this study, children (20-151 mo.) and adults searched for food rewards in a 8-arm RAM. Experimental conditions were: (1) one day of search in 8 open, baited arms followed by (2) 15 days of forced-choice search. In condition 2, four of the arms were closed and the subject searched the four open arms; then, after a delay (30-120 sec.) all arms were opened and the subject searched for the remaining baited arms. In condition one, there was a developmental progression: youngest children (20-40 mo.) performed at chance levels and older children (approx. 100 mo.) and adults performed at near perfect levels. In condition 2 adults significantly improved (trials 5-8) over the first 4 days of forced choice testing, but the youngest children failed to improve (trials 5-8) in 15 days of testing. However, since all subjects performed nearly perfectly on trials 1-4 of forced choice testing, this indicates that even the youngest children can remember at least four locations for a short period of time.

414.5

USE OF AN ODDITY TASK IN CHILDREN TO ANALYZE THE SOLUTION OF DELAYED NON-MATCH TO SAMPLE (DNMS). W. H. Overman, M. Miller*, K. Moore*, W. Kilpatrick*, C. Rogers* Psychology Department, UNC-Wilmington, Wilmington, N.C. 28403

The DNMS task has been used to measure hippocampal function in animals and man. Infant monkeys (Bachevalier and Mishkin, Beh. Neurosci. 98, 1984) and infant humans (Overman, Ann. N.Y. Acad. Sci.608,1990) are impaired in learning a DNMS task which has both a recognition memory and rule learning component. The trial-unique, simultaneous oddity task has a rule similar to DNMS but requires no recognition memory. Children (16-74 mo.) and adults were tested longitudinally and non-verbally on oddity. Oddity required significantly more trials to learn than DNMS, suggesting that impaired rule learning played a major role in the previous findings with DNMS. Subjects who failed to learn simultaneous oddity were then tested on two-part oddity (which was learned quickly), followed by retesting on simultaneous oddity (which was not learned quickly). Given the ABA design, the results suggest that two solutions are available for two-part oddity: win-shift and rule-learning (go to "odd").By inference, DNMS may be learned by infants on the basis of "win-shift", not on the basis of a rule "go to novel".

414.6

MEMORY SKILLS IN YOUNG SUBJECTS WITH CEREBELLAR TUMORS. J.A. Lazareff* and E. Castro-Sierra. Dept. of Neurosurgery and Lab. of Psychoacoustic Hospital Infantil de Mexico. 06720 Mexico, D.F. MEXICO.

Children (N=4; avg. age=10:7) with tumors of different etiologies of the cerebellum were studied for their abilities for carrying out visually- or auditorily-presented instructions and for naming and remembering visual images. These studies were undertaken before and after surgical treatment of the tumors by ablation. Results obtained were compared with those from children (N=2; avg. age=10:6) with cerebral tumors of different etiologies, both before and after ablation, and with those from normal children (N=2; avg. age=10:11). Subjects in the cerebellar tumor group manifested a distinct impairment for remembering and carrying out auditorily-presented instructions after surgical intervention. This impairment was especially noticeable in one female subject with an astrocitoma of the cerebellar vermis who suffered from total aphasia for 2 months after surgery. The abilities of the different groups for carrying out visually-presented instructions were at a par among all the groups tested. The results suggest the existence of auditory processing in cerebellar structures.

414.7

IMPAIRED MEMORY CAUSED BY HERPES SIMPLEX VIRUS INFECTION.
D.R. Beers*[1], J.S. Henkel*[2], W.G. Stroop*[2], S.F. Basinger[2]. [1]Program of Neuroscience, University of Utah, Salt Lake City, UT 84112; [2]Department of Ophthalmology, Baylor College Medicine, Houston, TX 77030.

Deficits of impaired intellectual function associated with herpes simplex encephalitis (HSE) have not been extensively investigated. Survivors of HSE experience intellectual impairment and an inability to store and recall information. These deficits are postulated to correlate with the stereotypic post mortem temporal lobe necrosis seen in this disease. Because olfactory and limbic system structures are central to storage and retrieval of memories, injury to these areas is believed to cause behavioral and learning disabilities. Therefore, an animal model is needed to explore the correlation between memory impairment and temporal lobe pathology. We have developed a rat model that mimics the physical and behavioral aspects of human HSE and does not involve invasive delivery of virus to the brain. Fifty-one percent of rats shed virus from their nose but rarely from their eyes following intranasal inoculation with 1.4 X 10^6 $TCID_{50}$ of syncytial forming herpes simplex virus type-1. Similar to HSE patients, these animals developed behavioral abnormalities and motor seizures. Memory dysfunctions were evaluated using a radial arm maze test to quantitate the acquisition of information of a learned task. Infected rats (N=8) performed at chance levels which was statistically different than controls (N=8). At different times post infection, rats showed histopathologic changes within the amygdala, hippocampus, and the entorhinal cortex. Viral antigens were also detected within these structures by immunohistochemistry. This model will allow neuroanatomic correlation between memory dysfunction with site-specific HSV replication, and provide a system to test compounds with potential antiviral or cognitive enhancing properties. *Supported by the Department of Veteran Affairs.*

LEARNING AND MEMORY—PHYSIOLOGY IV

415.1

LONG TERM RETROGRADE AMNESIA ON INHIBITORY AVOIDANCE AND CONDITIONED TASTE AVERSION LEARNING TASKS BY INSULAR CORTEX LESION. C.E. Ormsby*, A.L. Piña and F. Bermúdez Rattoni. Instituto de Fisiología Celular, UNAM. México, D.F. México. 04510

Previous works have described deficits in acquisition and consolidation of inhibitory avoidance (IA), spatial learning, and acquisition of conditioned taste aversion (CTA) produced by pre- and post-training reversible lesions of the insular cortex (ICx) (Bermúdez-Rattoni et al. PNAS. In press). Male Wistar rats were tested to observe the effects of ICx lesions on long-term memory of IA and CTA tasks. Twenty-six animals were trained in CTA and 48 hr later in IA, then assigned to 3 subgroups: one group (LxG) received bilateral electrolytic lesions of the ICx 48 hr after the IA training to allow consolidation, another, was sham operated the same day (ShG) and the third group served as control (CtlG). All animals were tested four days later for IA, and 11 days later for CTA. Results showed that the lesion of LxG significantly impaired IA, assessed by response latency and time spent in either compartment when compared with both the ShG and CtlG groups. Similarly, the CTA test showed significant impairment of the LxG in the recall of the aversive reaction to saccharin. These results confirm that ICx is necessary for the storage of long-term memory of previously learned avoidance conditionings.

415.2

Temporally graded retrograde and anterograde amnesia following ibotenic entorhinal cortex lesion in mice.
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The purpose of the present experiment was to study **retrograde amnesia** in mice by using a **spatial discrimination task** performed in 2 different 8-arm radial mazes. Animals learned successively, one at a time, and every 2 weeks, 5 pairs of adjacent arms (2 in the first maze and 3 in the second one). Bilateral ibotenic acid lesion of the entorhinal cortex (E. C.) was performed 1 hour after subjects reached the criterion on the 5th pair.

As compared to sham-operated animals, E.C. lesioned mice were severely impaired at remembering the 2 last pairs learned within **2 weeks before surgery** but had normal performance on those learned beyond this time period (**Retrograde Amnesia**).

Animals were trained on the second maze until they reattained the criterion on the each of 3 pairs learned before surgery. They were also trained on the 4th pair not previously used. Experimental subjects learned this 4th pair at the same rate as controls but when tested 2 weeks later, a significant forgetting was observed on the 3 pairs relearned post-operatively (**Anterograde Amnesia**).

These results confirm previous findings (Zola-Morgan and Squire, Science, 250, 288-290, 1990) and suggest that the hippocampal formation is required for **only a limited period of time after learning**. Beyond this critical period, normal performance would be supported by extra-hippocampal structures, thus suggesting a "**translocation**" of the internal representation. In addition, as shown by additionnal behavioral data, it seems that a **change in the code** of its representation might occured. This research was supported by Centre National de la Recherche Scientifique and by the Fondation pour la Recherche médicale Française.

415.3

INTRACELLULAR RECORDINGS FROM NEURONS IN THE FRONTAL CORTEX OF RATS PERFORMING LICKING CONDITIONED TO A LIGHT CUE. M.P. Kristensen and C.D. Woody. UCLA Medical Center, Los Angeles, CA 90024.

Male Sprague-Dawley rats were trained to lick for water when prompted by a light cue. Intracellular as well as extracellular recordings were obtained from neurons in the frontal cortex of animals performing the conditioned behavior in response to the light stimulus. The electrophysiological characteristics of the neurons were investigated with regard to discharge pattern for all cells and furthermore resting potentials and input resistances for intracellularly studied units, and the correlation of these parameters with the conditioned stimulus, the behavioral response or the reinforcement was analyzed.

Neurons showing increased discharge during and preceding licking were found. Intracellular recording disclosed depolarization of the baseline prior to the onset of licking and hyperpolarization at the cessation of licking.

(Supported by HD05958)

415.4

INSTRUMENTAL CONDITIONING FOLLOWING SELECTIVE LESIONS OF THE NUCLEUS ACCUMBENS. B.W.Balleine* and A.S.Killcross* (SPON: Brain Research Association). Dept of Experimental Psychology, University of Cambridge, Cambridge, CB2 3EB, UK.

Several investigators have suggested that the nucleus accumbens mediates the effects of the instrumental reinforcer and, as such, is involved in the control of instrumental performance. This hypothesis was assessed by examining the effects of bilateral ibotenic acid lesions (0.06M; 0.7 µL) of the nucleus accumbens on the instrumental performance of hungry rats in a free operant situation. In support of this proposal it was found that lever press responding for a food reward on a variable interval (VI) schedule was significantly impaired in lesioned animals relative to sham controls. Two potential accounts for this deficit were then examined, one associative and the other motivational. The associative account assumes that the lesion interferes with learning the response-reinforcer contingency. However, shifting reinforcer delivery from a contingent VI schedule to a noncontingent variable time (VT) schedule was found to produce a comparable decline in performance over trials in both the lesion and sham groups, suggesting that the lesion did not affect sensitivity to the instrumental contingency. Secondly, motivation to respond was assessed by shifts in primary motivation achieved by varying the number of hours of food deprivation prior to test sessions. This manipulation induced deprivation dependent changes in performance which were, again, similar in both the lesion and sham groups - indicating that the deficit in performance in lesioned animals was also not due to a change in the motivational significance of the instrumental reinforcer. These findings support the hypothesis that the nucleus accumbens is involved in instrumental conditioning but suggest that this involvement is not derived from a sensitivity to the instrumental contingency or the motivational significance of the reinforcer.

415.5

ACTIVATION OF THE VENTRAL TEGMENTAL AREA AFFECTS HIPPOCAMPAL EEG. K.B. Austin, Department of Psychology, McGill University, Montreal, Canada, H3A 1B1.

Dopamine (DA) neurons in the ventral tegmental area (VTA) project to the basal forebrain, and are known to mediate brain stimulation reward. The septal nuclei receive input from the VTA and also strongly influence the electrical activity of the hippocampal formation. In intact rats, lesions of the medial septum will abolish hippocampal theta rhythm and disrupt performance on spatial memory tasks. In addition, infusions of DA into the medial septum of chloral hydrate anesthetized rats will result in the generation of hippocampal theta rhythm. In freely-moving rat preparations, VTA activation using the GABA antagonist picrotoxin (PTX) produces compelled, stereotypical locomotion which is accompanied by hippocampal theta rhythm. To dissociate the behavior from the EEG changes, we recorded hippocampal EEG from chloral hydrate anesthetized rats and used local injections of PTX to activate the VTA. Intra-VTA PTX (15 ng in 0.5 uL) injections produced in an increase in EEG synchrony. FFT analysis revealed an increase in EEG power in the 3-5 Hz frequency band. This theta rhythm could be abolished by increasing the anesthetic infusion rate. Subsequent applications of PTX (up to 300 ng) could not reestabish theta, however, numerous sharp-wave complexes were observed at higher doses of PTX. This data demonstrates that the hippocampal EEG can be modulated by activation of the VTA. The VTA may play an important role in the generation of hippocampal theta and, thus, this experiment represents an important step in determining the interaction between reward and memory systems in the brain.

415.6

SUBJECTIVE REWARD MAGNITUDE AS A FUNCTION OF TRAIN DURATION AND STIMULATION STRENGTH

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We measured the subjective reward magnitude of electrical brain stimulation as a function of train duration and stimulation strength (where strength is defined as current x pulse frequency), using the matching law in two different ways. In the first method (trade-off method), the relative magnitudes of the rewards received from two different levers was estimated by the magnitude of the difference in the mean interreward intervals required to offset the difference in stimulation parameters. When the difference in mean interreward intervals offset the difference in reward magnitudes, the rat allocated its time equally between the levers. In the second method (direct method), the ratio of the reward magnitudes at the two levers was assumed to be directly given by the ratio of the rat's time allocations (after correction for side bias). The results of the two methods agree. They show that: i) Reward magnitude grows steeply up to train durations of about 1 s, then saturates (stops increasing). ii) The stronger the stimulation, the steeper the growth and the higher the asymptote. iii) The saturating duration is approximately the same at different strengths of stimulation. [Supported by NSF Grant BNS 89-96246.]

415.7

Subjective Reward Magnitude as a Function of Stimulation Strength Varies Within Rats as a Function of Site of Stimulation.

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Gallistel and Leon (in press) measured the subjective magnitude of a brain stimulation reward by manipulating the mean interreward interval on one lever and determining the current or pulse frequency required on a second lever to produce equal preference between the levers. At equipreference, the difference in the subjective magnitudes of the two rewards compensates for the difference in the mean interreward intervals. They found that subjective reward magnitude increases approximately as a power function of the strength of stimulation (current x pulse frequency), but the power varied from 2 to 10 between subjects and/or sites of stimulation (subjects and sites were confounded). We have now determined subjective reward magnitude as a function of stimulation strength for electrodes in the ventral tegmental area and right and left lateral hypothalamus in the same rats. We find that the exponent of the power function varies between stimulation sites within a rat, suggesting that the subjective scaling of reward magnitude is a characteristic of individual anatomical sites. [Supported by NSF Grant BNS 89-96246.]

415.8

HABITUATION OF THE ACOUSTIC STARTLE RESPONSE IS NOT FREQUENCY SPECIFIC. W. P. Jordan, L. H. Poore*, J. M. Clayton*, and J. Belcher*, Psychology, St. Mary's College of Maryland, St. Mary's City, MD 20686.

The acoustic startle reflex is mediated by a four synapse reflex circuit in the caudal brainstem. Short-term habituation of startle probably occurs within this reflex arc, but long-term habituation (LTH) relies on an accruing inhibition of the reflex by brain areas extrinsic to the reflex arc. Damage to the midbrain reticular formation either before or after habituation training severely attenuates LTH, placing the site of plasticity underlying LTH outside of the reflex arc. It is unknown whether this inhibition is activated by each stimulus presentation (phasic) or whether it chronically damps the startle reflex. A chronic mechanism likely would be unable to support frequency specificity of LTH. A phasic LTH mechanism poses no such restrictions.

Fifteen male rats received 10 trials per day for 8 days of either a 10k-Hz or a 22-kHz pure tone stimulus (97dB SPL, 400ms). Animals habituated equally well to either stimulus across days. When the stimulus was switched to the other frequency on Day 9 or Day 10, no recovery was found. A subsequent same/difference test of the two frequencies under varied interstimulus intervals revealed no specificity for short-term habituation.

The lack of frequency specificity does not rule out either a chronic or a phasic mechanism for LTH.

415.9

DISCRIMINATIVE CONDITIONING OF ORIENTING HEAD MOVEMENTS IN CATS. M. Penttonen* and T. Korhonen, Dept. of Psychology, Univ. of Jyväskylä, P.O. Box 35, SF-40351 Jyväskylä, Finland.

Habituation of orienting responses to auditory stimuli has been studied quite extensively. The purpose of this study was to develop a paradigm for studying the neural mechanisms of orientation. Miniature loudspeakers were attached at a distance of 2 cm from both ears in cats. During a habituation session two tones of different pitches were presented randomly either to the left or right ear. The cats oriented with head turns towards the tones at 10 trials before the habituation. Discriminative conditioning began on next day and lasted for 5 days. CS+ tone was randomly presented to the left or right ear and was paired with the medial forebrain bundle stimulation. Left or right ear CS- tones were not paired with the stimulation. The cats learned discrimination between these two tones: they turned their head significantly more to the CS+ than CS- and increased responding to the CS+ over sessions and maintained the responding at an asymptotic level during last 2 sessions. Evoked responses recorded from the cingulate cortex also showed a differentiation between discriminative stimuli. It is concluded that with this paradigm it is possible to modify orienting responses and maintain these responses at a constant level so that the analysis of underlying neural systems becomes feasible.

415.10

EFFECTS OF REPEATED MEASURES AND STIMULUS INTERRUPTIONS ON INDUCING PERSISTING HINDLIMB FLEXION (SPINAL FIXATION) IN RATS. M. J. Bartelt, E.S. Johnson*, and M.M. Patterson, Dept. of Psychology and College of Osteopathic Medicine, Ohio Univ., Athens OH 45701.

Steinmetz *et. al.*, (JCPP, 1981) demonstrated that persisting hindlimb flexion may be induced by 40 minutes of stimulation (2-4 mA) to a spinalized rat's hindlimb. The present study investigated the effect of repeated asymmetry measurements (within individual animals) and interruptions of the inducing stimulus on the fixation process. If feasible, a repeated measures design would require fewer animals.

Fifty rats were anesthetized (Nembutal 50 mg/kg i.p.), spinalized at T7, randomly assigned to one of five groups, and stimulated (3.5 mA, 100-pps, 7msec repetitive dc pulses) on the right hindlimb. Immediately following each stimulation we assessed the amount of weight needed to remove the persisting hindlimb flexion. Group 1 was assessed at 20, 30, and 40 minutes; group 2 at 20, 25, 30, 35, and 40 minutes; group 3 had the stimulus terminated for 2 minutes at 20 and 30 minutes and was assessed at 40 minutes; the control groups 4 and 5 were assessed only at 30 and 40 minutes.

We found the following: 1) both the 5 and 10 minute repeated measures groups displayed significantly less flexion than the corresponding control groups, 2) the repeated measures group displayed significantly less flexion than the stimulus interruption group, 3) the variances of the repeated measures groups were greater than the variances of the corresponding control groups or the stimulus interruption group.

The repeated measures study of the persistent spinal reflex alteration may show potential but the greater variability induced makes it's use questionable. From the present study a number of questions arose regarding the lessening of the flexion; for example, what mechanism lowered the persisting flexion, muscular or neural? Further investigations utilizing neural preparations are required to address these questions. Supported by American Osteopathic Association grant 90-08-319.

415.11

INHIBITION OF PERSISTING RAT HINDLIMB FLEXION (SPINAL FIXATION) BY CONTRALATERAL HINDLIMB STIMULATION. M.M. Patterson, M.J. Bartelt, and E.S. Johnson*, Dept. of Psychology and College of Osteopathic Medicine, Ohio Univ., Athens, OH 45701.

Steinmetz *et. al.*, (JCPP,1981) demonstrated that persisting hindlimb flexion may be induced by applying 40 minutes of stimulation (2-4 mA) to a spinalized rat's hindlimb. We explored the possibility that the spinal reflex alteration producing persisting hindlimb flexion could be inhibited by sensory input from the contralateral hindlimb.

Twenty-four rats were anesthetized with Nembutal (50 mg/kg i.p.) and spinalized at T7. Twelve animals were randomly assigned to each of the experimental and control conditions and all then received stimulation (3.5 mA, 100-pps, 7msec repetitive dc pulses) to the right hindlimb for 40 minutes. The amount of weight needed to remove the ensuing persisting hindlimb flexion was then measured. The experimental animals' then received stimulation to the left hindlimb for 40 minutes while control animals received no stimulation. Weight necessary to remove persisting flexion of the right hindlimb was then again measured in both groups.

The results indicated that the contralateral stimulation appeared to lower the persisting flexion by a small (2.1 grams) but consistent and significant ($p< 0.01$) amount. This finding suggested that the spinal fixation produced by the stimulation could be altered at least slightly by presumably the crossed extensor reflex pathways from stimulation of the opposite limb. The results suggest that there may be some possibility of reversing the persisting effects of altered reflex excitabilities by utilizing opposing inputs. Support: Am Osteo Assn 90-08-319

415.12

DOSE-RESPONSE EFFECTS OF d-AMPHETAMINE ON PERSISTING HINDLIMB FLEXION (SPINAL FIXATION) IN RATS. E.S. Johnson*, M.J. Bartelt, D.A. Johnson and M.M. Patterson, Dept. of Psychology & College of Osteopathic Med., Ohio Univ., Athens, OH 45701.

Steinmetz *et. al.*, (JCPP,1981) demonstrated that persisting hindlimb flexion may be induced by applying 2-4 mA of electrical stimulation for at least 40 minutes to the hindlimb of spinalized rats. In 1989 and 1990 Bartelt *et.al.(Soc. Neuro. Abs.)* found that d-amphetamine significantly increased the persisting hindlimb flexion. This study examined the effects of various doses of d-amphetamine on persisting hindlimb flexion.

Forty rats were anesthetized with Nembutal (50 mg/kg i.p.) and randomly assigned to one of four groups receiving either 0.0, 2.0, 5.0 or 10.0 mg/kg i.p. of d-amphetamine. The d-amphetamine was injected 15 min. prior to stimulation and the rats were subsequently spinalized at T7. Immediately following 40 minutes of 3.5 mA. (100 pps) hindlimb stimulation, we assessed the amount of weight needed to remove the persisting hindlimb flexion.

The results showed an increase in hindlimb flexion which generally increased with increasing dosage. This outcome supports Bartelt *et.al.*, 1989, and suggests that d-amphetamine effects may be maximal at 10.0 mg/kg. However, it may be that greater doses of the d-amphetamine accentuate the flexion even more but doses greater than 10.0 mg/kg were not used due to the difficulty of maintaining anesthesia. This work further supports involvement of catacholamine in the persisting alteration of spinal reflexes. Further studies will evaluate specific centrally acting NE and DA agonist effects on persisting postural asymmetry. Supported by American Osteopathic Association grant 90-08-319.

415.13

SYNAPTIC PLASTICITY IN THE ELECTROSENSORY LOBE MAY MEDIATE A MODIFIABLE COROLLARY DISCHARGE IN MORMYRID FISH. C. Bell, A. Caputi, K. Grant, and J. Serrier*. R.S. Dow Neurol. Sciences Inst., Portland, OR 97209 and Lab. de Physiol. Nerv., C.N.R.S., Gif sur Yvette, France 91190.

The mormyrid electrosensory lobe may be a useful site for studying the mechanisms and roles of synaptic plasticity. Corollary discharge signals in the lobe are associated with the electric organ discharge (EOD) motor command and are modified by pairing the command with sensory stimuli. The purpose of this study was to determine whether the plasticity takes place within the lobe.

Single cells were recorded intracellularly in the ampullary region of the electrosensory lobe. Intracellularly injected current pulses (instead of sensory stimuli) were paired with the EOD command. Clear changes in the synaptic effect of the corollary discharge were observed following pairing with depolarizing current pulses. Such pulses evoked both short (1 msec) and long (15 msec) duration spikes. The short duration spikes are presumed to be axonal or somatic and the long duration spikes are presumed to be dendritic. The long duration spikes appeared to be critical for plastic change but the short duration spikes did not. Only the short duration spikes are probably propagated down the axon to other cells.

Thus, modifications in the synaptic effect of the corollary discharge appear to result from pairing with postsynaptic events that are restricted to the cells of the electrosensory lobe. Supported by NSF Grant BNS-8919627.

415.14

CHRONIC FLUMAZENIL (RO 15-1788) FACILITATES HABITUATION TO NOVEL ENVIRONMENT AND ANTAGONIZES SCOPOLAMINE INTERFERENCE WITH ACQUISITION OF PASSIVE AVOIDANCE BEHAVIOR. T.J. Marczynski and M. Urbancic*, Dept. of Pharmacol. Univ. of Illinois Coll. of Med., Chicago, IL. 60612.

Adult rats were treated with flumazenil (FL; 4 mg/kg/day in drinking water) or drug vehicle (0.5% water solution of ethylen glycol). On day 12 of treatment, there was no effect on locomotor activity, but pairs of rats (n=5) spent more time in active, non-aggressive, social interactions, as scored in 1 min intervals ($p<.008$) than the control pairs (n=6). When retested 24 hrs later in the same and now familiar environment, the control group matched the behavioral scores of the FL group. However, the introduction of a 3 min lasting "white" noise inhibited social interactions in the control group, relative to the pre-noise condition ($p<.008$), while the FL-treated rats, after initial inhibition, resumed their interactions, which was higher than in the control pairs ($p<.007$). In another group of animals, on day 22 of FL treatment, the drug protected the animals against amnesic action of scopolamine HBr (0.4 mg/kg s.c.) injected 15 min prior to tests for acquisition of behavior avoiding a dark compartment, in which, every 5 sec, the animal received a mild 0.85 mA electric shock. The FL group (n=11) needed less shocks ($p<.03$) and less often re-entered the dark compartment (1.0±0.6 SE) than the controls (2.5±0.6; n=11; $p<.03$). FL had no effect on retention of avoidance behavior, as tested 24 hrs later. Since habituation to novel stimuli (noise) depends on cognitive functions and muscarinic cholinergic mechanisms (Carlton, Prog. Brain Res. 28: 1968, 48), and since scopolamine has amnesic actions, these results indicate that chronic FL increases the tone of the cholinergic systems, by uncoupling the postsynaptic GABA-A receptors from the benzodiazepine recognition sites (Urbancic & Marczynski, Europ. J. Pharmacol. 171:1989, 1-7) that control these systems. Supported by USAF grant 87-0364.

415.15

RELATIONSHIPS BETWEEN CARDIAC RESPONSE VARIABLES, URINARY CATECHOLAMINES AND MEASURED INTELLIGENCE IN EIGHT-YEAR-OLD CHILDREN. C. J. McCallister, N.-S. Jiang*, N. H. McArthur, W. R. Nash* and C. R. Reynolds*. Depts. of Educational Psychology and Veterinary Anatomy, Texas A&M University, College Station, TX 77843 and Dept. of Laboratory Medicine, Mayo Clinic, Rochester, MN 55905.

As part of a larger study, 24 eight-year-old subjects (12 males, 12 females) were administered a standardized intelligence test, the Kaufman Assessment Battery for Children (K-ABC). Height, weight, blood pressure and heart rate were measured immediately before testing. Catecholamines were measured by high pressure liquid chromatography in one 24-hr unstressed sample at least two days before or after the testing and these data were normalized using creatinine values. Diastolic (DBP) and systolic blood pressure (SBP) and heart rate (HR) were all significantly different by gender, as were norepinephrine and epinephrine. Dopamine, age, IQ, height and weight were not significantly different by gender although weight approached significance. Correlations between SBP and IQ for both boys and girls were negative ($r=-0.60$, $p<.03$, $r=-0.59$, $p<.04$ respectively). DBP correlated negatively with IQ for boys ($r=-0.67$, $p<.01$) and positively for girls ($r=0.48$, $p<.10$). No significant correlations were found between any urinary catechols in the 24-hr unstressed sample and IQ although epinephrine exhibited a trend toward significance for girls only. No significant correlations were found between any urinary catechols in the 24-hr unstressed sample and cardiac response variables although HR and norepinephrine showed a trend toward significance for girls only. Exploratory data analyses using stepwise regression revealed that blood pressure was the best predictor of test performance for both boys and girls; further regression analyses will be utilized following the completion of the larger sample (n=100).

415.16

DEVELOPMENT OF A BRAIN-COMPUTER PARALLEL INTERFACE. D.J. McFarland, G.W. Neat, and J.R. Wolpaw, Wadsworth Labs, New York State Department of Health and State University of New York, Albany, NY 12201.

Individuals can learn to modify the amplitude of the 9-12 Hz mu rhythm in the EEG recorded over primary sensorimotor cortex in order to move a cursor quickly and accurately to a target located at the top or bottom of a video screen (Wolpaw et al, Electroenceph clin Neurophysiol 78:252-259, 1991). We are now exploring development of two-dimensional control.

Bipolar EEG is recorded from the scalp over central sulcus of each hemisphere. Right and left mu rhythm amplitudes are assessed 5 times/sec by fast Fourier transform. A cursor appears in the middle of the screen and a target appears in one corner. The cursor moves in two dimensions as a function of the right and left mu rhythm amplitudes. The individual's task is to move the cursor to the target.

Initial results suggest that individuals can develop two-dimensional control and use it to reach targets with greater accuracy than accounted for by one-dimensional control.

An EEG-based brain-computer interface may provide a significant new communication and control channel for severely disabled individuals.

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415.17

A COMPUTER-BASED ANALYSIS OF PAVLOVIAN BEHAVIOR USING DORSAL MIDBRAIN STIMULATION AS THE UNCONDITIONAL STIMULUS. M.G. Laubach, R.C. Gonzalez*, and E. Thomas. Depts. of Biology & Psychology, Bryn Mawr College, Bryn Mawr, PA 19010

We have developed a method for the direct quantification of conditioned behavior. A CCD camera reports the position of an LED above the animal's head every 0.1s. Movement, defined as a change in LED position, was determined during pre-CS, CS, US, and post-CS periods in a light/tone discrimination paradigm, using Sprague-Dawley rats. "Freezing" was defined as less movement during the CS than during the pre-CS period. Electrical stimulation (EBS) of the dorsal midbrain (DM) was the US. The UR was movement: jumping forward or sideways, circling. Animals consistently "froze" on CS+ trials and were active on CS- trials. The difference between CS+ and CS- period behavior was significant. The difference between pre-CS activity and activity during the CS+ was significant from 0. These results, together with a measure of EBS efficacy in a conflict paradigm, suggest that DM EBS is an effective stimulus for aversive Pavlovian conditioning. The method will be expanded with a second camera and will quantify the different qualitative aspects of conditioned responding, currently assessed by judgement using video-tapes.

MOTIVATION AND EMOTION III

416.1

NEUROLEPTICS BLOCK HIGH DOSE, BUT NOT LOW DOSE, HEROIN CONDITIONED PLACE PREFERENCES. K. Nader, A. Bechara, F. Harrington* and D. van der Kooy. Dept. of Anatomy, University of Toronto, M5S 1A8.

We hypothesize that two separate motivational systems in the brain underlie the rewarding effects of opiates, one that functions in drug naive rats and the other that dominates in drug dependent animals. These two systems can be double dissociated by lesions of the brainstem tegmental pedunculopontine nucleus (which block conditioned place preferences for environments paired with opiates only in drug naive rats) and by neuroleptic induced dopamine blockade (which abolishes conditioned place preferences for opiates only in drug dependent animals). Recently, we found that the neuroleptic pimozide (0.5 mg/kg i.p.) blocked the conditioned place preferences produced by 500µg/kg(s.c.) heroin in drug naive rats, but had no effect on similar preferences produced by a 50µg/kg (s.c.) dose of heroin . Are four place conditioning trials with 500µg/kg sufficient to produce a dependent motivational state? We independently tested for dependence induced motivational effects of withdrawal in rats receiving only four 500µg/kg injections of heroin using a modified place conditioning procedure. Rats received four exposures to only one of the two place conditioning environments, an environment which was paired with the absence of opiates 23 hours after the animals' most recent heroin injection . When tested in a drug free state by providing free access to both environments, dependent rats show an avoidance of the withdrawal paired environment, while naive rats show no significant conditioned preference or aversion to the environment paired with the absence of opiates. Rats given four 500µg/kg heroin injections showed significant avoidance of the environment paired with the absence of heroin, however no such avoidance was seen in rats given four 50µg/kg heroin injections or one 500µg/kg injection. We conclude that after only four 500µg/kg heroin injections animals are in a dependent state and therefore, as our model predicts, their preferences were subject to neuroleptic blockade. Animals trained with one 500µg/kg or four 50µg/kg heroin doses are non dependent and their preferences were therefore immune to neuroleptic challenge. These findings further support a two system model of the motivational properties of opiates.

416.2

Neuroleptics Block The Aversiveness Of Withdrawal In Opiate Dependent Rats, But Not The Aversiveness Of Naloxone In Drug Naive Rats. A.Bechara and D.van der Kooy. Neurobiology Research Group, Dept. of Anatomy, Univ. of Toronto, Toronto,Ont.,Canada,M5S 1A8.

Morphine produces rewarding effects (as assessed by place conditioning) in drug naive animals,that are blocked by bilateral lesions of the tegmental pedunculopontine nucleus (TPP) of the brainstem, but not by dopamine blockade (i.e. neuroleptics). However, in drug dependent animals (chronically treated with morphine, a total of 60 mg/kg/day for a minimum of 14 days), similar doses of morphine produce rewarding effects that are blocked by neuroleptics, but not by TPP lesions. These results double dissociate two motivational processes mediating the rewarding effects of opiates. Just as the activation of opiate receptors in drug naive versus drug dependent animals produce fundamentally different mechanisms of reward, we now report that reduced activity on these opiate receptors produce fundamentally different mechanisms of aversion. Morphine dependent, but 16 hour abstinent, rats were injected with saline vehicle and then placed in a distinct environment. When later tested in a drug free and non withdrawan state, these rats avoided the environments previously paired with opiate withdrawal (i.e. saline). These conditioned place aversions were blocked by the neuroleptic alpha-flupenthixol (0.8 mg/kg i.p.) administered to animals prior to withdrawal conditioning. Morphine naive animals that were place conditioned with the opiate antagonist naloxone (10 mg/kg s.c.) avoided the places previously paired with naloxone. However,these conditioned place aversions were not blocked by neuroleptic treatment. Although spontaneous withdrawal in dependent animals and naloxone effects in drug naive animals both reflect an attenuated opioid action on opiate receptors, the motivational mechanisms underlying these two types of aversive effects may be fundamentally different. The important neurobiological fracture line in motivational processes may not lie between reward and aversion, but between the drug dependent and drug naive states.

416.3

HALOPERIDOL, QUININE ADULTERATION, AND EXTINCTION PRODUCE ELEVATIONS IN THE FORCE EXERTED DURING OPERANT RESPONDING BY RATS

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Previous studies have shown that animals responding in a food-rewarded paradigm exert increased force in the presence of neuroleptic drugs. Is the neuroleptic-induced elevation in emitted force a result of reward attenuation or, as others have suggested, a motoric-postural phenomenon? In this study, 3 groups of rats were trained to press a force-sensing operandum for 2.5 sec access to sweetened condensed milk. Testing consisted of two 5 min sessions separated by a 50 min intertrial interval. When animals responded during the second session under conditions of 0.075 mg/kg haloperidol, no reward, or quinine-adulterated (0.025%) reinforcer, all experimental animals emitted significantly greater levels of peak force than controls. These preliminary results suggest that neuroleptic-induced increases in peak force may reflect reductions in the rewarding quality of food reinforcement.

416.4

EFFECTS OF HALOPERIDOL ON PROGRESSIVE-RATIO PERFORMANCE MAINTAINED BY LOW AND HIGH LEVELS OF SUCROSE REWARD. P. Skjoldager, P. Pierre* and G. Mittleman. Department of Psychology, Memphis State University, Memphis, TN 38152.

To determine whether haloperidol effects the reinforcing efficacy of sucrose reward apart from its effects on motoric function, Long Evans rats were reinforced with either a low (1 pellet) or high (3 pellet) magnitude of sucrose reward under an arithmetic progressive-ratio 5 schedule of reinforcement. Sessions terminated when subjects failed to respond for 5 minutes. Baseline performance of subjects in the low-reward condition was characterized by lower break points (the value of the last reinforced ratio), longer pauses following reinforcer delivery (postreinforcement pause), lower running rates (response rate exclusive of postreinforcement pause), and longer operant response durations than subjects in the high-reward condition. Haloperidol (0.0, 0.04, 0.08, 0.16, 0.32 mg/kg, injected ip 45 min before an experimental session) differentially affected performance depending on the magnitude of the reward maintaining behavior. Subjects in the low-reward condition evidenced a dose-dependent decrease in break point, whereas subjects in the high-reward condition had higher break points at the 0.04 mg/kg haloperidol dose followed by a steep monotonic decrease in break point as haloperidol dose increased. Surprisingly, haloperidol did not effect other performance measures including postreinforcement-pause time, running rates, or operant response durations. These results suggest that haloperidol attenuates the reinforcing effects of sucrose reward without affecting response measures that are believed to be largely motoric in nature.

416.5

IBOTENIC ACID LESION OF THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS PRODUCES OVERWEIGHT AND MODULATES THE PREFERENCE THRESHOLDS FOR SACCHARIN.

K.Touzani*, B. Cardo and L. Velley - Lab. Neurosciences comportementales et cognitives, CNRS URA 339, Univ. Bordeaux I, Av. des Facultés 33405 Talence France.

In previous studies we showed that bilateral ibotenic acid (IBO) lesion of the lateral hypothalamus (LH) in the rat results in an increase of gustatory preference-aversion thresholds for saccharin, associated with body weight and water intake deficits.

In the present experiment, we studied the preference for saccharin in rats after lesions of the hypothalamic paraventricular nucleus (PVH), which are known to produce certain changes (namely hyperphagia and obesity) opposite to those produced by LH lesion.

Bilateral injection of IBO into the PVH destroyed the parvicellular cells but not the magnocellular cells of the nucleus. As compared with the normal weight gain of the sham-lesioned, lesioned rats exhibited a significantly higher increase in body weight from the third day after surgery. The lesioned rats also showed decreased preference thresholds for saccharin. These findings together with the results obtained after LH lesions indicate that some gustatory signals are controlled in an antagonistic fashion by the LH and the PVH. This control may depend, at least in part, on descending LH and PVH inputs to the first two gustatory relay-stations, the nucleus of the solitary tract and the parabrachial area.

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416.6

LESIONS OF LATERAL HYPOTHALAMIC NEURONES ALTER THE EFFECTS OF MORPHINE INJECTED INTO THE PARABRACHIAL AREA ON PREFERENCE FOR SACCHARIN

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In previous studies, we showed that ibotenic acid lesion of the lateral hypothalamic neurones (LH) alters the effects of peripherally-administered morphine on rats' preference for saccharin.(Touzani & Velley, Pharmacol. Biochem. Behav., 36, 585, 1990). Furthermore, we identified the gustatory relay, the parabrachial area (PBA) as one of the central sites of the effect of morphine (Akarid et al., Soc. Nsci Abstr., 16, 600, 1990).

In the present study we sought to determine whether lesions of the LH could also modulate the effects of morphine injected into the PBA. During the preference tests, lesioned and sham-lesioned rats were placed either on ad libitum or restricted access to water. Increasing doses of morphine (50 ng to 800 ng) were bilaterally injected into the PBA 15 min before the preference test.

In parallel with our initial studies, we observed that (1) morphine injected into the PBA increased or decreased saccharin preference depending on sweetener concentration; (2) lesioned rats exhibited altered preference response in the presence of morphine. Furthermore, the effects of the LH lesion were stronger when rats were placed on restricted access to water.

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416.7

EXCITOTOXIC LESIONS OF THE RAT LATERAL HYPOTHALAMUS ENHANCE ACQUISITION OF SCHEDULE-INDUCED POLYDIPSIA.

G. Parker*, J. Clark*, A. Clark*, and P. Winn. Dept. Psychology, Univ. St Andrews, Fife KY16 9JU, Scotland.

Schedule-induced polydipsia (SIP) is seen when hungry animals are exposed to the intermittent presentation of food. Electrolytic lesions of the LH abolish SIP. In the present experiment, the ability of rats bearing fiber-sparing NMDA lesions of the LH to acquire SIP was examined. Following surgery, 11 rats lesioned with 1.0ul 0.06M NMDA lost weight and were hypophagic and hypodipsic compared to 6 sham lesioned rats. None of the lesioned rats required wet mash or intragastric intubation. LH-lesioned rats showed an impairment in drinking over 1 h following i.p. injection of hypertonic saline. For SIP testing all rats were placed on a food restriction regime to reduce body weight to 85% of normal. At the start of this regime the LH rats had a lower body weight than controls, but this was not statistically significant. Throughout the 21 days of SIP testing the LH rats were mildly (but significantly) hypodipsic in the home cage. SIP was examined in 60 min sessions every other day; one 45mg food pellet was delivered every 60 sec, while water was available ad lib via a burette protruding into the operant chamber. There were significant differences between the groups over the first 6 sessions (groups $F(1,14)=5.10$ $p<0.04$; interaction $F(5,70)=2.34$ $p<0.05$), LH lesioned rats acquiring SIP more quickly than controls. These data show that there is no motor impairment in LH lesioned rats; and that the LH may be involved in higher-order processes relating to the control of motivated behavior.

416.8

WATER DEPRIVED RATS REMAIN THIRSTY DURING DOPAMINE RECEPTOR BLOCKADE.

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In order to determine whether dopamine receptor blockade would attenuate thirst, the drinking behavior of rats under 23, 16, 12, 4, and 0 hr water-deprivation was compared to that of 23-hr deprived animals under the influence of either 0, 0.5, 0.75, or 1.0 mg/kg of pimozide. Reductions in levels of water-deprivation produced a) increased latencies to initiate drinking, b) changes in the pattern of licking over the course of the session, and c) decrements in the total number of licks during the session. In contrast, administration of pimozide to 23-hr deprived animals produced no effect on either a) initiation latencies or b) lick patterns, and c) only marginally reduced the total number of licks during the session. Further, individual lick durations and interlick intervals were not affected by the neuroleptic. These data suggest that neither thirst mecanisms not motoric processes underlying drinking behavior are disrupted by dopamine receptor blockade.

416.9

REINFORCING PROPERTIES OF DRINKING WATER: ROLE OF OPIOIDS AND DOPAMINE.

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We have previously reported that sucrose drinking in non-deprived rats produces conditioned place preference. Consumption of the sucrose solution was taken to represent its rewarding properties and the place preference was taken to represent reinforcing effects. A dopamine antagonist blocked reinforcement but not reward, while an opiate antagonist blocked reward but no reinforcement.

The purpose of the present experiment was to evaluate whether drinking water in deprived rats and the reinforcement produced by this event responded in the same way to dopamine and opioid antagonists as sucrose drinking and reinforcement. Male rats were deprived of water for 24 h and then allowed to drink for 8 min in a lickometer. Immediately thereafter they were placed in place preference cages. The reinforced cage was the initially non-preferred cage. After a total of three reinforced and three non-reinforced sessions, the test was made.

Drinking produced a place preference that was blocked by pimozide, 1 mg/kg, administered before the reinforced sessions. A slight reduction of drinking was also observed. Naloxone, 16 mg/kg, administered before the reinforced sessions produced place aversion and considerable and progressive reduction of drinking. These data suggest that dopamine affects reinforcement and opioids reward.

416.10

SCOPOLAMINE BLOCKADE OF AMPHETAMINE-INDUCED CPP.

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Neurochemical and behavioral investigations support the existence of a DA-ACh reciprocal interaction in the neostriatum, which is apparent from opposite changes in turnover and in behavioral consequences of pharmacological challenge. While data have been reported implicating ACh in DA-mediated behaviors from from dorsal (catalepsy) and ventral (hyperactivity) striatum, exact nature of the DA-ACh limbic interface is not known; reports can be cited suggesting either a reciprocal relationship or no interaction. Amphetamine-CPP arises from DA activation in the (limbic) NAS region. DA in this structure has been repeatedly implicated in reinforcement/motivational processes. In the present study, 4 groups of (n=6-8) rats were conditioned with 1.5 mg/kg d-Amp to the nonpreferred side of a CPP chamber. Amp conditioning induced a significant increase in time spent on this NP side (6.33% to 26.8% over 15-min). Conditioning with 0.1 mg/kg scopolamine co-administration blocked the increase, although failing to induce a significant aversion itself. Locomotor activity data suggested that Sc also blocked conditioned hyperactivity but the magnitude of these changes failed to reach significance. These findings implicate a cholinergic component in the limbic circuitry generating Amp-CPP, and suggest the possibility that this component opposes DA as with striatally-mediated behavior.

417.1

AUDITORY FEEDBACK MAINTAINS CRYSTALLIZED SONG IN ADULT ZEBRA FINCHES. K.W. Nordeen, and E.J. Nordeen. Dept. Psych., U. Rochester, Rochester, NY 14627.

Birds use auditory feedback in learning song, but differ in their use of this feedback in maintaining adult song. Earlier work suggested that zebra finches, and other birds that learn song during critical periods, eventually attain songs that are independent of auditory feedback. It had been presumed that these species establish a central motor program as adult song patterns "crystallize". However, we have found that the crystallized songs of zebra finches change substantially following long-term deafening.

We recorded the songs of 25 adult (>120d) male zebra finches and then removed the cochleae from 11 of these birds. Songs were recorded up to 16 weeks following surgery (deaf) or after 15-68 weeks (control). By 16 weeks after surgery deaf birds accurately reproduced only 36% of the song syllables produced initially. In contrast, control birds retained 90% of their syllables. Also, the percentage of syllables that were unmatched or only slightly similar in phonology to previously recorded syllables was higher in deaf birds than in controls. Usually, these effects of deafening took 6-8 weeks to develop. We do not yet know where auditory feedback acts to maintain the neural circuits supporting adult song behavior, but preliminary data suggest that lesioning the lMAN, which disrupts song development, does not compromise the long-term maintenance of adult song.

417.2

BRIEF EXPOSURE SONG LEARNING IN ZEBRA FINCHES: ACQUISITION AND PRODUCTION OF A MODEL WITH ONE DAY OF TUTORING. A. Lombardino. Rockefeller University, Field Research Center, Millbrook, NY 12545

Juvenile male zebra finches, *Taeniopygia guttata*, normally learn the complex vocalizations of courtship song from an adult conspecific male with whom they can interact. Song learning in this species consists of two overlapping phases: an early sensitive period for acquisition of a model, and an extended period of vocal development during which the model is imitated. It is not known what minimal amount of song exposure is sufficient for imitation to occur. Here the auditory and social environments of male zebra finches were manipulated from one week after hatching until sexual maturity (day 90) in an attempt to narrow the period of model acquisition. Six zebra finch males ("scholars") were raised by their mothers until day 28; on that day each bird was placed singly with an unrelated adult female. On day 35 an adult male ("tutor") was placed in the scholar's cage for 24 hours. The scholars heard no other male zebra finch until day 90. Six control juveniles were treated in the same manner but had no access to a tutor male. The process of song development in both groups was followed by tape recording vocalizations at days 45, 60, 75, and 90. Visual inspection of sonagrams of each juvenile's song and his respective tutor revealed moderate to marked imitation of the model in four of the six scholars, whereas controls produced none of the complex syllables found in the tutors' songs, often incorporating the female companion's calls into a male song structure. The song of scholars and controls had similar introductory notes, which probably are not learned from male tutors. Experimentally restricting the time frame in which zebra finches acquire their song should greatly facilitate study of the mechanisms of song learning.

417.3

SONG INDUCES "IMMEDIATE EARLY" GENE EXPRESSION IN SONGBIRD FOREBRAIN. C. Mello*, D. S. Vicario and D.F. Clayton. Lab of Animal Behavior, The Rockefeller University, NY, NY 10021, & Beckman Institute, University of Illinois, Urbana, IL 61801

We identified a gene induced in songbird associative forebrain in response to the sound of birdsong. The gene ("ZENK") encodes the canary homolog of an "immediate early" transcription factor known to be induced by signals for neuronal growth and depolarization (Mello & Clayton, Soc Neurosci Abstr 16:657, 1990). By in situ hybridization, ZENK mRNA levels increased ~10-fold in the forebrain of canaries or zebra finches that heard tape-recorded birdsong after 1 day of acoustic isolation. The most marked induction occurred in the medial caudal neostriatum (NCM) when birds were exposed to song of their own species. Little or no induction occurred in response to pure auditory tones, or in the defined song control circuit or Field L. NCM may represent a site for: 1) auditory discrimination; 2) song storage; or 3) multimodal associations involving the song stimulus.

417.4

DEVELOPMENT OF AUDITORY RESPONSES IN NUCLEUS HVc WITH REDUCED AUDITORY FEEDBACK DURING SONG ACQUISITION. S.E. Volman. Dept. of Zoology, The Ohio State University, Columbus OH 43210.

The sensorimotor stage of song learning in white-crowned sparrows lasts for about 1 month. During this period, birds match their own "plastic song" to a stored memory of a song model. Before this stage, auditory neurons in the song-control nucleus HVc show no evidence of selectivity for the song model, and auditory responses habituate easily. During plastic song, HVc neurons become more responsive to song in general, and they also become preferentially responsive to a bird's own song (Volman and Konishi, Soc. Neurosci. Abst., 1987). This study asked: Is auditory feedback necessary to produce these changes in the auditory responses of HVc, or might they, at least in part, just be a consequence of maturation and activation of the song system? Four juvenile sparrows were individually isolated, and starting from when they first produced early plastic song, they were devocalized for at least 30 days. Devocalization was accomplished by puncturing the interclavicular air sac, which surrounds the syrinx. The air sac heals in 5-7 days, so vocalizations were monitored every day to determine both when repeat operations were necessary and what sounds the birds were producing. Each bird was able to hear himself sing for a total of about 4 days during this time. The responses of HVC neurons, recorded after the 30+ days of devocalization, were compared on a variety of measures to those in normal birds at an equivalent time after vocalization begins. The HVc neurons in devocalized birds gave robust responses to song and showed some song selectivity, but this was markedly less than that in normal birds. These results suggest that auditory responsiveness may develop in HVc in the absence of auditory feedback, but the mature pattern of song-selectivity requires such feedback, and does not simply reflect the activation of response patterns established during the acquisition of the song model.

Supported by NIH and the Deafness Research Foundation

417.5

A TEST OF THE AERODYNAMIC WHISTLE HYPOTHESIS FOR THE PRODUCTION OF BIRDSONG. R. A. Suthers and M. X. Zuo*. Medical Sciences, Indiana Univ., Bloomington, IN 47405.

Birdsong is often assumed to be generated by the vibration of tympaniform membranes in the avian vocal organ--the syrinx. However, an alternate mechanism of sound production based, on the principle of an aerodynamic whistle, has also been hypothesized to generate certain vocalizations in some species such as pigeons and doves (Columbidae) (Gaunt and Gaunt & Casey, Auk 99:474, 1982). According to the whistle hypothesis, sound is produced by a series of stable vortices in the expiratory air downstream from a constricted slot which acts like a hole-tone whistle in the vocal tract.

We have attempted to distinguish between these two mechanisms of sound generation by inserting a bundle of small diameter, parallel, straight stainless steel tubes into the trachea of male hubble pigeons immediately craniad to the syrinx. These tubes were designed to produce laminar airflow downstream from the syrinx and eliminate any turbulent vortices in the trachea. Vocalizations in which the syrinx functions as an aerodynamic whistle should be eliminated by this "flow straightener" whereas sounds produced by vibrating membranes should be little affected. Subsyringeal air sac pressure and tracheal pressure were monitored with miniature piezoresistive pressure transducers during vocalizations with and without this flow straightener. The rate of airflow through the trachea and through the mouth and nostrils was also measured using heated microbead thermistors. Insertion of the tracheal flow straightener had no significant effect on the coo-like vocalizations produced by male pigeons when presented with a female. This result suggests that cooing is not produced by an aerodynamic whistle in the syrinx. (NSF BNS 8720192)

417.6

INTRINSIC OSCILLATORY PROPERTIES OF VOCAL PREMOTOR NEURONS IN THE ZEBRA FINCH FOREBRAIN. R. Mooney. Div. of Biology 216-76 Caltech, Pasadena, CA 91125.

The zebra finch brain contains several nuclei that are specialized for vocal control during singing. Nucleus RA, located in the caudal forebrain, contains neurons that project directly onto the motoneurons controlling the syringeal musculature used in song. To learn how these neurons contribute to vocal motor control, their intrinsic electrophysiological properties were studied in an *in vitro* brain slice preparation. RA neurons fire highly regular trains of action potentials upon depolarization. These action potential trains exhibit little or no frequency adaptation even when depolarized for hundreds of milliseconds; the spike discharge rate increases as a linear function of the injected current pulse amplitude (ca. 63 Hz/ nA). Subthreshold membrane potential oscillations are a regular feature of the RA neuron's behavior near spike threshold, and increase in frequency with depolarization. Several ionic mechanisms contribute to the observed electrophysiological behavior of RA neurons. When impaled with intracellular electrodes containing 75 mM QX-314 (a sodium channel blocker) in 2 M potassium acetate, RA neurons rapidly lose their ability to fire fast action potentials upon depolarization. However, depolarization still evokes broader oscillations of the membrane potential. The major frequency component of these QX-314-insensitive oscillations also increases with greater depolarization, but disappears in nominally calcium-free solution, suggesting that these oscillations are Ca^{2+}-dependent. When RA neurons are depolarized beyond spike threshold without QX-314 in the recording electrode, the firing frequency increases and the interspike hyperpolarization diminishes in the Ca^{2+}-free solution. These experiments indicate that RA neurons have features that would be well-suited to their role in vocal motor control: the lack of frequency adaptation could be essential for the control of vocal muscle tension during singing, and their linear frequency response could render them quite sensitive to modulation by synaptic inputs both from within RA, and to synaptic inputs arising from other forebrain song control nuclei. (Supported by the Lucille B. Markey Charitable Trust.)

417.7

NONLINEAR RESPONSE PROPERTIES OF AUDITORY NEURONS IN THE ZEBRA FINCH'S SONG SYSTEM: A QUANTITATIVE APPROACH.

D. Lim, A. J. Doupe, and M. Konishi. Division of Biology, California Institute of Technology, Pasadena, CA 91125.

Auditory forebrain song nuclei of the male zebra finch (*Poephila guttata*) contain neurons sensitive to the bird's own song. These neurons respond best or exclusively to a certain configuration of song acoustic elements (E. S. Fortune, D. Margoliash, 1989 Abstr Soc Neurosci). The response of these neurons to the effective configuration does not appear to be a simple addition of their responses to individual elements presented in isolation, indicating the involvement of non-linear processes. To quantify these nonlinear interactions, we used a matrix-based optimization method (D. Lim and R. R. Capranica, 1990 J Acoust Soc Am Sup). This procedure made it easier to see interactions between a neuron's responses to various acoustic stimuli. Furthermore, it lent itself well to generating the set of most meaningful acoustic components for a particular group of neurons. Thus, the selectivity of forebrain nuclei for a given set of stimuli could be represented in terms of their nonlinear dependencies. For example, we studied responses of single HVc neurons to harmonically related tones and their combinations such as f1, f2, f1+f2, f1+f3, f1+f2+f3 etc. The results showed different degrees of inhibitory and facilitatory interactions between the neuronal responses to different tones. This work was supported by the McKnight Foundation and the Lucille P. Markey Charitable Trust.

417.8

SOURCES OF AUDITORY INPUT TO THE VOCAL MOTOR PATHWAY IN THE ZEBRA FINCH. D.S. Vicario and K. Yohay*
The Rockefeller University, New York, NY 10021.

Songbirds learn their vocalizations by imitating auditory models. Auditory responses can be recorded in many vocal motor structures of the songbird brain, including the robust nucleus of the archistriatum (RA). The two principal inputs to RA are from forebrain nuclei HVC and LMAN, both of which also respond to auditory stimuli, especially to the bird's own song.

We assessed potential sources of auditory input to RA in adult male zebra finches. 1) Multi-unit auditory responses in RA of anesthetized birds were examined before and after lesioning nucleus LMAN or the pathway connecting it to RA. Following these lesions, robust auditory responses continued to be recorded from cells in RA, although in some cases the latency of the response increased by about 10ms; the characteristic spontaneous bursting activity in RA was not changed. 2) Retrograde tracer injections were made in RA in an attempt to identify a pathway into RA from the "cup" region rostro-ventral to RA (that may receive auditory input from areas near Field L); these injections failed to label cells in the "cup" region. 3) The auditory responses of single HVC cells identified as projecting to RA by antidromic activation were examined.

The results show that the pathway to RA from LMAN is not required for auditory information to reach RA. Consistent with the earlier demonstration by Williams that HVC lesions abolish auditory responses in RA, the results further suggest that the direct projection from HVC carries this information. However, it is not excluded that, under some circumstances, auditory information travels through both pathways and is compared in RA. We are currently examining this intriguing possibility, using recordings in RA of awake birds. (Supported by MH40900)

417.9

EFFECTS OF AREA X LESIONS ON SONG DISCRIMINATIONS IN ZEBRA FINCHES. J. Cynx, C. Scharff, and F. Nottebohm. Rockefeller Univ. Field Res. Ctr., Millbrook, NY 12545

Area X is part of the recursive pathway in the circuitry controlling song behaviors. Lesions in this pathway disrupt the learning of song, while having no effect on adult song production (Bottjer et al., 1984; Scharff & Nottebohm, 1991; Sorabji et al., 1990). The recursive pathway has thus been characterized as involved only in song development. We hypothesized the function of the recursive loop might involve the correct discrimination of songs. To test this, we compared the performance of intact and Area X lesioned birds in operant discrimination tasks. Twelve adult male zebra finches were divided into six pairs. In each pair, one bird was given a bilateral Area X lesion. The intact bird in each pair served as a control. They were then trained on a number of operant discrimination tasks. In the first task, birds were trained to discriminate between pieces of hetereospecific (canary) song. There were no consistent differences between Area X lesioned and control birds. In the second task, birds in each pair were trained to discriminate between their own two songs. All Area X lesioned birds required more trials than their respective controls. In the last task, the birds were trained on a second canary song discrimination. Again, there were no differences between Area X lesioned and control birds. We conclude that Area X plays a role in conspecific song discriminations in adult zebra finches.

417.10

LATERALIZATION OF BIRD SONG DISCRIMINATION IN THE ZEBRA FINCH. H.Williams[1,2] J. Cynx[1] and F. Nottebohm.[1]
(1) The Rockefeller University Field Research Center, Millbrook, NY 12545, (2) Biology Dept., Williams College, Williamstown, MA 01267.

Lateralization of bird song production has been documented for a number of species. This is the first report that song discrimination is also lateralized. Fourteen adult male zebra finches were divided into seven pairs. In each pair, a bird was given a right- or left-side lesion of a thalamic auditory relay nucleus (nucleus ovoidalis), effectively deafening the respective telencephalic song control circuitry. This operation did not affect song production, but it affected performance in song discrimination tasks. Left-lesioned birds in each pair required more trials to reach a learning criterion when trained on an operant discrimination task between the pair's two songs. However, when the same birds were asked to discriminate between songs varying only in the placement of one frequency component in a harmonic structure, then left-lesioned birds in each pair mastered the task much faster.

This suggests that the hemispheres of the songbird brain may process song signals in different ways, paralleling inter-hemispheric perceptual differences reported in humans and other primates.

417.11

SONG CONTROL NUCLEI OF THE HOUSE SPARROW (PASSER DOMESTICUS) ARE PHOTOPERIODIC AND PHOTOREFRACTORY. M.G. Rucker*and V.M. Cassone. Dept. Biology, Texas A&M University, College Station, TX 77843.

The house sparrow is a well-studied species for photoperiodism. Song control nuclei have been shown to be seasonal in several avian species. The following study was to determine whether the song control nuclei of house sparrow were dependent upon photoperiodism and/or gonadal growth.

Adult male sparrows were housed in three separate light cycles (N=8/group). Group 1 was maintained in LD 6:18 for 6 wks and in LD 18:6 for 14 wks. Group 2 was maintained in LD 6:18 for 12 wks and in LD 18:6 for 8 wks. Group 3 was maintained in LD 6:18 for 20 wks. Body weight and bill color were recorded weekly. At the end of 20 weeks all animals were anesthetized, intracardially perfused with saline, then Bouin's fixative, and their brains removed. Gonads were also removed, weighed and paraffin embedded. Brains were processed for paraffin embedding and sectioned at 10μm. Tissue samples were then Nissl-stained for morphometric analysis on a computer-image analysis system (JAVA).

As previously determined in this species, gonads were small in photoinhibited Group 3 (21.9 $\pm$ 4.0 mg), large in photostimulated Group 2 (254.1 $\pm$ 11.2 mg), and small again in photorefractory Group 3 (65.1 $\pm$ 65.1 mg). As shown in other species, song control nuclei HVC and RA were smaller in photoinhibitory Group 3 than in photostimulatory Group 2. These nuclei were also reduced in size in photorefractory Group 1, indicating that in the house sparrow, song control is both photoperiodic and photorefractory. Mechanisms of this photorefractoriness is currently under study.

417.12

MOTOR BASES OF BEHAVIORAL STEREOTYPY IN SWAMP SPARROW SONG NOTE PRODUCTION. S. Nowicki and S. Peters*. Depts. of Zoology and Neurobiology, Duke University, Durham, N.C. 27706.

Swamp sparrows (*Melospiza georgiana*) incorporate a limited set of minimal units ("note types") into their trilled songs. These note types are species-specific and highly stereotypic. We asked whether birds that share identical note types in their repertoires also use similar motor programs to produce these note types. In other words, does stereotypy in song production on the level of behavior correspond to a comparable degree of stereotypy on the level of motor control? We addressed this question by quantitatively comparing the effects on song notes of identical experimental manipulations, both between and within individuals. Twelve adult males were subjected to denervation of either the left or right tracheosyringealis nerve, and within-treatment comparisons were made for all levels of note identity. We found in a significant proportion of cases evidence that identical note types are produced by different motor patterns. This result suggests that multiple pathways may be taken in the motor development of this stereotypic, species-specific behavior. Supported by DC 00402.

417.13

REGIONAL LOCALIZATION OF MK-801 BINDING IN ZEBRA FINCH SONG NUCLEI S.M. Aamodt, M.R. Kozlowski[1], E.J. Nordeen, and K.W. Nordeen. Dept. Psych., U. Rochester, Rochester, NY 14627 and [1]Bristol-Myers Squibb, Wallingford, CT 06492.

Effects of early sensory experience on neural and behavioral plasticity have been linked to activation of N-methyl-D-aspartate (NMDA) receptors. Early auditory experience is crucial for normal song learning in zebra finches, and the physiology of one song control area, the robust nucleus of the archistriatum (RA), indicates the presence of NMDA receptors in young males (Mooney and Konishi, '89). To further localize NMDA receptors in the song system, we measured glycine/glutamate-enhanced binding of 3H-MK801, an NMDA receptor antagonist, in brain sections from adult (>120 day) male zebra finches using film autoradiography.

Specific MK-801 binding was quite low in RA, the higher vocal center (HVC) and the lateral magnocellular nucleus of the anterior neostriatum (lMAN). In fact, binding within all of these regions was distinctly less than in adjacent tissue. In contrast, specific MK-801 binding was high throughout the parolfactory lobe, including Area X, a region critical for song learning. We are currently examining MK-801 binding in 30d and 50d old juveniles to determine whether NMDA receptor densities in these song related nuclei are higher during the sensitive period for song learning than in adulthood.

417.14

IMMUNOREACTIVE ESTROGEN RECEPTORS IN A SONG NUCLEUS IN FEMALE CANARIES. E. Brenowitz, B. Nalls*, and G.T. Smith. Departments of Psychology & Zoology, University of Washington, Seattle, WA 98195.

In songbirds estrogen (E) is important for the growth of forebrain song nuclei, juvenile song acquisition, and the activation of adult song behavior, (Gurney 1981; Harding et al. '83; Nordeen & Nordeen '89; Marler et al. '87). The accumulation of sex steroids by cells in song nuclei is thought to mediate hormonal effects. Gahr et al. (1987) showed that cells in the caudal nucleus of the ventral hyperstriatum (HVc) of male canaries have E receptors (ERs). We asked if female canaries also have ERs in HVc. Because female canaries can sing, but do so only rarely and more simply than males, they provide a test of the hypothesis that the ability of both sexes of a species to sing requires E accumulation by HVc cells in both sexes (Brenowitz & Arnold '89).

The brains of 4 male and 4 female canaries in breeding condition were cryosectioned at 18-25 µm. Sections were incubated with the H222 monoclonal antibody raised against human ERs (Abbott Labs), processed by the ABC method, and visualized with DAB.

Cells immunoreactive with the ER antibody were observed throughout HVc in both sexes. The proportion of ER (+) cells in HVc did not differ between females ($X \pm SD = 29.4 \pm 5.9\%$) and males ($30.8 \pm 5.6\%$). The absolute number of ER (+) cells in HVc was greater in males, however, because they have a larger HVc than females. The nuclei of ER (+) cells did not differ in size between females ($33.6 \pm 6.4\ \mu m^2$) and males ($31.1 \pm 7.5\ \mu m^2$).

These results support the hypothesis that the presence of ERs in HVc cells of both sexes is a necessary preadaptation for bisexual song. HVc also acts in song perception as well as song production (Margoliash '86; Brenowitz '91). Therefore, sex steroids may influence the activity of neurons important for both the production and perception of song. (Supported by NIH DC487, Sloan Fellowship to EB, Howard Hughes Predoctoral Fellowship to GTS)

417.15

NEUROGENESIS IN CANARY FOREBRAIN IS INDEPENDENT OF GONADAL STEROID LEVELS. S. D. Brown, F. Johnson, M. L. Godfrey* & S. W. Bottjer. Dept. Biol., USC, Los Angeles, CA 90089

The number of neurons in vocal-control circuits of adult canary brain varies as a function of season and circulating levels of sex steroids. Although the rate of incorporation of newly generated neurons into vocal-control circuits varies seasonally, this difference could reflect a higher rate of neurogenesis, a lower rate of cell death, or an altered migration. We are interested in the factors that control the rate of cell proliferation, and have looked for changes in the incidence of thymidine-labeled cells in the forebrain ventricular zone of adult canaries as a function of variations in hormone levels.

Adult female canaries maintained on short days were anesthetized and gonadectomized. Four separate groups of birds received systemic exposure to either testosterone, estradiol, a combination of an anti-androgen (flutamide) and an aromatase inhibitor (ATD), or nothing. All birds were also implanted with an osmotic mini-pump that released ^{3}H-thymidine at the rate of 1 uL/hr (c. 1.5 uCi/g/day) for three days and were killed four days following the onset of treatment. Analysis of autoradiograms revealed no differences between groups in the incidence of labeling within the ventricular zone at forebrain levels around the anterior commissure. Preliminary analysis of similarly treated birds allowed to survive 7 days following onset of treatment yielded comparable results. These results suggest that sex steroids do not directly regulate the rate of cell division in the ventricular zone. Differences in the incorporation of labeled cells into vocal-control nuclei may therefore be due to regulation of neurogenesis by other factors or to some other cellular mechanism.

417.16

TESTOSTERONE REGULATES THE DISTRIBUTION AND NUMBER OF ANDROGEN TARGET CELLS IN CANARY HVc. F. Johnson & S. W. Bottjer. Dept. Biol., USC, Los Angeles, CA 90089

Male canaries modify their songs seasonally, adding new and losing old syllables at a time when serum testosterone (T) levels are low (Nottebohm et al '87). Evidence that serum T levels vary seasonally and correlate positively with seasonal variation in the Nissl-defined volume of HVc (a brain region necessary for song production), suggests that T may regulate the size of specific cell populations in HVc (Nottebohm '81). However, at least two sub-populations of HVc cells show no seasonal variation in their distribution: estrogen target cells and neurons which project to Area X (Gahr '90). We have measured the incidence of androgen target cells in HVc and asked whether the distribution and/or number of these cells could be influenced by exposure to T.

Adult males were maintained on short days, gonadectomized, and treated systemically with either T or a combination of an anti-androgen (flutamide, FL) and an aromatase inhibitor (ATD). One month later birds were processed for ^{3}H-dihydrotestosterone (DHT) autoradiography. T treatment increased both the Nissl-defined volume of HVc and the proportion of DHT target cells in HVc. Thus, FL/ATD birds have a smaller HVc with many fewer DHT target cells. Moreover, in contrast to estrogen target cells, the distribution of DHT target cells matched precisely the Nissl-defined borders of HVc in both T- and FL/ATD-treated males. These data suggest that T can regulate the size of a population of androgen target cells in HVc, and in this way might influence seasonal relearning and modification of song.

417.17

LESIONING AFFERENT INPUT TO A FOREBRAIN NUCLEUS DISRUPTS VOCAL LEARNING IN ZEBRA FINCHES. K.A. Halsema & S.W. Bottjer. Dept. Biol., USC, Los Angeles, CA 90089

Vocal behavior in songbirds is controlled by a system of interconnected brain nuclei. Previous studies indicate that discrete portions of this neural system are instrumental during song learning, but are not needed for maintenance of song. Lesions of either lMAN or Area X in juvenile birds disrupt vocal performance whereas ablations of either forebrain nucleus in adults do not disturb the stable song pattern. Area X projects trans-synaptically to lMAN through the thalamic nucleus DLM, which appears to provide the sole source of afferent input to lMAN. In order to examine the effect of lesions at different levels in the X-DLM-lMAN circuit, DLM afferents to lMAN were ablated by knife-cut in young males before 50 days of age and in adult males between 70 and 100 days old. Damaging only DLM afferents disrupts the developing vocal pattern in juvenile males but has no effect on the stereotyped song of adults, as is true for lesions of lMAN and X. However, the nature of the song deficits appears to vary in relation to the level of interruption in the X-DLM-lMAN circuit. Zebra finches receiving lesions of lMAN at a young age produce only a few grossly abnormal notes. In contrast, severing the DLM input to lMAN in juvenile males renders a poorly modulated song consisting of an unstable sequence of 5 to 7 notes. These results indicate that removing the input to lMAN is less disruptive to the development of vocal behavior than are lesions to lMAN itself. This difference in the nature of the behavioral disruption may reflect the neural processing within each portion of the X-DLM-lMAN circuit.

417.18

SONG ACQUISITION IN ADULT FEMALE CANARIES REQUIRES N. L-MAN. K.M.Hill*, A.J.Carlson* and T.J.DeVoogd, Dept. Psych. and Field of Neurobiol. and Behav., Cornell Univ., Ithaca, NY 14853

The avian song system can be divided into two subsystems: a caudal group of nuclei that has a large role in crystallized song production, and a rostral group that has a large role in song acquisition. Song acquisition typically consists of an early phase of auditory learning and a later phase of auditory-motor integration. In development, major changes in the morphology of the rostral system occur during the initial auditory phase, suggesting that these regions play a role in this phase of song acquisition.

We now find that one rostral nucleus, l-MAN is also needed for the later phase. Adult female canaries were implanted with testosterone (T). This makes them produce a stereotyped song like that produced by adult males (but with a repertoire of c. 8 syllables rather than 20-30). After the birds were singing, we lesioned l-MAN bilaterally. In a second set of females, l-MAN was lesioned first and T was then given. Vocalizations of both groups were analysed. In birds that were already singing, the lesions do not appear to alter the number of syllables in the repertoire, the stereotypy of syllable production or the patterning of syllables (although a few syllables appear modified after the lesion). In contrast, birds that received the lesion before T, put together a song with only 2-3 simple syllables. These are less stereotyped than in the other group, and the birds appear to perseverate in producing them. These data suggest that l-MAN plays a role in the auditory-motor phase of song acquisition, even when song is acquired as an adult. As in males, l-MAN is not needed for stereotyped female song. Thus auditory-motor integration can be induced and studied in adult animals, perhaps making it possible to determine neural changes responsible for this aspect of song learning. Supported by HD21033-05.

417.19

DOPAMINE RECEPTORS IN THE SONG CONTROL SYSTEM: AREA X IS DEFINED BY THE D2 BUT NOT THE D1 DOPAMINE RECEPTOR SUBTYPE.
J.M. Casto, G.F. Ball and J. Balthazart Dept. of Psychology, Boston College, Chestnut Hill, MA 02167; Univ. of Liège, Liège, Belgium

Songbirds possess a system of sexually dimorphic brain nuclei that mediate the production and acquisition of complex vocalizations (i.e. song). One of these nuclei, area X, is a subdivision of the parolfactory lobe (LPO), the caudate homologue. Area X is markedly sexually dimorphic and is known to receive a dopaminergic projection from the area ventralis of Tsai (AVT, Lewis et al. 1981 J.Comp. Neurol.). To better understand this dopaminergic input to area X and LPO we mapped by in vitro quantitative autoradiography the distribution of D1 and D2 dopamine receptors in two songbird species, the zebra finch (Taeniopygia guttata) and the European starling (Sturnus vulgaris) and one non-songbird, the Japanese quail (Coturnix coturnix japonica). D1 receptors were labelled using [^{3}H] SCH 23390 (SA 87 Ci/mmol) at 2.0 nM ± 2 mM (+) Butaclamol. D2 receptors were labelled using [^{3}H] Raclopride (SA 84.7 Ci/mmol) ± 2 mM (+) Butaclamol (to define non-specific binding). In all species the highest receptor density for both receptor types was observed in parts of the basal ganglia such as LPO and paleostriatum augmentatum (PA) the putamen homologue. No difference in the pattern of D1 and D2 binding was discerned within the quail LPO. In the songbird species, Area X, in the LPO, was clearly defined by a high density of D2 receptors as compared to the surrounding LPO. In the case of the D1 subtype there was no apparent difference between area X and the surrounding LPO. In female zebra finches area X is not visible in Nissl stained sections and it was not discernible when examining the pattern of D2 receptor density. Area X is known to be necessary in songbirds for the acquisition but not the production of song; quail do not learn their vocalizations and have no apparent differentiation of LPO into a subregion such as area X.

417.20

AUTORADIOGRAPHIC LOCALIZATION OF NMDA RECEPTORS IN THE AVIAN SONG CONTROL SYSTEM USING [^{3}H] MK-801
G.F. Ball and J.M. Casto, Dept. of Psychology, Boston College, Chestnut Hill, MA 02167

The N-methyl-D-aspartate (NMDA) receptor type has been implicated in the control of learning and memory in several vertebrate species. Songbirds possess a set of interconnected brain nuclei that are involved in both the acquisition and the production of vocal behaviors. To assess the possible involvement of this receptor subtype in song learning, we mapped, by in vitro quantitative autoradiography, the pattern of NMDA receptor binding in the songbird brain. The non-competitive antagonist [^{3}H] MK-801 was used to label the receptors (32 nM ± 5 mM ketamine to define non-specific binding). Two songbird species, the zebra finch (Taeniopygia guttata) and the European starling (Sturnus vulgaris) and one psittacine, the bugerigar (Melopsittacus undulatus) were used in this study. In the songbirds, 3 song control areas, the magnocellular nucleus of the anterior neostriatum (MAN), the caudal part of the ventral hyperstriatum (HVc), and the robust nucleus of the archistriatum (RA) were defined by a low density of MK-801 binding (e.g. 3 to 4 pmol/mg protein) in comparison to the surrounding structures (e.g. 7-10 pmol/mg p). The boundaries of Area X could be clearly discerned by the high density of binding (e.g. 4 to 6 pmol/mg p) in comparison to the surrounding parolfactory lobe (e.g. 2 to 3 pmol/mg p). The putative homologues of these nuclei could not be discerned in the budgerigar. The physiological significance of the relatively low density in three telencephalic nuclei mediating vocal learning and production remains to be investigated.

INVERTEBRATE LEARNING AND BEHAVIOR II

418.1

NEURAL CORRELATES OF EXPLORATORY BEHAVIORS IN FREELY-MOVING *NAVANAX INERMIS* (MOLLUSCA; OPISTHOBRANCHIA).
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Navanax is a predatory hermaphrodite whose normal sexual behavior involves bouts of copulation in which a pair of individuals repeatedly alternate sexual roles. In an attempt to elucidate the role of the CNS in these long-term behaviors, I have used 2-14 h observation periods on 2-4 consecutive days to record repeated instances of whole behavioral sequences from animals with chronically implanted bipolar cuff electrodes. Use of a split-screen technique makes it possible to record, visualize, and playback, CNS activity on intact nerves or commissures, simultaneously with behavioral data. The high degree of cryptic neural activity and the fact that *Navanax* behavioral states are not mutually exclusive, make it difficult to identify one-to-one correspondences between behaviors and neural patterns. Also, individuals with lesions of major nerves often show few behavioral deficits. However, recordings from the 5th pedal nerve show a pattern of low frequency bursts of medium amplitude (~0.2-0.5 mv) which appears to be associated with investigation (presumably chemosensory) of the substrate. It may be related to flaring of the Hancock's organ. These spikes are normally absent during contact with prey and may therefore be associated with recognition of, or searching for, conspecifics. There is little overlap between spikes making it possible to identify single units. This suggests that it will be possible to identify the neurons responsible for these spikes. More variable patterns of activity on P5 are associated with head-turning. Supported by NIMH and NSF.

418.2

INTERACTIONS BETWEEN MOTIVATIONAL STATE AND CHEMOSENSORY STIMULATION OF SKIN ACID SECRETION IN *Pleurobranchaea*.
S.D.Grunder and R.Gillette. Dept. of Physiology and Biophysics, University of Illinois, Urbana Il 61801.

Mechanical and chemical stimuli elicit defensive acid secretion from the skin of *P. californica* (Gillette *et al.*, J. Exp. Biol. 156, 335 (1991)). Skin acidification potentiates aversive withdrawal of the affected body part and potentiates aversive turns and locomotion. It was shown that stimuli that elicit acid secretion also suppress feeding behavior and raise feeding thresholds. We have now found that appetitive food stimuli also stimulate acid secretion at the oral veil, suggesting that acid secretion could interact with an animal's motivational state to regulate feeding behavior. To test this, skin acid secretion was recorded while appetitive stimuli (squid homogenate or trimethylglycine) were applied to the oral veil of animals before and after satiation. Feeding thresholds rose by an average of 1.75 log units, but oral veil acidification (avg. 1.19 ± 1.09) was not significantly affected by satiation. These data suggest that food chemosensory stimulation of acid secretion interacts with satiation state to limit meal size. Differential chemosensory conditioning may help to further clarify the role of acid secretion in regulation of feeding behavior.

418.3

NETWORK MODEL OF AVERSIVE DISCRIMINATIVE CHEMOSENSORY CONDITIONING IN *Pleurobranchaea*.
J.R.Payne and R.Gillette. Neuroscience Program, University of Illinois, Urbana Il 61801.

P. californica learns to discriminate chemosensory stimuli such as squid homogenate, sea anemone homogenate, and beer extract (Davis *et al.*, J. Comp. Physiol. 138, 157 (1980); Mpitsos and Cohan J. Neurobiol. 17, 487 (1986)). A simple computational model has been constructed to study possible mechanisms by which complex olfactory stimuli are encoded and discriminated. The model incorporates 1) broadly but distinctly tuned olfactory receptors, 2) simple contrast enhancement mechanisms, 3) documented interactions of neural circuitry for appetitive and aversive behavior, and 4) simple learning mechanisms tied to nociceptive and reward pathways. Results so far suggest that expression of a repertory of relatively complex behavior may be appropriately and specifically altered by simple heterosynaptic modification of sensory paths to specific motor network elements.

418.4

LEARNING CORRELATED CHANGES IN A CALCIUM-ACTIVATED K CURRENT IN HERMISSENDA TYPE A CELLS.
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Previous research has determined that Type A photoreceptors in the eyes of Hermissenda exhibit reduced light-induced depolarizing generator potentials and resting input resistances for several days following pairings of light and rotation, when compared to results from random control or untrained specimens (Behav. Neuroscience., 1990). Voltage-clamp studies indicate that a TEA- and 4-AP resistant calcium-dependent K current ($I_{K\text{-}Ca}$)is an important determinant of a Type A cell's light response. In the present study, generator potential amplitudes of synaptically-isolated Type A cells from trained and control specimens were measured in normal and calcium-free ASW on retention days following learning. The ~ 5.0 mV training-associated difference in generator potential amplitude in normal ASW (3 min post light-onset) was virtually eliminated when calcium was removed from the bathing media. Measurements of I_A failed to reveal any training-associated difference in this component of K current, while measurements of $I_{K\text{-}Ca}$ revealed greater current amplitudes (by ~ 25% for potentials > -25 mV) in A cells from trained vs. control specimens. Collectively, these results demonstrate that long-term training-associated differences in Type A cells' light responses are due in part to training-associated increases in $I_{K\text{-}Ca}$, a mechanism which is the converse of that which underlies learning-associated changes in Type B photoreceptors.

418.5

IDENTIFICATION OF A $GABA_B$ RECEPTOR THAT CONTRIBUTES TO VISUAL-VESTIBULAR INTERACTIONS IN *HERMISSENDA*

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Department of Psychology, Rutgers University, New Brunswick, NJ 08903

Stimulation of vestibular hair cells induces a hyperpolarization and cessation of firing in ipsilateral B photoreceptors in the mollusk *Hermissenda*. This response is mediated by a GABAergic synapse, the function of which cannot be exclusively attributed to an inward Cl^- conductance at the B cell soma (e.g., Matzel & Alkon, *Brain Research*, 1991). At resting membrane potentials (-50 to -60 mV), pressure microapplication of either GABA (12.5 μM) or the $GABA_B$ agonist baclofen (125 μM) to the terminal branches of the B cell induced a hyperpolarization analogous to that induced by haircell stimulation, though the hyperpolarizing response to baclofen was reduced relative to the hyperpolarizing response to GABA ($\approx$ 4 mV and 8 mV, respectively). The GABA-induced hyperpolarization was reduced, but not eliminated, by the $GABA_A$ antagonist bicuculline (300 μM) or the $GABA_B$ antagonist 2-hydroxysaclofen (450 μM), but was eliminated by the combined application of each antagonist (150 μM and 225 μM, respectively). Moreover, the potential at which GABA induced no net voltage response shifted from -90 mV to -70 mV when either bicuculline (300 μM) or 2-hydroxysaclofen (450 μM) were added to the extracellular solution. To determine the conductance changes underlying these voltage responses, the B cell was voltage-clamped at -55 mV, and GABA application was found to induce a net inward current. Following application of 2-hydroxysaclofen (450 μM), the magnitude of the inward current increased, while application of bicuculline (300 μM) revealed an outward current in response to GABA, consistent with the persistence of an outward K^+ flux induced via stimulation of a $GABA_B$ receptor. In total, these results indicate that the voltage response of the B photoreceptors to GABA is the sum of an inward and an outward current, which arise from dual activation of both $GABA_A$ and $GABA_B$ receptor subtypes.

418.6

CORRELATES OF CONDITIONING IN PAIRS OF IDENTIFIED TYPE A & B PHOTORECEPTORS IN HERMISSENDA: EFFECTS OF ADAPTATION, LIGHT INTENSITY AND SYNAPTIC INTERACTIONS.

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Type A & B photoreceptors have been shown to exhibit intrinsic cellular modifications in conditioned *Hermissenda*. Here we report that the effect of conditioning on the light responses of identified pairs of Type A and B photoreceptors depends on light intensity and light adaptation. The light-elicited spike frequency during the first 30s of a bright light (-2.0, -1.0 N.D.) was enhanced for lateral A cells from conditioned animals ($\bar{x}$= 7.67 spikes/s, $\bar{x}$= 5.51 spikes/s) compared to backward (BW) controls ($\bar{x}$= 5.5 spikes/s, $\bar{x}$= 2.84 spikes/s). However, the discharge frequency during the last 30s of a five min light step was less for conditioned animals (-2.0 N.D.)($\bar{x}$= 0.77 spikes/s) compared to BW controls ($\bar{x}$= 1.44 spikes/s). Medial Type A cells from conditioned animals exhibited a decrease in light response to bright light (-1.0 N.D.)($\bar{x}$= 4.46 spikes/s) compared to the BW control group ($\bar{x}$= 7.06 spikes/s) during the first 30s of the light step. The response of medial Type A cells from conditioned animals during the last 30s of a 5 min light step was decreased to bright light ($\bar{x}$= 0.64 spikes/s) compared to BW controls ($\bar{x}$= 1.80 spikes/s). The amplitude of the transient peak of the generator potential of intact medial A's from the conditioned group was enhanced in response to the brightest light intensity ($\bar{x}$= 43.8 mV) compared to normal controls ($\bar{x}$= 38.9 mV) and BW controls ($\bar{x}$= 40.0 mV). However the transient peak of the generator potential of lateral A's was reduced in conditioned animals ($\bar{x}$= 36.3 mV) compared to both normal controls ($\bar{x}$= 42.7 mV) and BW controls ($\bar{x}$= 47.0 mV). Preliminary studies of mutually inhibitory synaptic connections between pairs of A and B photoreceptors measured by Type B spike evoked IPSP's in Type A cells did not reveal significant changes in IPSP amplitude produced by conditioning.

418.7

EVALUATION OF MALE REPRODUCTIVE FUNCTION IN THE PULMONATE SNAIL MELAMPUS BIDENTATUS AFTER PENIAL COMPLEX EXCISION AND NERVE AND GANGLION LESIONS.

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The penial complex of Melampus is innervated by two clusters of neurons, a right cerebral cluster that is excitatory, and a right pedal cluster that is inhibitory (Moffett, Neurosci. Abs. 1989). Specific reinnervation of the penial complex occurs after the axons are severed (Ridgway et al., Neurosci. Abs. 1988) and the penial complex regenerates if at least part of its innervation is intact. The copulatory success in these hermaphroditic animals is tested in snails with 1) regenerated penial innervation or 2) regenerated penial complex (vas deferens, penis, penial sheath and prepuce). Snails with the same lesion history are maintained together and egg development is observed. Physiological and anatomical evidence of reinnervation appears within 2 weeks and snails with the penial nerve transected 3 weeks before onset of egglaying produced fertile eggs. Regeneration of the penial complex requires 4-6 weeks; snails did not produce fertile eggs at three weeks postoperative but will be monitored for the remaining 2 months of the reproductive season.

418.8

ODOR RESPONSES AND OUTPUTS OF AN OSCILLATING OLFACTORY PROCESSING NETWORK IN A TERRESTRIAL MOLLUSK.

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Odor processing networks in a variety of species are being explored from both a cellular and computational perspective to determine how molecular recognition and categorization are accomplished and how the resulting information is interfaced to behavioral subsystems which require processed odor information, such as navigation and odor learning. The procerebral (PC) lobe of the terrestrial slug *Limax maximus* is such an odor processing network. The PC lobe receives direct input from both inferior and superior noses and displays coherent network oscillations at 0.7 Hz which are modulated in waveform and frequency by natural odor input (**Nature** 345:437, 1990). The natural odorant 2-ethyl-3-methoxypyrazine (potato odor) produces comparable changes in the PC lobe oscillation when applied to the neuroepithelium of the superior nose in solution (10^{-4} % in saline) or as the vapor phase of a 10^{-5} % aqueous solution. Candidate outputs of the PC lobe were identified by application of the fluorescent carbocyanine dye $DiIC_{12}(3)$ to the neuropil of the PC lobe and incubation of the tissue at room temperature for 12 to 36 hours. A small number of labelled fibers (4 $\pm$ 2, N = 15)(mean $\pm$ std.dev.) appeared in the cerebropedal connective as did labelled cells in the pedal ganglion (8 $\pm$ 5, N = 13). The pedal cells appeared in 1 - 3 clusters. By analogy to similar cells in *Achatina* described by Chase and Tolloczko, the *Limax* pedal cells with processes in the PC lobe are likely to carry output from the PC to locomotor control centers in the pedal ganglion.

418.9

FOOD DEPRIVATION INCREASES THE CURVATURE OF SYNAPSES CONTACTING A MOLLUSCAN FEEDING INTERNEURON.

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The purpose of this study was to investigate the plasticity of the synapses on the Giant Cerebral Neuron (GCN), a molluscan serotonergic cell that is known to respond to food stimuli and to facilitate feeding behavior. Snails (Rumina decollata) commonly experience extended periods of time without food. Therefore, we fed one group of snails ad lib, while a second group was starved for 5 days. Synapses converging onto the GCN were identified, within the brain, after injecting the GCN with hexamminecobalt. The principal result was that the ratio of curved synapses to flat synapses was significantly greater in the starved animals than in the sated animals. There were no significant differences between the groups in regard to the numerical densities of labelled synapses, the vesicle numerical densities, or the synaptic lengths. The results imply that sensory information about food is transmitted more effectively to the GCN in hungry snails than in sated snails.

418.10

BEHAVIOURAL ANALYSIS OF INTERACTIONS BETWEEN TWO ANTAGONISTIC REFLEXES IN *C. ELEGANS*.

C. H. Rankin, Department of Psychology, University of British Columbia, Vancouver, BC V6T 1Z4.

In response to a mechanical tap the nematode *Caenorhabditis elegans* shows a withdrawal reflex, swimming backwards for some distance. This reflex is capable of habituation, dishabituation and sensitization, as well as both short and long term memory. In addition, we have discovered two antagonistic reflexes in *C. elegans*.

Antagonistic reflexes that use the same final common path cannot be activated simultaneously; as a consequence one reflex often inhibits the expression of the other. In adult *C. elegans* tail-touch normally elicits forward movement, while tap normally elicits backward movement. When tail-touch was delivered one second before a tap, reversals to the tap were inhibited and the magnitude of any reversal that did occur was reduced. The relative magnitude of the two stimuli, tail-touch and tap, affected the amount of inhibition observed. Habituating the response to tail-touch decreased the inhibition of reversal to tap following a tail-touch. The tail-touch induced inhibition of reversal to tap diminished over an interval of at least 10 seconds; however, following the inhibition an enhancement of responding to tap was seen. Inhibition of reversal to tap was present in worms of all stages of development including newly hatched worms.

418.11

CIRCUIT ANALYSIS OF INTERACTIONS BETWEEN TWO ANTAGONISTIC REFLEXES IN *C. ELEGANS* . S. R. Wicks and C. H. Rankin, Department of Psychology, University of British Columbia, Vancouver, BC V6T 1Z4.

In order to investigate the mechanisms underlying a behavior, it is first necessary to determine the neural elements involved in the behavior. In this research we have focussed on the neural circuit underlying the withdrawal reflex made by the nematode worm *C. elegans* in response to a mechanical tap. Using mutant analysis and laser ablation techniques we have determined that the touch withdrawal circuit described by Chalfie and colleagues also mediates the tap withdrawal reflex.

Our behavioral experiments showed that the tail-touch withdrawal reflex inhibits the tap withdrawal reflex. Investigations into how these antagonistic responses can be reconciled within the touch circuit followed three lines. First, mutants specific for identified neurons within the circuit have been identified. For example, *deg-1* is a mutant in which the interneuron PVC degenerates between the first and second larval stages. This mutant failed to show the tail-touch induced inhibition to tap suggesting that plasticity is afferent to PVC. Second, laser ablating individual neurons within the circuit confirmed and extended the genetic studies. Finally, many of the worms neurons arise postembryonically. That the worm shows inhibition prior to the formation of some synapses argues against the possibility of those synapses mediating the response.

418.12

THE EFFECTS OF AGING ON NON-ASSOCIATIVE LEARNING IN THE NEMATODE *CAENORHABDITIS ELEGANS*. C. D. O. Beck and C. H. Rankin, Department of Psychology, University of British Columbia, Vancouver, BC, Canada V6T 1Z4.

In *Caenorhabditis (C.) elegans*, there are changes with age in habituation and recovery from habituation. Worms were tested at 4 days (peak of egg-laying), 7 days (after completion of egg-laying), and 12 days (shortly before death) of age. While the frequency of reversal withdrawal responses to single taps to the holding dish (vibrational stimuli) did not change with age, the magnitude of the reversals diminished.

In post-reproductive development, worms at all ages habituated to trains of taps delivered at a 10s interstimulus interval. However, worms tested at 7 and 12 days continued to make small responses throughout stimulation while 4 day olds often stopped responding entirely. All ages showed dishabituation in response to a mild electric shock. However, spontaneous recovery from habituation (measured 30s, 10min, 20min and 30min after the last habituating stimulus) was seen only in the worms tested at 4 days and 7 days. The deficit in recovery at 30min in worms 12 days of age cannot be accounted for by motor fatigue as worms of the same age dishabituated after habituation training.

Describing the changes in non-associative learning with age in normal wild-type worms will allow an analysis of the effects of treatments which alter the aging process in *C. elegans*.

418.13

AN ANALYSIS OF THE TAP WITHDRAWAL REFLEX IN MALE *CAENORHABDITIS ELEGANS*. K. B. Mah and C. H. Rankin, Department of Psychology, University of British Columbia, Vancouver, BC, V6T 1Z4.

Caenorhabditis elegans is a simple soil dwelling nematode which exhibits sexual dimorphism. The male *C. elegans* is differentiated from the hermaphrodite by the addition of 14 sensory structures in the tail. In this study, we compared the behavioural responses of the male to that of the hermaphrodite. It was hypothesized that the anatomical difference in mechanosensory structures might result in behavioural differences in the reversal response to vibratory stimulation (a tap to the side of the holding dish).

The adult male *C. elegans* shows a higher incidence of spontaneous reversals than the hermaphrodite. In response to differing intensities of tap there were no apparent differences between the responses of males and hermaphrodites. Further, the male was shown to be capable of simple non-associative learning: it demonstrated habituation, dishabituation and recovery from habituation in much the same manner as the hermaphrodite. Tail-touch induced inhibition of the reversal response appeared to be similar in males and hermaphrodites.

Thus, there appeared to be no behavioural differences between the male and hermaphrodite in the tap withdrawal reflex despite the presence of additional neuronal structures in the male.

418.14

Behavioral Development in the Embryonic Medicinal Leech, *Hirudo medicinalis*. S.A.Reynolds*, G.K.Bryan, A.Baader, and W.B.Kristan Jr., Department of Biology, U.C.S.D., La Jolla, CA 92093-0322.

The neuronal bases of several behaviors have been characterized in the adult medicinal leech. As a preliminary to studying the development of these behavioral circuits, we have been studying the normal course of behavioral development in the embryos between developmental days 10 and 30. We have determined the time of onset and the maturation of 14 different behaviors, both spontaneous and evoked, using microscopic observations and micro-cinematography with frame-by-frame analysis of behavior. We have found that the behaviors develop along a consistent course. For instance, spontaneous lateral movements of the head appear earlier than shortening can be evoked, and both appear earlier than swimming. There is also a rostrocaudal gradient of development for some behaviors, particularly the ones, such as lateral bending, which develop first. Complex behaviors develop in stages. For instance, in the development of crawling, the embryo masters the coordinated elongation and contraction phases of the step cycle before it can use the front and rear suckers to initiate and complete each step. In leeches, as in other poikilotherms, the overall rate of development depends on temperature. We are investigating whether the sequence of behavioral development is also temperature-dependent. This work was supported by USPHS research grant MH43396 to WBK.

418.15

CELLULAR ANALYSIS OF PREDICTABILITY IN THE LEECH. A. M. Horgan * and C. L. Sahley. Dept. Bio Sci, Purdue Univ., W. Lafayette, IN 47907.

Pairing and predictive relationships between stimuli are critical variables in associative learning for both vertebrate and invertebrate species including the leech, *Hirudo medicinalis*. We previously reported that the Retzius cell (R) may be implicated in the phenomenon (1988,*Soc. Neurosci. Abs.,14*, 838). That is, the R cell, driven by the US, continues to fire to throughout training for leeches experiencing paired CS-US presentations. In contrast, the response of the R cell decrements to repeated US alone presentations as well as to paired CS-US presentations with added unpredicted US alone trials. Learning only occurs following paired training.

Previous experiments have indicated that the long term inhibition of the R cell by serotonin (5HT) occurs via enhancement of IA (1989, *J. Exp. Bio., 145*, 403-17). We now report that pretreatment with FMRF replaces the 5HT induced inhibition of the R cell with an excitatory response consisting of a depolarization and barrage of action potentials. Voltage clamp analysis suggests that inward current is activated at a more negative potential in the presence of FMRF than in the control.

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418.16

KILLING A SINGLE INTERNEURON ELIMINATES SENSITIZATION AND REDUCES DISHABITUATION IN THE LEECH. B. K. Modney#, K. J. Muller# & C. L. Sahley*. Dept. Physiol. & Biophysics#, U. Miami Sch. Med., Miami, FL 33136 and Dept. Bio. Sci.*, Purdue Univ., West Lafayette, IN 47907

Shortening, a defensive reflex of the leech, exhibits dishabituation and sensitization in response to a noxious stimulus. A chain of coupled interneurons (S cells) that forms the Fast Conducting System of the leech has an uncertain role in this reflex. In this study an intracellular pronase injection was made in vivo to kill a single S cell in segmental ganglion 4 to establish the role of the S cell in sensitization and in dishabituation of the shortening reflex. Control leeches underwent surgery without S-cell pronase injections. The effect of the lesion was assessed in semi-intact leeches using the protocols described previously (*J. Neurosci.*, 1988, 8, 4621).

No differences were found between control and S-cell killed animals in the shortening reflex or its habituation. S-cell kills disrupted, but did not eliminate dishabituation; i.e. animals showed significantly less dishabituation than control animals ($F_{(4,52)}=3.7$, $p<0.01$). In contrast sensitization was completely eliminated by S-cell kills ($F_{(1,12)}=5.62$, $p<0.05$). Thus, the S cell appears to be important in non-associative learning of the shortening reflex, but is not required for the reflex itself. The differential effect of S-cell killing on dishabituation and sensitization is added evidence that these types of learning may operate by different mechanisms.

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418.17

GIANT FIBER ACTIVATION OF AN INTRINSIC MUSCLE IN THE MESOTHORACIC LEG OF *DROSOPHILA*. J.R. Trimarchi and A.M. Schneiderman. Section of Neurobiology and Behavior, Cornell University, Ithaca NY 14853.

Many of the muscles and neurons recruited during the escape response in *Drosophila* have been characterized. In particular, the tergotrochanteral muscle (TTM) which adducts the femur provides the force which propels the fly from its substrate during the initial jumping phase of the behavior (Mulloney, 1969, *Z. vergl. Physiol.*, **64:** 243-253). The present study focuses on the synergistic role in jumping of the tibial levator (TL), an intrinsic leg muscle.

During activation of the escape response by stimulation of the giant fiber pathway (Tanouye & Wyman, 1980, *J. Neurophysiol.* **44:** 405-421), muscle potentials are recorded from the TL with characteristic latencies of 1.3 ± 0.01 msec. They occur 0.48 and 0.04 msec after muscle potentials appear in the TTM and the dorsal longitudinal indirect flight muscles (DLMs), respectively. The threshold voltage of brain stimulation required to elicit a response in the TL is the same as that required for the TTM and DLMs. As stimulus voltage increases, the responses of the TTM and DLMs undergo a distinctive shift from long to short latency (Levine & Tracy, 1973, *J. Comp. Physiol.* **87:** 213-235). We observed that the response of the TL maintains its temporal delay with respect to the responses of the TTM and DLMs during this shift.

HRP backfills of the TL motor neuron indicate that it has both a dendritic arborization in the leg neuromere and a medially directed neurite. We are extending these studies to characterize the neuronal circuitry underlying activation of the TL by the giant fiber pathway.
(NSF BNS-90-09833, NIH 5-T32-GM07469)

418.18

EFFECTS OF cAMP ON GROWTH CONE MOTILITY IN CULTURED CNS NEURONS: ANALYSIS OF *DROSOPHILA* MEMORY MUTANTS, *dunce* AND *rutabaga*. Yun-Taik Kim* & Chun-Fang Wu
Department of Biology, University of Iowa, Iowa City, Iowa 52242

Learning and memory capacity of animals depends on cellular mechanisms underlying functional plasticity of neurons. This plasticity may involve morphological modifications of nerve terminal arborization and synaptic contact between neurons. The *Drosophila* mutants *dunce (dnc)* and *rutabaga (rut)* perform poorly in learning tasks, and are known to have elevated or reduced levels of intracellular cAMP, respectively. Since the growth cone plays a critical role in axonal elongation, branching and synaptogenesis, morphological and behavioral characteristics of growth cones may be altered during neural development in these learning mutants. The dissociated larval CNS culture system (Wu *et al.*, *J. Neurosci.* 3:1888, 1983) was employed in video microscopy examinations of mutant growth cones to investigate the role of cAMP in growth cone morphology and motility. We found that growth cones in *dnc* and *rut* were similar to wild type in several morphometric parameters, including the number and length of filopodia, and area and roundness of lamellipodia. However, the motility of growth cones, characterized by motility index and boundary flow plots (Kim & Wu, *J. Neurobiol.* 22: 263, 1991), was significantly lowered by *dnc* and *rut* mutations. Since *dnc* and *rut* affect cAMP-specific phosphodiesterase or Ca^{2+}-calmodulin activation of adenylate cyclase, respectively, we examined the effects of 10-50 μM forskolin or dibutyryl cAMP in the perfusion medium. The treatment resulted in retraction and thickening of lamellipodia which led to lower motility of growth cones and triggered the transformation of lamellipodia into club-shaped neurite endings. The fact that either elevated or lowered cAMP in *dnc* or *rut* had similar effects on growth cones suggests that an optimal level of cAMP is required to maintain growth cone motility, which may be important to neural plasticity underlying learning behavior.

418.19

SUPPRESSION OF ACTIVITY-DEPENDENT SYNAPTIC PLASTICITY IN NERVE TERMINAL ARBORIZATION BY MEMORY MUTANT *rutabaga* IN *DROSOPHILA*. Y. Zhong and C.-F. Wu Dept of Biology, Univ. of Iowa, Iowa City, IA 52242

Previous studies on hyperexcitable and *dnuce* mutants suggest that the cAMP cascade may be a crucial biochemical pathway in mediating activity-dependent enhancement of motor nerve terminal arborization in *Droasophila* larvae (Budnik et al., 1990, J. Neurosci. 10: 3754; Zhong et al., 1990, Neurosci. Abst. 16: 594). We investigated the effect of the *rutabaga* (*rut*) mutation, which eliminates Ca/CaM activation of adenylate cyclase, on hyperexcitable mutation- and temperature-induced enhancement in larval motor terminal arborization.

In larvae reared at 25 °C, the numbers of motor terminal branches and varicosities were increased in hyperexcitable *Shaker* (*Sh*) mutants with a reduced or eliminated transient K current (mean±SD of branches/varicosities in fiber 12 and 13, abd. segment 3, from 8-12 larvae: Sh^{120}; 22+3/332+51; Sh^{M}; 22±3/392±56; normal; 14±3/243±29). When rearing temperature was increased to 30 °C, expanded nerve terminal projection was also seen in normal larvae (26±5/392±38) as compared to those reared at 25 °C. This hyperexcitability- and temperature-induced enhancement in terminal arborization was suppressed by the *rut* mutation as shown in *rut* Sh^{120} (15±3/255±36) and *rut* Sh^{M} (19±3/298±28) double mutants reared at 25 °C as well as in *rut* single mutant reared at 30 °C (18±45/276±57).

Elevated cAMP in *dnc* mutants has been correlated to enhanced terminal arborization. Our results further support that the activation of cAMP cascade is a critical biochemical step in mediating synaptic plasticity.

418.20

LATHEO, A NEW SINGLE-GENE MUTANT AFFECTING LEARNING AND MEMORY IN *DROSOPHILA MELANOGASTER*. S. Boynton* and T. Tully. Department of Biology, Brandeis University, Waltham, MA. 02254

We have begun a screen for new mutations affecting classical conditioning or retention of olfactory avoidance responses in *Drosophila*. Use of P-elements as the mutating agent will ultimately expidite molecular cloning of the relevant genes. One strain isolated from this mutagenesis, $latheo^{P1}$, shows reduced learning and memory. Behavioral analyses indicate that the behavioral deficit of $latheo^{P1}$ flies is not caused by an inability to smell the odors (conditioned stimulus) used during training or by an inability to sense and escape from electric shock (unconditioned stimulus).

Genetic analyses of *latheo* indicate that the behavioral deficit in *latheo* flies is attributable to a single gene. Flies heterozygous for the $latheo^{P1}$ insertional mutation and for chromosomal deficiencies of the second chromosome show abnormal memory of olfactory avoidance conditioning. Two percent of *latheo* alleles generated by P-element excision were lethal, and the lethality mapped to a single, lethal complementation group in the *vestigal* region. More severe alleles of *latheo* show pleiotropic effects on several behaviors reflecting the fact that the normal *latheo* gene product is necessary for survival. In particular, locomotor ability is reduced in $latheo^{P1}$ flies hemizygous for $latheo^{P1}$ or $latheo^{P1}$ flies heterozygous with a lethal allele. The isolation of several hypomorphic excision alleles, however, allows a specific link between *latheo* and learning/memory to be established.
Supported by grants from NIH (GM 33205), the McKnight Foundation, the John Merck Fund, and NIMH (MH09946).

418.21

THE RHYTHMIC MOTOR PATTERN CONTROLLING OVIDUCT CONTRACTIONS IN *LOCUSTA MIGRATORIA*: DESCENDING NEURAL CONTROL AND TRACING OF EFFERENT TARGETS. G. Facciponte* and A. B. Lange. Dept. of Zoology, University of Toronto, Erindale College, 3359 Mississauga Road, Mississauga, Ontario, Canada, L5L 1C6.

The oviduct and associated muscles of the reproductive system of *Locusta migratoria* are innervated by neurons in the VIIth abdominal ganglion which project along the oviducal nerves (OVNs). Interruption of *Locusta* during egg-laying results in rhythmic neural activity recorded extracellularly from the OVNs.

The rhythmic neural activity consists of up to five discernable efferent units and no afferent units. All units fired with temporally interrelated patterns. The motor pattern persists in isolated VIIth abdominal ganglia of disturbed egg-layers indicative of a central pattern generator. In non-egg-laying females, where neural activity at the OVNs is normally very low, the rhythmic motor pattern was initiated by transection of the VIth-VIIth abdominal connectives.

Units within the motor pattern were traced to their respective muscle targets. One unit was recorded from a branch of the OVN innervating the lower lateral and common oviduct. Other units were recorded from branches of the OVN innervating skeletal muscles in the VIIIth abdominal segment lying in close association with the ovipositor muscles. The motor pattern therefore coordinates the activities of both a visceral muscle and skeletal muscle groups.

These results suggest that contractions of the lower lateral and common oviduct as well as associated skeletal muscles are controlled, at least in part, by motor pattern generating circuits located in the VIIth abdominal ganglion. These circuits appear to be under descending inhibitory control with the inhibition being removed during the disruption of egg-laying.

418.22

DIFFERENTIAL SENSITIZATION OF THE HONEYBEE'S PROBOSCIS EXTENSION REFLEX. M.Hammer and G.Braun-Weninger*. Inst. für Neurobiologie, Freie Universität Berlin, FRG

We examined the effects of three different stimuli (sucrose solution to the antenna, to proboscis, or to both) on the response to an odor (carnation) in a sensitization paradigm. Responses to the control stimulus (carnation), the sensitizing stimulus (sucrose solution), and test stimuli (.5, 1, 2, 5 min later) were monitored by extracellular recordings from a muscle involved in proboscis movement. The number of muscle spikes per trial served to judge the response-intensity. Also, the percentage of successfully sensitized animals was compared among the different experimental groups. During the control trial we could discriminate animals which showed a response (spontaneous animals) and non-responsive animals (not spontaneous). In both groups a sensitizing effect was apparent with either treatment at the test trial at .5 min, but among the different experimental groups we found no differences for spontaneous animals. The results reported below refer to not-spontaneous animals.

At the first test trial the percentage of successfully sensitized animals was significantly greater when sensitized by antennal stimulation. In contrast, the response-intensity (i.e. muscle-spikes per trial) to the sucrose solution stimulus and during the first test trial was lowest. The experimental groups receiving stimulation to proboscis or to both proboscis and antenna did not show profound differences.

The combined results reveal that intensity and probability of the sensitizing effect are not linked in a trivial way. As a functional interpretation we suggest that antennal sensitization facilitates the sensory components of the reflex, while the procedures involving stimulation of the proboscis facilitate components of the motor program.

418.23

DIFFERENTIAL TONIC INHIBITION OF CRAYFISH LATERAL GIANT ESCAPE TO STIMULI IN DIFFERENT SENSORY FIELDS. F.B. Krasne, E.T. Vu, and S.C. Lee. Neuroscience Program, Dept. of Psychology, and Brain Research Institute, University of California, Los Angeles, CA 90024.

Crayfish lateral giant escape is subject to suppression by a "tonic inhibitory" pathway that descends into the abdomen from more rostral ganglia. Recent physiological evidence (Vu and Krasne, these abstracts) indicates that tonic inhibition is due to remote postsynaptic inhibition of the lateral giant command neurons. Remote postsynaptic inhibition theoretically has the capability to inhibit selected regions of a dendritic tree. As a preliminary test of whether the tonic inhibitory pathway can suppress responses to selected stimulus fields, we simultaneously monitored restraint-induced inhibition in two different abdominal segments to see if differential onsets or offsets could be observed. We found that inhibition tends to turn on at about but not exactly the same time in different segments. Thus, the different segments are subject to independent control. Experiments to search for differential inhibition of different fields within a hemisegment are planned.

Supported by USPHS grant NS08108 (FK) and a Predoct. NSF Fellowship (EV).

418.24

OCTOPAMINE INCREASES EFFICIENCY OF COMMISSURAL TRANSMISSION BETWEEN CRAYFISH LATERAL GIANTS. S.C. Lee and F.B. Krasne. Dept. of Psychology and Brain Research Institute, University of California, Los Angeles, CA 90024.

Octopamine is known to increase transmission between primary afferents and first order interneurons of the crayfish lateral giant escape reflex circuit and has been conjectured to mediate behavioral sensitization of the lateral giant escape reflex. Evidence that it also affects the lateral giants directly is now provided by the finding that octopamine ($8x10^{-5}$ - $3x10^{-4}$ M) decreases the time needed for one lateral giant to recruit its contralateral homologue by up to 20%. This effect is antagonized by the octopamine antagonist, phentolamine (10^{-4} M). The presence of functional octopamine receptors on LGs may facilitate analysis of octopamine's mechanism of action in this system.

Supported by USPHS grant NS08108 (FK).

HORMONAL CONTROL OF BEHAVIOR II

419.1

THE ONTOGENY OF BEHAVIORAL ASYMMETRY IN THE RAT: INFLUENCE OF SEX & PRENATAL TESTOSTERONE. K.J. Schultz and J. Quinn*. Psychology Department, University of Winnipeg, Winnipeg, MB, Canada R3B 2E9.

Behavioral asymmetry exists in rats and tends to be more strongly exhibited by females than males. Tail posture asymmetries assessed at Day 1 (birth=Day 0) have been found to be predictive of adult turning preference. This study assessed the development of behavioral asymmetries from Day 1 to Day 21 in male (N=44) and female (N=52) Sprague-Dawley rats with varying levels of prenatal testosterone exposure. Testosterone levels were inferred from ano-genital (AG) distance measurements taken on Day 0. The development of behavioral laterality was evaluated using measures of tail posture and negative geotaxis turing direction, while adult patterns of behavioral laterality were assessed on a circling, a swimming and a head turn task. Laterality measures taken on Day 1 revealed no turning preferences and did not reliably predict adult laterality. A rightward turning preference did emerge over the next two weeks, particularly for negative geotaxis, and over this period tail posture varied with sex and AG as well as with age. Measures taken on Days 3 to 15 were significant predictors of adult turning preference and adult behavior also varied as a function of sex and AG distance. Neural mechanisms underlying these changes are being explored.

419.2

RAPID NEUROPHYSIOLOGICAL ACTIONS OF CORTICOSTERONE RELATED TO STRESS-INDUCED INHIBITION OF SEXUAL BEHAVIOR IN AN AMPHIBIAN. J. D. Rose, F. L. Moore and M. Orchinik. Dept. of Psychology, University of Wyoming, Laramie, WY 82071 and Dept. of Zoology, Oregon State University, Corvallis, OR 97331.

Acute stress inhibits courtship clasping by male rough-skinned newts due to a rapid effect of corti-costerone (CORT). A binding site in neuronal membranes from newt brain, with properties correlated with the CORT inhibition of clasping has recently been characterized (Orchinik, Murray and Moore, Science, in press.) In the present study, neural actions of CORT were examined in male newts by microelectrode recordings from medullary reticulospinal neurons that were backfired (antidro-mically-activated) by spinal cord stimulation. Systemic administration of a behaviorally-effective dose of CORT produced an increase in antidromic spike amplitude within 5 min that was followed immediately by a progressive de-cline in excitability, in which spikes exhibited slowed rise rates and fragmentation of components. Topical medullary CORT application also depressed neuronal exci-tability, but more rapidly than systemically-administered hormone. The time course of CORT effects on these neurons corresponds well with the rate of onset of the hormone's inhibition of clasping and is consistent with a membrane mechanism of action. Supported by NIH grant NS13748 (J.R.) and NSF grants BNS8901500 and BNS8909173 (F.M.).

419.3

STEROID HORMONE MEDIATION OF THE EFFECTS OF ENERGETICS ON MALE FROG REPRODUCTIVE BEHAVIOR. C.A. Marler*, M. Ryan* and W. Wilczynski. Depts. of Zoology and Psychology, University of Texas, Austin, TX 78712.

Energetic factors might influence reproductive success in male túngara frogs (*Physalaemus pustulosus*) by limiting the best predictor of male mating success: time spent in chorus activity. We manipulated food intake and found that males with lower food intake called for fewer hours, even though their lipid reserves were not greatly decreased. Average lipid stores in males provided energy for calling approximately six hours a night for six nights.

Hormonal mechanisms potentially mediating this effect of energetics on behavior were also examined. One potential mechanism for decreasing calling is through lower testosterone (T) levels, possibly through increases in corticosterone (B) levels. Males with lower food intake had lower T-levels. Male reproductive behavior was associated with T-levels: calling males had higher T-levels than noncalling males. Two lines of evidence suggest that B might influence T levels; (1) B-levels were negatively correlated with T-levels, and (2) B-implants caused a decrease in T-levels. B may inhibit reproductive behavior directly. B-implants decreased calling behavior, but there was no difference in B-levels between fed and unfed males. However, levels were high in all groups, potentially masking a difference. These data suggest that plasma steroid levels may be mediating effects of energetic factors on reproductive behavior. (supported by Smithsonian Tropical Research Institute and NIMH grant T32 MH18837-01)

419.4

POSSIBLE ROLE OF ANDROGENS IN THE LOSS OF SECONDARY SEX CHARACTERISTICS IN BORNEAN VOICELESS FROGS C.N. Rowsemitt, S.B. Emerson*, and D.L. Hess*. Dept. of Biology, Univ. of Utah, Salt Lake City, UT 84112, and Oregon Reg. Primate Ctr, Beaverton, OR 97006

Four species of Bornean frogs lack a suite of behavioral and morphological secondary sex characteristics which occur in breeding north temperate and tropical frogs (enlarged fore-limbs, advertisement calls, vocal sacs, nuptial pads). We have examined plasma steroid levels in reproductively active males of one species of voiceless frogs (Rana blythi) to test the hypothesis that decreased circulating androgens could be at least part of the explanation for the unusual loss of these characteristics. Frogs were captured in Sabah, Malaysia, in 1989 and 1990 and immediately bled. Plasma androgens were lower in R. blythi than those reported for other species in the literature where comparable blood sampling techniques were used. We suggest that lowered androgen levels may be at least part of the explanation for the loss of behavioral and morphological secondary sex characteristics in voiceless frogs. [NSF grant #BSR-8822630 (SBE) and NIH grant #HD18185(DLH)]

419.5

TESTICULAR SECRETIONS MASCULINIZE VOCALIZATIONS IN FEMALE *X.LAEVIS*. J T. Watson and D. B. Kelley, Dept. Biol. Sci., Columbia Univ., NY, NY 10027

Male *Xenopus laevis* frogs produce a song consisting of alternating fast and slow trills while adult females do not sing. We examined the ability of testicular hormones to induce singing in females. Adult male and female frogs were gonadectomized and implanted with Silastic tubes which contained either testosterone propionate (TP) or nothing, and then tested after 13-17 months with sexually unreceptive females. While TP restored singing in castrated males, it did not induce singing in ovariectomized females. Since previous studies have shown that long-term TP treatment fully masculinizes laryngeal muscle contractile properties, these females' larynges were capable of song. Male and female frogs at various ages from metamorphosis to early adulthood (20-50 g B.W.) were gonadectomized and implanted with testes, grown to sexual maturity (as evidenced by growth of nuptial pads), and tested for singing. All testes-implanted females sang. The number of songs and total duration of singing were not significantly different from identically-treated or intact adult males. Females implanted early in post metamorphic development produced fully masculinized trills (53-70 Hz), but females implanted after 15 g B.W. produced trills which were slower (45-50 Hz). This study provides the first evidence that testicular hormones can induce singing in female *X. laevis*. Supported by NS23684 and NS08304.

419.6

CASTRATION DISRUPTS MALE RAT'S ABILITY TO RECOGNIZE FEMALE CONSPECIFICS. T. Mencio Wszalek and V.D. Ramirez, Beckman Institute, University of Illinois, Champaign, Illinois, 61820

We have previously reported that adult male rats are capable of recognizing sexually receptive adult female rats with whom they have previously interacted. Using the time spent in female-directed olfactory investigation as an index of recognition, we have observed that recognition is significantly disrupted 2, 22, and 44d post-castration; i.e., castrate males, in contrast to intact males, fail to exhibit less olfactory investigation of a familiar female. (▲T=Change in time spent investigating female from the first to the second interaction period, sec±SEM ; ▲T = 2±9, 10±14, and -5±13 sec for the 2, 22, and 44d castrates respectively; n=6 in each group.) Testosterone propionate (TP) (100ug/rat) replacement via subcutaneous injections at 24 and 1 hour prior to interaction test restored the 2 and 44d castrate males ability to recognize a familiar female conspecific (▲T= -35±7 sec for the 2d castrate + TP, n=5; and ▲T= -32±10 sec for the 44d castrate + TP, n=6). The recognition exhibited by the 2d castrates + TP persisted for more than three hours, while that of the 44d castrates + TP began to deteriorate after 2h. In addition, the 44d castrates + TP failed to exhibit mounting behavior during any of the interaction trials, while the 2d castrates + T mounted the familiar female significantly more rapidly during the second, third, and fourth interaction periods as compared to the first, (latency to mount in seconds±SEM = 255±29, 97±29, 129±70, and 127±71.) Thus, it appears that testosterone is a necessary requirement for the adult male rat's exhibition of recognition of a familiar female conspecific.

419.7

DISASSOCIATION OF JUVENILE PLAY FIGHTING AND ADULT DOMINANCE IN MALE RATS TREATED NEONATALLY WITH TESTOSTERONE PROPRIONATE. S. M. Pellis and V. C. Pellis. Dept. Psychology, Univ. Lethbridge, Lethbridge, AB. T1K 3M4

It is known that juvenile female rats play less than juvenile males and that neonatal treatment with testosterone proprionate (TP) elevates the frequency of play of females to that of males. Such treatment to males increases their play to levels above that shown by controls. Studies have indicated that the effects of such neonatal exposure to androgens have a masculinizing effect on brain and behaviour. In the present study, neonatal male rats were injected with TP (two injections of 0.05 ml each, one/day, of 250 µg/ml, s.c. in the nape) and a control group received an equivalent volume of the oil vehicle. At weaning, each injected male was paired with a male littermate, and they were housed together until 100 days old. At 30-40 days and at 80-90 days, they were isolated from one another for 24 h and then re-paired in a test exclosure, and their play behaviour was videotaped. This was repeated twice at each age. At the 30-40 day age class, TP treated rats played 2-3 times more frequently than the oil injected controls. This is comparable to other studies. When housed together, male pairs form a dominant-subordinant relationship which becomes more clearly asymmetrical in the weeks following sexual maturity (i.e., 50-60 days). Behavioural and physical measurements at 80-90 days clearly identified the dominant pairmate in each pair. TP treated rats were no more likely to become the dominant pairmates than were their untreated pairmates. Therefore, while neonatal exposure to additional androgens masculinizes play behaviour, it does not appear to influence other masculine behaviours related to aggression and dominance.

419.8

EMG ACTIVITY OF THE SEXUALLY DIMORPHIC SPHINCTER CLOACAE MUSCLE DURING COPULATION AND FOAM PRODUCTION. J.S. Park,* C.M. Seiwert, and E. Adkins-Regan, Departments of Psychology and Neurobiology and Behavior, Cornell University, Ithaca, NY 14853.

The foam gland complex (FGC) of Japanese quail (*Coturnix japonica*) is a large sexually dimorphic structure located in the dorsal wall of the cloaca which produces foam that is transferred to the female during copulation. The FGC consists of an aggregate gland whose units are interdigited with fibers of the large, circular sphincter cloacae muscle (mSC; Klemm et al., 1973). The major muscle of the cloaca, mSC is involved in copulation and defecation and may play a role in foam production by mechanically whipping the clear fluid secreted by the gland into its final form. Chronic EMG recordings from freely moving male quail were used to study potential mSC involvement in foam production and copulation, and to determine if the muscle is differentially active during these behaviors. A silastic "tophat" containing a female connector was implanted in the lumbosacral area of the back. Electrode wires were passed subcutanously to the cloacal region, where the wires were inserted into dorsal mSC. Different mSC movements accompany copulation and foam production. The mSC is responsible for elevation of the cloaca and extension of the vent during the cloacal contact movements (CCM; King, 1981) that characterize copulation. A series of spontaneously occuring rhythmic waves (SRW) of contraction are associated with foam production. EMG activity during CCM and SRW is composed of a series of high frequency waves, with bursts during CCM possessing a greater amplitude than those during SRW. SRW activity is rhythmic, although bursts occur more frequently immediately prior to mounting. Thus mSC exhibits different patterns of activity during copulation and the SRW associated with foam production in male Japanese quail. Future work will address whether mSC activity is necessary for the production of foam and whether manipulation of androgen levels alters mSC activity during either of these behaviors. [Supported by NSF # BNS 88-09441]

419.9

A SEX-DEPENDENT INFLUENCE OF TESTOSTERONE ON THE DORSO-MEDIAL NEURONAL POPULATION OF THE JAPANESE QUAIL INTERCOLLICULAR NUCLEUS. G.C.Panzica, N. Aste, J.Balthazart and C.Viglietti-Panzica, Dept. Human Anatomy & Physiology, Univ. of Torino, I-10126 Torino, Italy and Lab. Biochemistry, Univ. Liège, B-4020 Liège, Belgium.

The nucleus intercollicularis in quail is implicated in the control of a number of vocalizations. It is also a target structure for steroids and includes high numbers of cells containing androgen and estrogen receptors. We recently demonstrated that in another steroid target, the medial preoptic nucleus, the effects of testosterone are paralleled by significant changes in the neuronal size (J. Comp. Neurol., 303, 443-456, 1991). A morphometrical analysis of the nucleus intercollicularis was therefore undertaken in male and female Japanese quail (*Coturnix coturnix japonica*) that were either gonadectomized or gonadectomized and treated with testosterone or left intact as controls. This showed that, in males, testosterone increases the cellular size in the dorso-medial part of the nucleus: the mean neuronal area was larger in intact and testosterone-treated males than in castrates. Such an effect was not observed in females nor in the adjacent nucleus mesencephalicus dorso-lateralis which was taken as control structure because it is devoid of steroid receptors. The changes observed in the nucleus intercollicularis of males represent a morphological marker for the cellular actions of the steroid. They could provide a useful tool to analyze the mechanisms by which testosterone activates vocalizations in male quail. *Supported by EEC (SC1-0230C/TT), CNR (89.03043.04, 90.02456.04), MURST 40%, and FRFC (9.4601.90 and 2.9003.91).*

419.10

LOCALIZATION OF MOTONEURONS INNERVATING THE MAJOR MUSCLES OF THE CLOACA IN JAPANESE QUAIL. C.M. Seiwert and E. Adkins-Regan, Department of Psychology, Cornell University, Ithaca, NY 14853.

Four major striated muscles are associated with the dorsal cloaca in birds: the sphincter cloacae (mSC), the transversus cloacae (mTC), the levator cloacae (mLC), and the constrictor cloacae (mCC). By far the largest of these is the sexually dimorphic, hormone-sensitive mSC. Motoneurons responsible for the innervation of mSC lie in the lateral motor column of synsacral segments 7, 8, and 9 or 8, 9, and 10 (Seiwert and Adkins-Regan, 1987). The variability in rostral-caudal location reflects individual differences in which segments of the cord contribute to the pudendal nerve. We now report that the motoneurons supplying mTC, mLC, and mCC also lie in the lateral motor column in the same segments as those innervating mSC. A single small injection of cholera-toxin conjugated to horseradish peroxidase (CT-HRP) was made into the dorsal musculature of the cloaca [mSC-- 0.5 µl, n=7; mTC-- 0.3 µl, n=8; mLC-- 0.25 µl, n=2; mCC-- 0.25 µl, n=3]. Animals were sacrificed 48±3 hours later. Regardless of the muscle injected, labelled somata with multiple primary dendrites were located in the lateral motor column of synsacral segments 7, 8, and 9 or 8, 9, and 10. Bilateral label was observed after injections into mSC and mCC, while in mLC and mTC labelled somata were present only ipsilateral to the injection site. No differences existed in the proportion of labelled somata present in any given segment. Thus the motoneuron pools which innervate mTC, mLC, and mCC are distributed thoughout the larger pool of motoneurons innervating mSC. Work in progress addresses the following questions: 1) Are cloacal muscles other than mSC sexually dimorphic?; 2) Does a sex difference in muscle fiber type exist in cloacal musculature?; 3) Are cloacal motor neurons, like cloacal muscle, sensitive to sex steroids? [Supported by NSF # BNS 88-09441.]

419.11

ASYMMETRIC LESIONS OF THE SEXUALLY DIMORPHIC AREA AND RETRORUBRAL FIELD IMPAIR SEXUAL BEHAVIOR IN MALE GERBILS. P.D.Finn and P.Yahr. Dept. of Psychobiology, University of California, Irvine, CA 92717.

Bilateral radiofrequency (RF) lesions of the lateral part of the sexually dimorphic area (lSDA) of the gerbil hypothalamus disrupt male sex behavior (Commins & Yahr, '84). The lSDA projects to two midbrain areas, the retrorubral field (RRF) and the ventrolateral central gray (CGvl; Finn et al., unpub.) that are implicated in the control of male sexual behavior in rats (Brackett & Edwards, '84; Hansen et al., '82). To determine if pathways between these areas and the lSDA are involved in male sexual behavior, asymmetric lesions were used. Gonadally intact male gerbils were given unilateral RF lesions of the lSDA and contra- or ipsilateral RRF or CGvl, or sham lesions at both sites. They were tested for 3 weeks for male sexual behavior. The lesions were small and rarely destroyed the entire target area. An analysis of variance revealed that males with unilateral lesions of the lSDA and contralateral RRF were impaired on most parameters of copulation. Thus, pathways between the lSDA and RRF appear important in the control of male sexual behavior. Since RF lesions destroy both cell bodies and fibers of passage, we are now studying sexual behavior in male gerbils given unilateral RF lesions of the SDA combined with cell body lesions of the contralateral RRF. (Supported by ADAMHA grants MH26481, MH00478 and MH14599).

419.12

PELVIC AND HYPOGASTRIC NERVES CO-PARTICIPATION IN THE MEDIATION OF MALE RAT SEXUAL BEHAVIOR. R.A. Lucio, J. Manzo and P. Pacheco. CIRA, Univ. Auton. Tlaxcala; CIB Univ. Veracruzana; IIB-UNAM México, D.F.

Erection is controlled by the pelvic nerve (PN) while hypogastric nerve (HgN) controls emission and the fertility potential. Transection (Tx) of PN viscerocutaneous branch (PNVB), but not of HgN, alters some copulatory parameters. We analyzed the co-participation of both nerves in male rat sexual functions. Copulatory behavior and penile reflexes were tested before and after PNVB, HgN or PNVB+HgN Tx. One ejaculatory series plus penile reflexes or two ejaculatory series plus seminal plug presence (SPP) were scored. RESULTS: a) PNVB Tx increases the number of mounts and the intromission latency, while decreases the number of intromissions, the hit rate, the SPP, and the frequency of penile reflexes. All but SPP values recover progressively. b) HGN Tx reduces even more the SPP (to 20±10% vs to 50±8% of PNVB Tx); again this value never recovers. c) PNVB+HGN Tx produces similar effects than PNVB Tx, but eliminates SPP and penile reflexes; also with this Tx procedure neither parameters nor SPP recover. CONCLUSIONS: a) HGN participates in the regulation of copulatory parameters by compensating the alterations produced by PNVB Tx. b) HgN besides controlling seminal emission, participates in erection. c) PNVB besides controlling erection participates in emission. d) both nerves plays a decisive role on SPP. SEP C91 (P.P.); SEP C91; CONACyT D111903850 (J.M.).

419.13

THE MALE RAT LEVATOR ANI: EVIDENCE FOR A SEXUAL FUNCTION FOR A MODEL ANDROGEN-SENSITIVE MUSCLE. G.M. Holmes and B.D. Sachs. Dept. of Psychology, Univ. of Connecticut, Storrs, CT 06269-1020.

The levator ani (LA) of most species is involved in defecation. The sexual dimorphism and androgen sensitivity of the male rat LA has implied a reproductive function for the LA, but there has been no direct evidence for such a function. These experiments addressed the role of the LA in sexual and defecatory reflexes of the male rat. Penile bulb and rectal pressure changes were monitored during contraction of the LA and proximal bulbospongiosus (pBS). LA stimulation produced moderate increases in penile bulb pressure and negligible rectal pressure changes. LA denervation produced a 48% decrease in the pressure generated by the pBS relative to pBS muscle contraction with the LA intact. Removal of the denervated LA muscle reduced pressure further. EMG recordings from the LA and pBS during copulation revealed similar and coordinated activity in the two muscles. These data show that the levator ani (a) is an active component in a highly coordinated muscle system augmenting penile erection in the rat and (b) is unlikely to participate in defecation.

419.14

EFFECTS OF DISCRETE CELL BODY LESIONS ON SEXUAL AND SCENT MARKING BEHAVIOR IN MALE GERBILS. H. Hershey, T.R. Akesson, and C. Ulibarri, Dept of VCAPP, College of Veterinary Medicine, Washington State University, Pullman, WA 99164-6520.

Previous research demonstrates that the medial preoptic area accumulates gonadal steroids and plays a major role in the control of male scent marking and sexual behavior in Mongolian gerbils (Meriones unguiculatus). Other areas of the brain that also accumulate estrogens and/or androgens are suspected to be involved in the control of scent marking and sexual behavior in gerbils. This study focuses on the roles of the nucleus accumbens (ACC), bed nucleus of the stria terminalis (BST), medial nucleus of the amygdala (MeA), and ventral premammillary nucleus (PMv).

Adult male gerbils were evaluated for scent marking and sexual behavior in test arenas containing four raised blocks to elicit scent marking. The number of marks in the five minutes preceding sexual behavior testing were assessed. During sexual behavior testing the time to initiate a mount, the number of mounts, the time to show an intromission, the number of intromissions, the time required to ejaculate, and post-ejaculatory interval were also recorded. Gerbils were tested several days apart until each completed 3 ejaculations or 5 test sessions, whichever came first. Bilateral cell body lesions were then made by infusion of excitotoxin into the following areas (N = 10/group): ACC, BST, MeA, PMv, or control lesions. Behavior testing was repeated 2 days, 5 days, 1 wk and 2 wk after lesions were produced. Gerbils were then castrated and implanted with testosterone (T) capsules and tested 3 days, 1 wk and 2 wk to determine if behavior changes were the result of decreased T secretion following brain lesions. After behavior testing, lesion placement was confirmed histologically. Analysis of data was performed utilizing analysis of variance followed by orthogonal comparisons. Supported by HD 22869.

419.15

INDUCTION OF c-fos IMMUNOREACTIVITY WITHIN THE NEURONAL CIRCUITRY UNDERLYING COPULATORY BEHAVIOR IN THE MALE SYRIAN HAMSTER. S.S. Kollack and S.W. Newman. Department of Anatomy & Cell Biology, University of Michigan, Ann Arbor, MI 48109-0616.

We have analyzed the patterns of c-fos immunoreactivity within the neuronal circuitry underlying mating behavior in the male Syrian hamster (*Mesocricetus auratus*) in order to identify specific subpopulations of neurons involved in the mating behavior response. Three groups of adult male hamsters were studied: (1) sexually-naive hamsters as controls, (2) mated hamsters killed immediately after behavioral testing, and (3) mated hamsters killed one hour after behavioral testing. All hamsters were perfused with 4% paraformaldehyde and 40 um frozen sections were processed for immunocytochemistry using a polyclonal c-fos antibody (Oncogene Sciences). Compared to controls, mating induced at least a five-fold increase in the number of c-fos immunoreactive neurons in the caudal aspect of the medial preoptic nucleus, the magnocellular medial preoptic nucleus, and the medial subdivision of the bed nucleus of the stria terminalis. No difference was observed in the patterns of c-fos immunoreactivity between the two mated groups. These data provide a dynamic demonstration of the functional significance of specific neuronal populations to the mating behavior response. We are currently analyzing patterns of c-fos immunoreactivity in other brain areas to obtain the neuroanatomical distribution of neurons activated with mating behavior. (Supported by NIH NS 20629 to SWN.)

419.16

IMMEDIATE EARLY GENES INDUCED FOLLOWING SEXUAL BEHAVIOUR IN MALE RATS: DIFFERENTIAL EXPRESSION OF C-FOS IN LIMBIC STRUCTURES. M.J. Baum[1] and B.J. Everitt. Dept. of Anatomy, Cambridge University, Cambridge CB2 3DY, England & [1]Dept of Biology, Boston University, Boston, MA 02215.

The protein products of immediate early genes (IEGs: *c-fos, egr-1, c-jun)* were visualized immunocytochemically in the brains of male rats following different degrees of sexual interaction.

One hour following ejaculation, FOS was expressed prominently in the medial preoptic area (mPOA), bed nucleus of the stria terminalis, the medial amygdala (especially a cluster of cells in the posterodorsal part of the nucleus) and in a large group of neurons in the mesencephalic central tegmental field (CTF). Restricting interaction to 1 or 5 intromissions resulted in proportionally fewer FOS-immunoreactive (IR) neurons in these structures. Prolonged olfactory investigation during interaction with oestrous females induced FOS-IR generally in the medial amygdala, but not in the posterior dorsal nuclear cluster, minimally in the mPOA and not at all in the CTF. Olfactory investigation of females caged in a perforated Perspex compartment did not result in the appearance of FOS-IR in these structures. FOS-IR was detectable as quickly as 15min after ejaculation; the response increased through 30min and was maximal at 1h, but began to dissipate by 3h and was virtually undetectable by 6h.

The results suggest that IEG induction may be used to visualise a widespread neural system associated with the display of sexual behaviour in male rats. This activity-dependent response may provide a means by which to analyse the hormone-dependence and sensory determinants of such neural activity.

419.17

MOTONEURONS OF THE GUINEA PIG RETRACTOR PENIS ARE SEXUALLY DIMORPHIC IN SIZE BUT NOT NUMBER. Louise M. Freeman & S. Marc Breedlove, Psychology Dept., U.C. Berkeley, Berkeley CA 94720.

The retractor penis (RP) attaches between the penis and the pubis in male guinea pigs; a much smaller counterpart, the retractor clitoris (RC), is present in females. We previously reported that the associated motoneurons occupy the central region of the ventral horn from the L5 to S1 regions of both sexes (Freeman & Breedlove, Soc. Neurosci. Abstr. 115.4, 1989). To determine whether these motoneurons were sexually dimorphic in number or size, we counted and measured cells labeled by large muscle injections of horseradish peroxidase (HRP) into the muscle.

Under ketamine anesthesia, six adults of each sex were injected bilaterally with HRP (Sigma VI, 30%). After 3 days, we perfused the animals intracardially with saline, followed by 1% paraformaldehyde. Cords were frozen sectioned longitudinally at 50 µm, mounted and counterstained with neutral red. All HRP-labeled neurons with a visible nucleus were counted, and raw counts were corrected for split nuclei error by the method of Konigsmark. Nuclear and soma area were measured from camera lucida drawings of 12 motoneurons/animal.

	SOMA AREA ± SEM (μm^2)	MOTONEURON # ± SEM
Males	1292 ± 181	35±7
Females	908 ± 127	32±8

ANOVA shows a sex difference in MN size ($p<.002$) but not number ($p=.413$). The presence of a sex difference in size but not number is in agreement with our findings for the motoneurons innervating the guinea pig bulbocavernosus (BC) and ischiocavernosus (IC) (Freeman & Breedlove, Soc. Neurosci. Abstr. 380.15, 1990.

Supported by a NSF predoctoral fellowship (LMF) and March of Dimes (SMB).

419.18

EFFECTS OF BRAIN LESIONS ON PENILE REFLEXES. Edward P. Monaghan, Jamshid Arjomand & S. Marc Breedlove, Psychology Department, Univ.Calif., Berkeley CA 94720.

Motoneurons in the spinal nucleus of the bulbocavernosus (SNB) innervate striated perineal muscles which mediate reflexive cup-like erections of the penis. Midthoracic spinal transections potentiate these reflexes, indicating that brain regions modulate their expression (Sachs & Garinello, 1980). Brain areas which were previously implicated as afferents of the SNB (Monaghan & Breedlove, in press) were ablated to determine their role in mediating penile reflexes. Lesioned sites included the raphe, paraventricular (PVN), and lateral vestibular (LVN) nuclei of adult male rats. Ablation of the median raphe nucleus ($n=8$) increased the display of penile cups and flips ($p<.02$). PVN lesions ($n=6$) increased the latency to the first erection ($p<.05$) but did not otherwise affect penile reflexes. In animals with complete lesions of the LVN ($n=4$), penile reflexes were eliminated, however these lesions had a generally debilitating effect on the animals. These results suggest that the raphe, LVN, and PVN provide spinal projections which influence the expression of penile reflexes.

Supported by NIH NS28421.

419.19

EFFECTS OF INTRATHECAL TESTOSTERONE ON PENILE REFLEXES. Jamshid Arjomand, Edward P. Monaghan & S. Marc Breedlove, Psychology Department, Univ. Calif., Berkeley CA 94720.

The spinal nucleus of the bulbocavernosus (SNB), spanning L5 and L6 of the spinal cord, innervates perineal muscles involved in penile reflexes. Castration decreases the reflexes (Hart, 1967) and changes the morphology of motor neurons (Breedlove & Arnold, 1981; Kurz et al, 1986). To begin an investigation of the site of androgen action for these effects, we examined the concentration of spinally administered androgen required to maintain penile reflexes in castrated rats. Osmotic mini-pumps attached to intrathecal catheters administered testosterone (T) to the lumbosacral spinal cord in castrated adult males. T treatments were 4.8, 9.6, and 14.4 µg/day for two weeks. The 4.8µg/day dosage was not sufficient to maintain the reflexes, whereas the animals receiving 14.4µg/day dosage displayed reflexes comparable to precastration levels. Animals receiving 9.6µg/day of T displayed an intermediate response level. These results indicate that the T concentration necessary for maintaining these behaviors may be higher than previously suggested.

Currently underway is a study of the efficacy of intrathecal versus systemic T administration. In addition to gathering behavioral data, we will examine whether the various T treatments affect the morphology of SNB cells.

Supported by NIH NS28421.

419.20

INTRACRANIAL ANDROGENIC AND ESTROGENIC STIMULATION OF SIGNALING BEHAVIORS IN MALE RATS. J. A. Matochik and R. J. Barfield. Dept. Biol. Sci., Rutgers Univ., New Brunswick, NJ 08903.

Previous research in our laboratory has identified neural sites of steroid hormone action regulating copulatory behavior in the diagonal band of Broca (DBB)-anterior hypothalamic (AHA) continuum. The present study investigated the effects of central implants of testosterone (T) or estradiol (E2) in the restoration of scent marking and 50 kHz ultrasonic mating vocalizations (US) in castrate male rats. Signaling behaviors were tested for 10 min in the presence of estrous odor cues. After the pretest, males were castrated and tested for the absence or reduction of behavior; the animals then received stereotaxic implants of T or dilute E2 (1/100 cholesterol) with systemic DHT and were tested over four weeks for restoration of behavior. Central sites investigated with T were: DBB, AHA, and ventromedial hypothalamus; E2: DBB, lateral preoptic area, and medial preoptic area. Only implants of T or E2 in or near the DBB were effective in restoring scent marking (94% of pretest levels for E2 and 110% for T), an area we previously identified as important for E2 restoration of copulatory behavior. No significant restoration of US was observed in any of the implant groups. Blank cannulae or cannulae filled with cholesterol were completely ineffective in restoring either behavior. This preliminary study suggests that the neuroendocrine substrates for marking and US are different in the male rat. **(Supported by NIH grant HD-04484)**

419.21

PASSIVE ELECTRICAL PROPERTIES OF BULBOSPONGIOSUS MOTONEURONS IN GONADALLY INTACT AND CASTRATED ADULT MALE RATS. W.F. Collins, III Dept. of Neurobiology & Behavior, SUNY at Stony Brook, NY 19104.

Bulbospongiosus motoneurons (BS MNs) are smaller in castrated, as compared to gonadally intact, adult male rats (Kurz, et al., Science 232:395,1980). The present study was undertaken to evaluate possible changes in the passive electrical properties of BS MNs following castration. Gonadally intact and castrated (30-45 days) Sprague-Dawley rats (350-400 gm) were anesthetized, and *in vivo* intracellular recordings were obtained from BS MNs (1-8/rat) using conventional techniques. All lumbar and sacral dorsal roots were cut bilaterally. Passive membrane properties were assessed by injecting 1 nA of hyperpolarizing current (30-50 ms) and averaging ($n=32$-64) the resulting change in membrane potential. Following correction for anomalous rectification, input resistance (Rn) was measured. Estimates of membrane (T_0) and equalizing (T_1) time constants were made from the voltage transient following cessation of the current and used to calculate the electrotonic length (L). The data were analyzed using a two-level nested ANOVA, and the results are summarized below. Although mean Rn was larger in castrates, no significant difference was observed in any of the parameters. These results indicate that the passive electrical properties of BS MNs are relatively unaffected by castration. Supported by NIH NS24206 (WFC) and NS16996 & NS14899 (L.M. Mendell).

	#rats	#MNs	Rn (MΩ)	T_0 (ms)	T_1 (ms)	L
Intact	7	13	2.46 (0.42)	3.51 (0.43)	0.48 (0.08)	1.27 (0.12)
Castrate	5	19	3.29 (0.46)	3.71 (0.48)	0.59 (0.08)	1.38 (0.12)

unweighted mean (SE)

419.22

FEMALE HAMSTER VAGINAL SECRETION STIMULATES C-FOS EXPRESSION IN THE VOMERONASAL AND OLFACTORY MATING BEHAVIOR PATHWAYS IN THE MALE GOLDEN HAMSTER. J.M. Fiber and J.M. Swann. Dept. of Biological Sciences, Rutgers University, Newark, N.J. 07102.

Olfactory and vomeronasal chemosensory input is essential for arousal and execution of mating behavior in male golden hamsters. Precopulatory anogenital investigatory behavior exposes the male to Female Hamster Vaginal Secretion (FHVS) which stimulates copulatory behavior in males. FHVS is composed of volatile and hormonally regulated non-volatile components that stimulate the main olfactory system and the accessory olfactory system respectively. The chemosensory pathway regulating mating behavior and precopulatory anogenital investigation include the vomeronasal organ, the accessory olfactory bulb (AOB), medial (ME) and anterior cortical (C1) nuclei of the amygdala, medial preoptic area (MPOA), and the bed nucleus of the stria terminalis (BNST). Destruction of these nuclei abolish mating in male hamsters.

In the current experiment we determined which brain regions were stimulated by FHVS using c-fos immunocytochemistry as a marker of cell stimulation. Adult male golden hamsters were given FHVS on a swab 1-1.5 hours prior to sacrifice. Controls were given no stimulus. Exposure to FHVS stimulated regions that regulate mating behavior, including, the AOB, bed nucleus of the accessory olfactory tract, medial nucleus of the amygdala, BNST, and MPOA. Exposure to FHVS also stimulated regions of the main olfactory system, not directly involved in the regulation of mating behavior. These include the main olfactory bulb, anterior olfactory nucleus, endopyriform nucleus, horizontal and vertical limbs of the diagonal band of Broca (HDB and VDB), and pyriform cortex. In addition, regions which receive projections from the main and accessory olfactory systems or which are motor output regions also stimulated by FHVS include the anterior and lateral hypothalamus, paraventricular, reuniens and anterodorsal nuclei of the thalamus, cingulate and orbital cortex, frontal and insular cortex, raphe nuclei, central grey, and tegmentum. Brains of control animals showed very light diffuse staining in the main and accessory olfactory bulbs, medial preoptic area, anterior olfactory nucleus -medial, BNST, HDB, VDB, central, basolateral, C1 and ME, and frontal, pyriform, and parietal cortical regions. Our results show that exposure to FHVS stimulates regions known to regulate mating behavior as well as regions involved in arousal, motivation and motor function.

419.23

UNILATERAL LESIONS OF THE SEXUALLY DIMORPHIC AREA (SDA) OF THE GERBIL HYPOTHALAMUS DISRUPT MALE SEXUAL BEHAVIOR WHEN COMBINED WITH CONTRALATERAL LESIONS OF THE MEDIAL AMYGDALA-AMGYDALOHIPPOCAMPAL AREA (MA-AHi) OR MEDIAL BED NUCLEUS OF THE STRIA TERMINALIS (mBST). N. Sayaq* & P. Yahr. Dept. Psychobiol., U. Calif., Irvine, CA 92717.

N-methyl-d,l-asparate (NMA) lesions of the SDA impair mating in male gerbils given exogenous testosterone (T; Yahr & Gregory, unpub.). To learn more about pathways that may mediate male sexual behavior, we identified the afferents (DeVries et al., '88) and efferents (Finn et al., unpub.) of the SDA. Here we wanted to determine if any of three areas that are reciprocally connected to the SDA influence sexual behavior via these connections. Male gerbils given exogenous T were infused with NMA in the left SDA. Some received contralateral infusions in the MA-AHi, mBST or ventrolateral septum (vLS). Controls received both lesions on the left. Every control (13/13) mounted during at least one of four tests given 1-3 weeks after surgery; overall, they mounted in 75% of the tests. Males with lesions aimed at the SDA and contralateral vLS behaved similarly (13/14; 75%). In contrast, only half of the males with lesions aimed at the SDA and contralateral MA-AHi (6/10) or mBST (5/12) mounted (% tests with mounts = 40% and 29%, respectively). Thus the MA-AHi and mBST may affect male sexual behavior via projections to the SDA or by relaying the effects of SDA efferents. (Supported by MH26481 and MH00478).

NEUROPEPTIDES AND BEHAVIOR I

420.1

FUNCTIONAL CHANGES INDUCED BY HABENULA CELL TRANSPLANTS FOLLOWING LESION OF THE FASCICULUS RETROFLEXUS. E.W. Thornton*†, M. Murray, T.C. Eckenrode* and F. Haun (SPON: European Neuroscience Association). †Dept. of Psychol., Univ. of Liverpool, U.K. and Dept. of Anat., Medical Coll. Penn., Philadelphia, PA 19129.

Lesions of the fasciculus retroflexus (FR) de-afferent the interpeduncular nucleus (IPN) of both its cholinergic and peptidergic (Substance P) input from the habenula. Cell suspension transplants of embryonic habenula cells have been shown to restore normal Substance P staining in target sub-nuclei of the IPN and to restore normal REM-stage sleep behavior. We now show FR-lesioned rats are hyperactive relative to sham-operated controls in open-field tests over 3 months of testing, an effect potentiated in lesioned rats with transplants. In contrast, motor behavior assayed in a rotating-rod and a forced swimming test was similar to controls for both the lesion-only and transplant animals. The data indicate that transplants providing partial reinnervation of target IPN result in improvement or impairment of behaviors depending on the specific test paradigm. Supported in part by NATO, Burroughs Wellcome Fund and Wellcome Foundation.

420.2

LHRH AND SUBSTANCE P MODULATE *IN VITRO* ELECTROPHYSIOLOGICAL RESPONSES OF PERIAQUEDUCTAL GRAY NEURONS TO GABA AGONISTS AND NOREPINEPHRINE IN ESTROGEN-TREATED FEMALE RATS. Sonoko Ogawa, L.-M. Kow, S. Schwartz-Giblin & D.W. Pfaff. The Rockefeller University, New York, NY 10021.

LHRH and substance P (SP) are both known to facilitate lordosis behavior of estrogen-primed females rats by acting on periaqueductal gray (PAG). Previously, we have reported that SP excites PAG neurons through a neurotransmitter-like action whereas LHRH rarely acts this way (Ogawa et al., Soc. Neurosci. Abst., 16, #114.7, 1990). In the present study, we searched for modulation of responses by PAG neurons to lordosis-relevant neurotransmitters, as could be involved in behavioral effects of these neuropeptides. Extracellular single-unit activity was recorded from dorsal PAG in brain tissue slices prepared from estrogen-primed ovariectomized female rats. Effects of $GABA_A$ (THIP) and $GABA_B$ (baclofen) agonists and norepinephrine (NE) on firing rates (spikes/sec) were compared before and after LHRH (10^{-7}M) or SP (10^{-8}M: this dose of SP rarely excited PAG neurons) applications.

It was found that both LHRH and SP can modulate PAG neuronal responses. LHRH could either potentiate or attenuate inhibitory responses to THIP and baclofen, and tended to potentiate both excitatory and inhibitory responses to NE. These LHRH effects persisted over a long period of time (up to 60min). SP also had long-lasting neuromodulatory actions. It tended to potentiate excitatory action of NE and attenuate inhibitory action of NE. Finally, SP potentiated THIP and baclofen effects, consistent with facilitatory effects of SP and GABA on lordosis behavior in the PAG.

420.3

EFFECTS OF OPIATE-AMPHETAMINE CROSS-SENSITIZATION IN CONDITIONED REINFORCEMENT ARE MEDIATED BY MU- BUT NOT DELTA-SELECTIVE OPIATE RECEPTORS IN THE NUCLEUS ACCUMBENS. S.T. Cunningham and A.E. Kelley. Dept. of Psychology, Northeastern University, Boston, MA. 02115.

Chronic peripheral administration of opiates induces sensitization in various behavioral responses such that following repeated treatment, subsequent treatment elicits a potentiated response. Repeated intra-cranial injections of opiates into distinct midbrain regions also induce behavioral sensitization. In the following experiments, we investigated opiate sensitization and opiate cross-sensitization to *d*-amphetamine in the conditioned reinforcement (CR) paradigm, following infusion into a striatal subregion. In the CR paradigm, hungry rats (N=38) were trained to associate a compound stimulus (light/click) with delivery of a food pellet (primary reinforcement). During the test phase, a lever is introduced and when depressed, results in the compound stimulus (secondary reinforcement) alone. Each test session is 45 min. in duration, in which total lever presses for secondary reinforcement is recorded. All drugs were delivered into the nucleus accumbens (N. Acc.) over a volume of 0.5 µl per side. Microinfusion of drugs occurred over 6 test sessions, each separated by one day. The first 4 test days consisted of infusion of 0.9% saline, morphine (µ-selective, 0.5 µg), DAGO (µ-selective, 1.0 µg) or DPEN (∂-selective, 2.0 µg), and on the 5th and 6th test days, 2.0 and 10.0-µg doses of *d*-amphetamine were administered, respectively. CR responding was not enhanced by any of the opiate infusions, nor was there any indication of behavioral sensitization. However, animals given either of the µ-selective opiates for chronic treatment showed potentiated lever responding following *d*-amphetamine (2.0 and 10.0 µg), relative to either the chronic DPEN or saline-treated animals. The current results suggest that multiple injections of opiates in the N. Acc. induce an alteration in dopamine transmission, and that this effect is mediated by the mu-selective opiate receptor subtype.

420.4

REGULATION OF VENTRAL PALLIDAL-STIMULATED LOCOMOTION BY THE NUCLEUS ACCUMBENS DEPENDS ON ENKEPHALIN AFTER DOPAMINE DEPLETION. L. Churchill and P.W. Kalivas. Department of VCAPP, Washington State University, Pullman, WA 99164-6520.

Locomotor activity initiated by picrotoxin in the ventral pallidum (VP) is blocked by inhibiting dopamine receptors in the nucleus accumbens (NA). However, blocking opioid receptors with naloxone did not affect the picrotoxin-initiated locomotion in the VP. In this study, we depleted dopamine levels in the NA by >80% and analyzed the locomotor responses to pictrotoxin (0.17 nmol/0.5 µl/side) in the VP at least 10 days after 6-hydroxydopamine lesions (5 µg/3 µl/side/12 min) in the NA or a sham lesion with 0.2% ascorbic acid in sterile saline (3 µl/side/12 min). Locomotion was measured as horizontal photocell counts using an Omnitech photocell cage for a maximum of 6 trials with a minimum intertrial interval of 3 days. Surprisingly, after dopamine lesions, picrotoxin still increased locomotion from the VP. This locomotion was blocked by haloperidol (0.1 mg/kg, i.p.) or naloxone (1 mg/kg, s.c.). To demonstrate that the opioid receptors in the NA were responsible for the block by naloxone, naltrexone (0.6 µg/0.5 µl/side) was microinjected into the NA while picrotoxin was injected into the VP. Locomotor activity returned to control levels in the presence of naltrexone in the lesioned rats, but not in the sham-lesioned rats. These results suggest that <20% of the normal dopamine levels in the NA is sufficient to regulate VP-induced locomotor activity, but that enkephalin in the NA also regulates the VP-induced locomotion after dopamine lesions.

420.5

DIFFERENTIAL EFFECTS OF A CCK_A and CCK_B ANTAGONIST AGAINST MORPHINE CONDITIONED PLACE PREFERENCE (CPP). G.A. Higgins and E.M. Sellers. Departments of Pharmacology, Medicine and Psychiatry, University of Toronto and Clinical Research and Treatment Institute, Addiction Research Foundation, Toronto, Ontario, Canada M5S 2S1.

Experimental evidence consistently supports the involvement of the dopamine (DA) mesolimbic pathway in behaviours underlying drug-induced reward (e.g. morphine CPP). CCK is also closely associated with this system and accordingly may modulate a number of DA dependent behaviours. We have previously reported (Higgins et al, Eur J Pharmacol, 1991 [in press]) that pre-treatment with the CCK_A antagonist, devazepide, blocked the acquisition of a morphine CPP. In the present report we describe the effects of L365-260 (L), a selective CCK_B antagonist, on this behaviour. (L) was administered (0.001-10 μg/kg s.c.) 30 min prior to morphine (1.5 mg/kg s.c.). The CPP procedure was as described previously (Higgins et al, Eur J Pharmacol, 1991 [in press]). At no dose did (L) block morphine CPP, instead a mild potentiation was recorded. To study this further we examined the interaction between (L) (10 μg/kg) and a subthreshold dose of morphine (0.3 mg/kg). At these doses neither drug elicited CPP alone, however, when co-administered, a CPP was recorded ($p < 0.01$). In addition, a higher dose of (L) (1 mg/kg) also induced a significant CPP when administered alone ($+120 \pm 45$ sec, $p < 0.05$).

It is concluded that antagonism of CCK_A receptors may attenuate a behaviour indicative of opiate reward, while antagonism of the CCK_B subtype potentiates this response. These effects might be related to the differential roles of these receptors on DA release within the nucleus accumbens (Marshall et al, J Neurochem 56: 917-922, 1991).

420.6

MEDIAL PREOPTIC AREA CHOLECYSTOKININ INFUSIONS BLOCK BETA-ENDORPHIN INDUCED DISRUPTION OF MATERNAL BEHAVIOR IN LACTATING RATS. L.F. Felicio, P.E. Mann and R.S. Bridges. Dept. Comparative Medicine, Tufts University School of Veterinary Medicine, North Grafton, MA 01536.

Recent work has demonstrated that intraventricular infusions of cholecystokinin octapeptide (CCK-8) prevents the disruptive actions of beta endorphin (βE) on maternal behavior in lactating rats. Maternal care is also blocked when βE is infused directly into the medial preoptic area (MPOA). In the present study we investigated the possibility that CCK-8 might antagonize βE's action on maternal behavior at the level of the MPOA. Day 5-6 lactating rats previously implanted with bilateral cannulas in the MPOA were infused on Day 5 with saline and on Day 6 with βE (0.145 nmol) or βE plus CCK-8 (1.45 nmol). Testing for maternal behavior began 30 minutes after each infusion and responses were scored throughout the 1 hr test session and again at hourly intervals until all females were fully maternal. Approximately 50% of the βE plus CCK-8 rats were fully maternal within 60 min., whereas none of the βE alone rats responded during this period. In addition, animals treated with βE plus CCK-8 had significantly shorter latencies than βE animals to retrieve, group, and crouch over their pups. These results demonstrate that the administration of CCK-8 directly to the MPOA blocks the inhibitory actions of βE, and suggest that the MPOA is one site of dual peptidergic regulation of maternal behavior. [Supported by NIDA Grant #DA04291 to RSB].

420.7

ESTROGEN REGULATION OF PREPROENKEPHALIN-A IN TACHYKININ-CONTAINING CELLS IN THE RAT VENTROMEDIAL NUCLEUS OF THE HYPOTHALAMUS. C. A. Priest, P. Popper and P. E Micevych. Dept. of Anatomy and Cell Biology and the Laboratory of Neuroendocrinology, Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024-1763.

Regulation of lordosis behavior by the ventromedial nucleus of the hypothalamus (VMN) involves the interaction of estrogen with steroid-sensitive neuropeptide circuits, such as those which contain enkephalin (ENK) and the tachykinin substance P (SP). Estrogen induces ENK expression in the VMN but doesn't appear to affect expression of SP, although populations of estrogen-concentrating cells have been described that show ENK-like or SP-like immunoreactivity. Additionally, a previous immunohistochemical study (Priest, Popper and Micevych, Soc. Neurosci. Abst., *16*:325, '90) identified a population of neurons in the VMN that contained both ENK-like and SP-like immunoreactivity. The commercially obtained antisera used in that study, however, showed extensive cross-reactivity with other opiate and tachykinin peptides. To verify the protein content of the doubly-labelled cells, the current study was performed using the combined techniques of *in situ* hybridization and immunohistochemistry. Preproenkephalin-A (PPE-A) mRNA was localized to SP immunoreactive neurons of the VMN using a ^{35}S-labelled single-stranded cRNA probe complementary to the entire coding sequence of the PPE-A mRNA. The probe was transcribed from a 935 bp PPE-A cDNA inserted into a pSP6 vector (gift from Drs. Yoshikawa and Sabol, NIH, Bethesda, MD). The study also examined the quantitative effects of ovariectomy and estrogen replacement on protein expression in the doubly-labelled population of neurons. Supported by NS21220.

420.8

ANTIBODIES RAISED AGAINST sCCK-8 MIMIC sCCK-8 INDUCED INHIBITION OF LORDOSIS BEHAVIOR. P.E Micevych, C.A.Priest, R. K. Bittner and P. Popper. Dept. of Anatomy & Cell Biology and the Laboratory of Neuroendocrinology, Brain Research Institute, UCLA, Los Angeles, CA 90024.

Microinjections of sCCK-8 into the ventromedial nucleus of the hypothalamus (VMH) inhibit the display of lordosis behavior in estrogen primed male and female rats (Babcock et al. Physiol Behav, *43*:195,'88; Ulibarri et al. Neuroendocrinol. 52:70, '90). Since CCK tissue levels are higher in males than in females and the levels of CCK binding sites in the VMH are similar in both males and females, we hypothesized that endogenous CCK may act at the VMH of the male to inhibit the display of lordosis behavior. To test this, we microinjected 1 μl of undiluted polyclonal anti-CCK-8 serum (R183E, R. Elde, Univ. MN) into the VMH of gonadally intact males and castrated males that had been injected with 30 μg estradiol benzoate/day for 7 days. R183E attenuated the display of lordosis behavior in castrated rats 48 hrs after estrogen priming, thus mimicking the effects on lordosis behavior of microinjections of exogenous sCCK-8 peptide into the VMH of male and female rats. To examine whether R183E contained anti-idiotypic antibodies that interact with the CCK receptor, we sequentially incubated VMH tissue sections with R183E and then with ^{125}I-sCCK-8. There was no effect on levels of CCK binding in the VMH, suggesting that R183E did not displace ^{125}I-sCCK-8 from its binding sites. In a parallel experiment, ^{125}I-sCCK-8 binding at the VMH was attenuated in a dose dependent manner after incubation with a cocktail of ^{125}I-sCCK-8 and 1:50, 1:100, 1:200, 1:400 or 1:800 dilutions of R183E antiserum. The levels of ^{125}I-sCCK-8 binding increased with greater antibody dilution, indicating that R183E bound the ^{125}I-sCCK-8 and prevented interaction of the ligand with its receptor. Together, the results of these experiments refute the hypothesis that R183E contains sufficiently high titers of anti-idiotypic antibodies to account for its behavioral effects. Supported by NS21220.

420.9

THE EFFECTS OF SEX HORMONES ON CHOLECYSTOKININ RECEPTOR BINDING IN THE RAT BRAIN. P. Popper, C. A. Priest and P. E Micevych. Dept. of Anatomy and Cell Biology and the Laboratory of Neuroendocrinology, Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024-1763.

We investigated the effects of sex hormones on cholecystokinin (CCK) receptor binding in the ventromedial nucleus of the hypothalamus (VMN) and cingulate cortex of male and female rats. Female rats were ovariectomized (OVX) for two weeks and assigned to the following treatment groups: untreated, rats given estrogen benzoate (EB, 5 μg) 24 h and progesterone (P, 500 μg) 4 h before decapitation or implanted at OVX with silastic capsules containing testosterone propionate (TP). Males were intact, castrated for 4 weeks or castrated for 4 weeks and implanted at castration with TP silastic capsules. Thirty μm sections through the VMN were obtained and were processed for CCK receptor autoradiography using ^{125}I sulfated CCK octapeptide. Consecutive sections were processed identically with the addition of 50 mg/ml EB to the incubation buffer. In the VMN of EB+P treated rats, the level of receptor binding was reduced compared to untreated or TP treated OVX rats, thus mimicking previously reported binding following EB treatment alone. The latter two treatment groups did not differ in receptor binding. Castration reduced receptor binding in the VMN compared to intact or castrate+TP males. In the cingulate cortex, CCK receptor binding was not influenced by any of the treatments. However, when EB was added to the buffer, receptor binding increased in both the VMN and cingulate cortex. These results indicate that EB modulates CCK binding in the VMN. *In vivo*, EB reduces CCK binding, suggesting that EB may affect CCK receptor expression, while the *in vitro* effects of EB suggest that EB affects binding of the ligand to its receptor. TP *in vivo* facilitates expression of CCK receptors in the VMN. Supported by NS21220.

420.10

REGULATION OF PREPROCHOLECYSTOKININ mRNA IN MOTONEURONS OF THE SPINAL NUCLEUS OF THE BULBOCAVERNOSUS IN THE RAT. L.A. Abelson, P. Popper and P.E Micevych. Dept. of Anatomy and Cell Biology, Laboratory of Neuroendocrinology, Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024-1763.

Motoneurons of the spinal nucleus of the bulbocavernosus (SNB) are known to be regulated by androgens. Castration of a male rat reduces the size of SNB somata as well as both the size and number of chemical and electrical synapses onto SNB somata. Castration also regulates the expression of calcitonin gene-related peptide (CGRP) in SNB motoneurons by causing an increase in both mRNA and peptide levels (Popper and Micevych, Neuroendocrinol., 50:338-343, 1989; Popper and Micevych, Mol. Brain Res., 8:159-166, 1990). In this study we determined the effect of androgen on expression of preprocholecystokinin (preproCCK) mRNA in SNB motoneurons. In situ hybridization was done with a ^{35}S-labelled single-stranded cRNA probe complementary to the entire coding sequence of the preproCCK. Adult male rats were castrated for four weeks and compared with normal males as well as with males that were castrated and implanted with a silastic capsule filled with testosterone propionate (TP) for four weeks. Analysis of the in situ hybridization was done with both bright and darkfield optics and silver grains were counted on the Cue-2 image analysis system (Olympus Corp.). Motoneurons were considered labelled if the density of silver grains was 3X background. Overall, treatment had a significant effect on the level of preproCCK mRNA as measured by the density of silver grains over SNB motoneurons ($p < 0.01$). The density of silver grains over SNB motoneurons (mean $\pm$ S.E.M. x $10^2 \mu m^{-2}$) in castrate + TP was 60.3 ± 12.85 which was significantly higher ($p < 0.005$) than in castrates (14.04 ± 3.64) or in intacts (22.34 ± 4.17). This research was supported by NS21220.

420.11

ANXIOLYTIC-LIKE EFFECTS OF THE CCK-B ANTAGONISTS LY 262691, LY 262684 AND LY 247348 ON PUNISHED RESPONDING OF SQUIRREL MONKEYS. J.E. Barrett[1], M.C. Linden*[1], H.C. Holloway[1], M.J. Yu*[2] and J.J. Howbert[2]. [1]Dept. of Psychiatry, Uniformed Services University of the Health Sciences, Bethesda, MD 20814 and [2]Lilly Research Labs., Eli Lilly & Co., Indianapolis, IN 46285.

Cholecystokinin (CCK) has been proposed as an etiologic factor in anxiety, since the tetrapeptide form (CCK-4) induces panic attacks in humans and CCK antagonists have demonstrated anxiolytic-like effects in animal models of anxiety. In this study, two structurally distinct series of potent, selective antagonists for the brain CCK receptor (CCK-B) were assessed for anticonflict effects in a primate model of anxiety. Lever press responses of squirrel monkeys were maintained under a two-component schedule. During one component, a response produced food after a 3-min interval elapsed; during the second component, every 30th response during the 3-min interval resulted in the delivery of a mild electric shock (punishment or conflict procedure). At 0.3-10 mg/kg, p.o., the CCK-B antagonists LY 262691 and LY 262684 (pyrazolidinone class) and LY 247348 (quinazolinone class) all increased punished responding, with peak increases reaching approximately 150% of control performance levels at the 1.0 to 3.0 mg/kg doses. These doses had little effect on unpunished responding occurring in the alternate component. Agents with clinical efficacy in anxiety have been shown widely to produce increases in punished responding in this type of model. These results suggest that CCK-B antagonists of diverse structural types may be useful in the treatment of anxiety. The lack of suppressed responding in either component further suggests that such treatment could be accomplished without accompanying sedative activity.

420.12

CHOLECYSTOKININ RECEPTOR SUBTYPE ANTAGONISTS MODULATE EXPLORATORY LOCOMOTION IN THE RAT MESOLIMBIC PATHWAY. J.N. Crawley. Unit on Behavioral Neuropharmacology, Experimental Therapeutics Branch, National Institute of Mental Health, Bethesda, MD 20892.

High affinity compounds selective for the central cholecystokinin (CCK-A) or for the peripheral (CCK-B) receptor subtype now permit direct pharmacological analysis of the receptor subtypes mediating the actions of CCK. These compounds also provide critical tools for investigating the functional role of endogenous CCK. L-364,718, a CCK-A antagonist, L-365,260, a CCK-B antagonist (both gifts from Merck, Sharp and Dohme), and CI-988, a water soluable CCK-B antagonist (gift from Parke-Davis), were administered to male Sprague Dawley rats in several behavioral paradigms previously used to analyze CCK-dopamine interactions.

1) Antagonists were microinjected 5 minutes before saline, dopamine, or dopamine+CCK, into the a) nucleus accumbens, or b) ventral tegmental area, immediately before a 15 minute session in the Digiscan exploratory activity monitor. 2) Antagonists were microinjected into the a) nucleus accumbens, b) ventral tegmental area, or c) intraperitoneally, to test for modulation of dark-induced hyperlocomotion. Comparisons of anterior versus posterior nucleus accumbens and ventral tegmental area will be drawn, in an attempt to delineate the postulated contribution of endogenous CCK to the activity of the mesolimbic dopamine pathway.

420.13

HEART RATE CHANGES FOLLOWING ADMINISTRATION OF PUTATIVE PANICOGENIC CHEMICALS IN SQUIRREL MONKEYS. J.D. Newman and L.J. Crepeau. Laboratory of Comparative Ethology, NICHD, NIH, Poolesville, MD 20837-0289.

Clinical studies have determined that intravenous sodium lactate and cholecystokinin tetrapeptide (CCK-4) induce panic episodes in susceptible human subjects. Studies of the physiological changes accompanying panic attacks in humans indicate that heart rate is accelerated (tachycardia) following both spontaneous and lactate-induced panic episodes. We used chair-restrained squirrel monkeys (Saimiri) to test the effects on heart rate of slow iv infusions of sodium lactate, CCK-4, and a muscarinic receptor antagonist (benactyzine) shown in previous work to enhance alarm responses in squirrel monkeys. Eight adults (four males and four females) were adapted to chair restraint following institutional ACUC guidelines. During weekly experiments, the monkeys were removed from their home cages and given a light sedative dose of ketamine, after which an intravenous catheter was introduced into the saphenous vein and cardiac electrodes were affixed to the chest. Lactated Ringers/5% dextrose solution was slowly dripped into the vein throughout the experiment. After recovery from ketamine, a 20 minute period of undisturbed heart rate data was collected, followed by infusion of 0.5 M DL-sodium lactate (High dose), 0.25 M sodium lactate (Low dose), or High lactate plus a high or low dose of CCK-4. Lactate was infused at a rate of 0.5 ml/ min. for 20 minutes. CCK was infused at a rate of 0.05 ml/min in a concentration of 20 or 100 ug/ml. We found that the higher dose of sodium lactate with or without CCK produced a similar pattern of heart rate change, consisting of a transient tachycardia over 2-5 min., followed by incremental bradycardia over the remainder of the 20 minute trial. The low dose of sodium lactate failed to produce transient tachycardia but did result in bradycardia. We conclude that DL-sodium lactate at a dose that triggers panic attacks in susceptible humans produces a reliable pattern of heart rate change in a nonhuman primate model consistent with expected changes during panic episodes.

420.14

INTRAPERITONEAL INJECTIONS OF CHOLECYSTOKININ INCREASE [^{14}C] 2-DEOXYGLUCOSE UPTAKE IN THE AREA POSTREMA AND SOLITARY NUCLEUS OF THE RAT. M. R. Alberro, J. A. Deutsch* and M. F. Gonzalez. Department of Psychology, UCSD, La Jolla, CA 92093

The gastrointestinal octapeptide cholecystokinin (CCK-8) has been hypothesized to act as a satiating agent. There is still some controversy regarding its putative mechanisms of action since there is also evidence that it can produce conditioned taste aversion learning. In this study we investigated the pattern of [^{14}C] 2-deoxyglucose (2-DG) uptake in brain areas involved in the perception of nausea following CCK-8 injections. Twenty-hour-fasted Sprague-Dawley rats (300-400 g) were used as subjects. CCK-8 (10 mg/kg, Bachem) or control saline injections were administered intraperitoneally and placed in a restraining cage. Ten minutes later the rats received an injection of 2-DG (167 µCi/kg) via the lateral tail vein. Forty-five minutes later the rats were sacrificed, their brains removed, sectioned, and autoradiographed using standard procedures. The brain sections of CCK-8-treated subjects exhibited an increase in 2-DG uptake in the area postrema (AP) and the nucleus of the solitary tract (NTS). The effect encompassed the entire the AP, but was obvious only in the most caudal region of the NTS. The brains of animals that received injections of a similar volume of isotonic saline did not show apparent increases in 2-DG uptake in these regions. The present findings suggest that the decreases of food intake observed by other investigators following systemic injections of this neuropeptide, can be attributed to the activation of neural substrata that mediate malaise. Whether lower doses of CCK-8 that produce reductions in eating also alter the metabolic activity of the area postrema and solitary nucleus is to be determined by further experimentation.

420.15

INTRA-ACCUMBENS NEUROPEPTIDE Y PRODUCES REWARD: A CONDITIONED PLACE PREFERENCE THAT IS BLOCKED BY PRE-TREATMENT WITH A DOPAMINE ANTAGONIST. S. A. Josselyn and R. J. Beninger. Dept. of Psychol., Queen's University, Kingston, K7L 3N6, CANADA.

Neuropeptide Y (NPY) has been localized in the nucleus accumbens (N. Acc.) and may influence dopamine (DA) neurotransmission. Extensive data implicate N. Acc. DA in the neurochemical substrate of reward. This raises the possibility that NPY microinjected into the N. Acc. may produce rewarding effects that are mediated by DA. Two experiments replicated the previous findings that systemic (2.0 mg/kg, ip) or central (10.0 ug in 0.5 ul, bilaterally in the N. Acc.) amphetamine produced a conditioned place preference (CPP). The third experiment examined whether NPY would produce a similar CPP and if this preference would be influenced by a dopamine antagonist. Each experiment consisted of three distinct phases; pre-conditioning, conditioning and test. An increase in the time spent in the drug-paired conditioning compartment from the pre-conditioning to the test phase was accepted as evidence for a CPP. Intra-accumbens NPY (0.1 ug in 0.5 ul, bilaterally) produced a CPP. Furthermore, this preference was blocked by pre-treatment with a dose of cis-flupenthixol (20.0 ug in 0.5 ul, bilaterally in the N. Acc.) that, alone, produced no effect. These results strongly suggest that NPY exogenously applied to the N. Acc. produces reward. In addition, the NPY reward effect may be mediated by DA.

420.16

HIPPOCAMPAL AND VENTRICULAR ADMINISTRATION OF NEUROPEPTIDE-Y MODIFIES DELAYED MATCHING-TO-SAMPLE ACCURACY.

J. R. Thomas and S. T. Ahlers. Naval Medical Research Institute, Bethesda, MD 20889.

There is increasing evidence that neuropeptide-Y (NPY), one of the most prevalent neuropeptides in the brain, is important in memory processing. As one of the highest concentrations of NPY and NPY receptors is in the hippocampus, a structure important in normal short-term or working memory, the purpose of the study was to examine the effects of central NPY administration on working memory in rats. A delayed matching-to-sample (DMTS) task was used to assess working memory. The subjects, Long-Evans rats, performed on the DMTS daily in an operant chamber for 180 trials. Each trial began with the illumination of a cue-light over one of two response levers on the front chamber wall. The rat had to press the lever under the cue-light which turned off that light and initiated a delay interval of 2 to 60 sec. During the delay interval the rat was required to respond on a lever located on the rear chamber wall. At the end of the delay interval both cue-lights were illuminated over the two front wall levers. A response to the previously responded to cued-lever was recorded as a correct response and was followed by delivery of a food pellet. NPY was administered bilaterally into the dentate gyrus of the hippocampus (0.06 - 4.0 µg) immediately before a session or into the lateral ventricle (0.3 - 20.0 µg) 45 min before a session. Low doses of NPY consistently increased accuracy of performance at longer delays while higher doses decreased accuracy. No NPY effects were obtained on acquisition or reference memory. These data shown that NPY can profoundly modulate short-term or working memory as measured by accuracy of performance on DMTS.

420.17

EFFECTS OF BENEXTRAMINE, A PUTATIVE NPY RECEPTOR ANTAGONIST, ON SEXUAL BEHAVIOR IN MALE AND FEMALE RATS. J.T. Clark and A. Keaton*. Dept. Physiol., Meharry Medical Coll., Nashville, TN.

Adrenergic transmitters and NPY have been implicated in the regulation of sexual behavior. We assessed the effects of benextramine (BXT), an α-adrenergic and NPY receptor antagonist, on sexual behavior. For males, sexually experienced rats received either benextramine (3 mg/kg, IP) or vehicle, 30 min prior to mating tests. BXT induced a selective increase in the copulatory rate (decreased ICI). In a second study, BXT pretreatment (3 mg/kg, 30 minutes prior) failed to attenuate the suppressive effects of clonidine (250 μg/kg, 5 min prior to mating tests). However, clonidine administration reversed the effects of benextramine on the copulatory rate. In ovariectomized females, progesterone (P) treatment typically exerts a biphasic effect on copulatory behavior, initially (4-10 h) there is a synergy with estradiol (E) which is followed (>20h) by a profound inhibition. We hypothesized that this inhibitory effect of P could be due to increased release of NPY. Rats were tested for female sexual behavior 4h post-P (52h post E) following which they were administered BXT (0, 3 or 15 mg/kg, IP). Subsequent tests were administered 24,48,72, and 96h post-BXT. BXT treatment attenuated the inhibitory effects of P on receptivity (lordosis quotients and percent of responding animals) without affecting either proceptive or rejection behaviors. These data indicate that blockade of NPY (and α-adrenergic) receptors is associated with selective enhancements of specific components of sexual behavior in male and female rats. Taken together with previous data on the effects of central administration of NPY, a physiological role for this peptide is suggested.

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420.18

HYPERAMMONEMIA DECREASES NPY-INDUCED FEEDING. W.T.Chance, F.S.Zhang*, T.Foley-Nelson and A.Balasubramaniam*. Dept. Surgery, Univ. of Cincinnati Med. Ctr. and VA Med. Ctr., Cincinnati, OH.

We reported that feeding to neuropeptide Y (NPY) is reduced in anorectic tumor-bearing (TB) rats (Ann. NY Acad. Sci., 611:497,1990) and that hyperammonemia may be a primary cause of experimental cancer anorexia (Brain Res. 486:316,1989), In this study we tested the effect of hyperammonemia on NPY-induced feeding in normal rats. Two weeks after the implantation of cannulae into the periventricular hypothalamic area, the external jugular vein of adult, male, F 344 rats was cannulated. Normal saline (SAL) was infused (2 ml/hr) for four days, after which half of these rats were switched to infusion of 0.2-0.3 M ammonium acetate + ammonium bicarbonate (pH = 7.7). On the 4th day of ammonia infusion, noninfused (n=5), saline-infused (n=5) and ammonia-infused rats(n=7) were tested for feeding to 1 ug NPY. Compared to saline-infused rats, food intake was reduced by 65% (1 hr) and 58% (4 hr) in ammonia-infused rats. Although 0.2 M ammonium salts had no significant effect on twenty-four hr feeding, infusing 0.3 M ammonium reduced ad lib. food intake by 44%. These results indicate that hyperammonemia reduces NPY-induced feeding and suggest that the increase in blood ammonia concentration may be responsible for decreased feeding to NPY in TB rats.

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ALZHEIMER'S DISEASE: EXPERIMENTAL MODELS

421.1

REAGGREGATING CULTURES OF MOUSE TRISOMY 16 BRAIN. D.E. Bredesen, D.J. Kane*, D.M. Holtzman*, C.J. Epstein*. Reed Neurological Research Center, UCLA, Los Angeles, CA 90024-1769; and Depts. of Neurology and Pediatrics, UCSF, San Francisco, CA 94143.

The development of an in vitro model of Alzheimer's disease would be advantageous in the analysis and treatment of neurodegenerative diseases. Because Down's syndrome (trisomy 21) patients over the age of 40 develop the pathology of Alzheimer's disease in virtually all cases, and mouse chromosome 16 is syntenic with HSA 21 over a large region of the long arm of HSA 21, we have been analyzing long-term reaggregating central nervous system cultures of trisomy 16 mice and control euploid littermates. Cultures were reaggregated at embryonal day 15, and followed for 11 months. Within four months after reaggregation, marked differences were noted between control and trisomy 16 cultures. Lipid-laden cells appeared in the trisomy, but not control, cultures. Immunohistochemistry, using an antibody directed against residues 1-28 of human beta-amyloid peptide (anti-BAP; a gift from D. Serban), produced vascular and parenchymal staining of trisomic but not control cultures. The parenchymal staining occurred in foci of amoeboid microglia and in a second, as yet unidentified, cell type. To distinguish between staining of beta-amyloid precursor protein and beta-amyloid peptide, electron microscopy was performed on sections of the reaggregating cultures. Abundant extracellular fibrils of approximately 10-15 nm diameter were detected in the trisomy 16 cultures, whereas only very rare extracellular fibrils were detected in euploid cultures. Immunoelectron microscopy demonstrated binding of anti-BAP antibodies to some, but not all, of the fibrils in the trisomy 16 cultures. Pre-adsorption with mouse beta-amyloid peptide prevented the binding. These findings are compatible with previous suggestions that cells of the macrophage lineage may play a key role in amyloid formation.

421.2

EXAMINATION OF ALZHEIMER-TYPE PATHOLOGY IN MOUSE TRISOMY 16 NEURONS MAINTAINED BY TRANSPLANTATION. J. Stoll, B. Ault, A. Balbo*, S. I. Rapoport and A. Fine, Laboratory of Neuroscience, National Institute on Aging, Bethesda, MD 20892 & Dalhousie Univ., Halifax, NS, CA.

Hippocampal tissue from day 15-17 fetal mice, trisomic for chromosome 16, or their normal littermates, was transplanted with or without dissociation into the striatum or the lateral ventricle of 6-8 week old female C57Bl/6 mice. After 2 weeks to 18 months of survival, host brains were sectioned and the grafts were examined by *in situ* hybridization or by immunocytochemistry for antigens present in pathological brain structures of Alzheimer's disease patients. The grafts contained a layer of large neurons similar to the pyramidal cell layer of the normal adult hippocampus. No obvious morphological difference was detected by cresyl violet staining between trisomic and control transplants. RNA's for amyloid precursor protein (with and without the KPI domain), for GAP-43 and for somatostatin, genes located on Mu16, were assayed by *in situ* hybridization at 2 weeks and 4 months of survival and showed similar patterns of expression. Antibodies against ubiquitin, neurofilaments and tau failed to demonstrate the presence of Alzheimer type pathology in the trisomic or control grafts. These results differ from those of Richards et al., EMBO J. (1991) 10, 297-303, who reported Alzheimer type degeneration in trisomy 16 transplants.

421.3

PHORBOL ESTER-INDUCED ABERRANT SPROUTING IN THE RAT NEOCORTEX AS A MODEL OF AD NEUROPATHOLOGY. E. Masliah, M. Mallory*, N. Ge*, R.D. Terry*, T. Saitoh, University of California, San Diego, La Jolla, CA 92093

Previous studies in Alzheimer disease (AD) have shown that synapse loss and aberrant sprouting are prominent features of AD neuropathology (*Neuron*, 1991, in press). To further explore these aspects of AD, we developed a rodent model by combining denervation and neurointoxication. The rationale is that AD pathogenesis may be a multi-step process, such as a widespread synapse loss followed by a neurotoxic event that eventually will lead to an ineffective and abnormal repair process in the denervated regions. The main neurotoxin that we tested was phorbol 12-myristate 13-acetate (PMA) which has been described to activate and eventually down-regulate PKC and promote the expression of amyloid genes in the rat. Preliminary studies have shown that the administration of multiple injections of 2mM PMA in the denervated neocortex of the rat promotes, in the first week post-injection, a widespread vacuolization of the neuropil with a subsequent disruption of the synapses in the injection site followed by aberrant sprouting and dystrophic neurite formation at day 15 (in 7 of 10 rats). These neuropathological changes are similar to the synaptic abnormalities observed in AD. Control experiments (n=7) where the vehicle was injected, with or without contralateral lesion, failed to show aberrant sprouting with dystrophic neurites. Immunohistochemical analysis indicated that the dystrophic neurites in the areas of denervation and PMA administration were anti-synaptophysin and anti-GAP43 positive with an increased APP immunoreactivity surrounding them. APP immunoreactivity was mostly associated with pyramidal neurons in the cortex. At the ultrastructural level the PMA-induced abnormal neurites contained abundant dense and laminated bodies as well as synaptic vesicles. These organelles displayed synaptophysin immunoreactivity in their outer membranes.

421.4

SELECTIVE EXCITOTOXIC NEURONAL LOSS IN THE RAT ENTORHINAL CORTEX CAUSES MEMORY DEFICIT AND ALZHEIMER-LIKE CHOLINERGIC SPROUTING IN THE DENTATE GYRUS OF THE HIPPOCAMPUS

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The entorhinal cortex (EC) is one of the most severely afflicted brain regions in Alzheimer's disease (AD), in Down's Syndrome, and in aging; it is the earliest to exhibit the classic neurofibrilary tangles (NFT's) and continues to exhibit the greatest number of NFT's throughout the progression of disease. While previous anatomical and behavioral research has included primarily surgical or electrolytic lesions, in the present study we used NMDA to cause selective bilateral neuronal loss in the EC, as an approach to model the early neurodegeneration of AD and aging. Lesioned, sham lesioned and intact control rats learned a novel reference memory task involving a brightness discrimination for water reward. Rats were trained over 1 week until reaching criteria and tested for retention after a 10 day interval. In the retention test, EC-lesioned rats were significantly impaired ($p<.05$) compared to control and sham lesioned groups. Anatomical analysis confirmed excitotoxic lesions of EC and also showed evidence of denervation plasticity in the outer molecular layer of the dentate gyrus, by extensive cholinergic sprouting into this region, similar to that seen in AD. This functional and anatomical study of the EC demonstrates EC's role in reference memory and raises the possibility of functional deficits resulting from partial and selective neuronal loss in this area.

421.5

AN ANIMAL MODEL OF ALZHEIMER'S DISEASE: IMPLICATIONS FOR SPATIAL DISORIENTATION, MEMORY LOSS AND WANDERING BEHAVIOR. D. Hoffman*, & J. P. Ryan, Department of Psychology, State University of New York at Plattsburgh, Plattsburgh, NY 12901

Spatial information from cortical areas is received and processed by the dentate gyrus of the hippocampus. We have found that degeneration of this area in rats results in spatial memory impairments and mimics the wandering behavior in Alzheimer's disease. The present study measured spatial memory impairments using the Morris water maze task. A baseline of maze performance was recorded for twenty-two Long-Evans hooded rats for a period of six days (36 trials). The experimental group (N = 11) received bilateral intradentate injections of colchicine (15 µg/µl), while a sham group received bilateral intradentate injections of saline. Ten days following surgery, both groups were behaviorally tested in the maze for a period of three days (18 trials). By changing the platform location of the maze (i.e. the goal) a new task was introduced and tested for three days (18 trials). In comparison to the control group, the lesioned animals learned the behavior equally as well during baseline. However, they failed to recover their memory for the maze after surgery and also failed to learn a new task, whereas the control group consistently located the platform across all testing conditions. The results indicate that disruption to the spatial information processing system (dentate gyrus) interrupts and impairs recently acquired spatial memory, while impeding new learning of a spatial task. This suggests that in Alzheimer's disease, spatial disorientation in familiar environments could be related to degeneration of the dentate gyrus. Furthermore, the introduction of a new environment may exacerbate an already disoriented condition.

421.6

BEHAVIORAL AND MORPHOLOGICAL EFFECTS OF ASTROCYTE TRANSPLANTS IN RATS WITH NUCLEUS BASALIS LESION. L. Lescaudron, Z. Fulop*, R. Sutton, H.M. Geller and D. Stein. Brain Research Lab., Rutgers Univ., Newark NJ 07102 and UMDNJ-Robert Wood Johnson Med. School, Piscataway, NJ 08854.

Adult male rats received transplants (TP) of dissociated 30-day old cultured cortical astrocytes [AST: labeled with bisbenzamide (BIS)] into the nucleus basalis magnocellularis (NBM) or into the frontal and parietal cortex (CX) immediately after ibotenic acid lesion of the NBM. Nine days after surgery rats with AST-TP into NBM or CX were as impaired in the acquisition of a passive avoidance (PA) task as untreated counterparts. No effects were found for PA retention. Brains were processed for BIS, GFAP-ICC, ACh-E and cytochrome oxidase (CO) 14 days after TP. Host brain gliosis, but no surviving AST-TPs, were observed in the NBM. AST-TPs had no effect on size of the lesions. Concentrations of ibotenic acid used to lesion the NBM killed AST in vitro within 24 hours, suggesting a similar fate for AST-TPs in NBM. BIS-labeled AST were observed in CX, but these AST-TPs produced severe laminar disruption, gliosis and decreased ACh-E and CO staining.

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421.7

BEHAVIORAL AND PHYSICAL DISTURBANCES OF RATS WITH IBOTENIC ACID LESION OF BASAL FOREBRAIN. C.Hara and N.Ogawa. Dept. of Pharmacol., Ehime Univ. Sch. of Med., Ehime-ken 791-02, Japan.

In this study, characteristics of behavioral and physical disturbances of rats with ibotenic acid lesions of the bilateral nucleus basalis of Meynert (NBM) were analyzed in connection with physical disability or abnormal behavior in patients of Alzheimer's disease. Male Wistar rats (9 weeks old) were used. They were maintained in the air-conditioned room with 12:12 LD cycle, and were housed in the running wheel or the activity meter (Scanet SV-10, Toyo-sangyo) cages. Ibotenic acid (Sigma; 5µg/0.5µl/2 min) was injected into the NBM (Paxinos & Watson;A:7.7,L:2.3,V:7.1) under pentobarbital anesthesia (50 mg/kg, i.p.). After the surgery, rats were forcedly given milk daily. In the results, the NBM-lesioned group revealed body weight loss, disruption of food-intake, and disturbance of circadian rhythm of activity with the increased daytime activity. These disturbances recovered within 1 week accompaying with recovery of body weight in most of the rats. However, several rats showed no recovery with urinary incontinence. The results suggest that these disturbances may be attributable to damage to the adjacent lateral hypothalamus, and are interested in the physical disability model.

421.8

MEMBRANE DEFORMATIONS IN A CELLULAR MODEL FOR ALZHEIMER AMYLOIDOSIS. G.E. Maestre, R.E. Majocha, B. Tate, E.M. Sajdel-Sulkowska, and C.A. Marotta. Massachusetts General Hospital, McLean Hospital and Harvard Medical School, Boston, MA 02114.

PC12 cells were permanently transfected with DNA corresponding to the ß/A4 to C terminal region of the amyloid precursor protein of Alzheimer's Disease. Integration of human amyloid DNA was confirmed by Southern blotting. Transfected clonal cell lines and controls were examined at both the light and electron microscopic levels. Many morphological parameters are similar between transfected and control cell lines, including number and size of mitochondria and number of lipofuscin inclusions. However, transfected cell lines exhibited numerous membrane extensions not seen in similar quantities in the control cell lines. Membrane extensions include ruffles and blebs. Extensions may be related to reorganization of membrane proteins and/or the cytoskeleton. Similar cellular deformations, if present in Alzheimer brain cells, may indicate disturbances in membrane-associated functions, including cell-cell interaction. Supported by NIH AG02126 and Metropolitan Life Foundation. GEM supported by University of Zulia.

421.9

Senile plaques do not support neurite outgrowth *in vitro*. M.K. Carpenter[1], K.A. Crutcher[2], S.B. Kater[1]. [1]Dept. of Anatomy and Neurobiology, Colorado State Univ., Fort Collins. CO 80523; [2] Dept. of Neurosurgery, Univ. of Cincinnati Med. Ctr., Cincinnati, OH 45267.

Although senile plaques represent a consistent neuropathological feature in Alzheimer's brains, it is not known what role plaques play in the etiology of the disease. Both growth-promoting and growth-inhibiting influences have been postulated. One of the major components of plaques, namely ß-amyloid, has been shown to affect neuron survival and neurite outgrowth *in vitro*. Since plaques consist of other components in addition to ß amyloid, we undertook the present study to determine whether neurite outgrowth is affected by senile plaques in culture. This was accomplished using a novel technique in which embryonic rat hippocampal neurons are cultured on cryostat sections of the postmortem-derived cerebral cortex of patients diagnosed with Alzheimer's disease. Using this paradigm, neurons growing on a substrate with a high density of plaques can be examined. The cultured cells were visualized using a vital fluorescent dye (the acetoxymethyl ester form of carboxyfluorescein). After imaging the cultured neurons, the section was then fixed and stained with thioflavin S to identify plaques. Using computer analysis of superimposed images, the morphology of neurons that associate with plaques was examined. Living neurons are found on senile plaques, suggesting that plaque components are not toxic under these culture conditions. However, such cells rarely extend processes or, and if processes are present, they are shorter in length than processes of cells attached to non-plaque regions. In addition, neurites that extend from neurons adjacent to plaques appear to turn away from the plaques.Supported by NIH-NS26819, MKC supported by NIH postdoctoral fellowship NS08842.

421.10

QUISQUALATE INJECTION INTO THE NUCLEUS BASALIS MAGNOCELLULARIS INDUCES SEIZURE-RELATED BRAIN DAMAGE. M.T. Price, D. F. Wozniak, S. Sun*, and J. W. Olney Washington Univ, St. Louis, MO 63110.

Ibotenate (Ibo), quisqualate (Quis) and N-methyl-DL-aspartate (NMA), are excitotoxins that have been used to destroy neurons in the area of the nucleus basalis magnocellularis (NBM) in adult rats. NBM lesions produced by Ibo and NMA are associated with reliable learning/memory deficits. This is consistent with the hypothesis that cholinergic neurons play an important role in memory, and that loss of these neurons may contribute to memory impairment in Alzheimer's disease (AD). However, recent reports that Quis produces at least as great a reduction in cortical choline acetyltransferase activity as Ibo or NMA, without producing consistent learning/memory deficits, raises the important question whether some factor other than loss of cholinergic neurons might account for the Ibo/NMA-induced memory impairment. In the brains of AD patients, a marked loss of somatostatinergic (Som) as well as cholinergic neurons has been reported, and we have recently found that Som-immunopositive neurons in the rat basal forebrain are readily destroyed by NMA (Wozniak et al., Neurosci Abs. '91). The present study was undertaken to determine what effect Quis has on basal forebrain Som neurons. Addressing this question proved rather difficult because unilateral injection of Quis into the NBM (n=10) caused sustained seizures and disseminated brain damage in all rats. While Som neurons were destroyed in these brains, it is impossible to know whether this signifies sensitivity to Quis or to sustained seizure activity. Since the dose of Quis and injection parameters we used were the same as those used by others who did not report seizure pathology as a complication, we are currently exploring relevant variables to clarify the discrepancy. Our results indicate that Quis poses the same kind of problem as kainic acid -- instead of producing a simple excitotoxic lesion confined to the injection site, it induces persistent seizures and seizure-mediated brain damage near to and far from the injection site. Supported by AG05681 (DFW & JWO) and RSA MH38894 (JWO).

421.11

SENSITIVITY OF BASAL FOREBRAIN SOMATOSTATINERGIC NEURONS TO N-METHYL ASPARTATE NEUROTOXICITY. D. F. Wozniak, G.R. Stewart, S. Sun*, and J. W. Olney. Dept. of Psychiatry, Washington Univ. Sch. Med., St. Louis, MO 63110.

Various excitotoxin agonists, including ibotenate (Ibo), quisqualate (Quis) and N-methyl-DL-aspartate (NMA), have been used as lesioning agents to destroy cholinergic nucleus basalis magnocellularis neurons in adult rats. Destruction of these neurons by Ibo and NMA is associated with similar learning/memory deficits. This is consistent with the hypothesis that cholinergic neurons play an important role in memory, and that loss of these neurons contributes to memory impairment in Alzheimer's disease (AD). However, recent reports that Quis deletes at least as many basal forebrain cholinergic (BFC) neurons as Ibo or NMA, without producing consistent learning/memory deficits, raises the important question whether some factor other than BFC neuronal loss might account for the Ibo/NMA-induced memory impairment. It is not known whether injection of excitotoxins into the basal forebrain destroys somatostatinergic (Som) as well as cholinergic neurons, but in the brains of patients with AD, both of these transmitter systems are reportedly affected. The present study was undertaken to determine what effect NMA has on basal forebrain Som (BFS) neurons. Injection of NMA into the basal forebrain caused a lesion which, by routine histopathological evaluation, appeared well confined to the injection site. The largest and most conspicuous group of BFS neurons are those in the entopeduncular nucleus (EpN) located 1-1.5 mm from the center of the injection site. The NMA injection destroyed 100% of these BFS-EpN neurons but only ≤ 70% of the BFC neurons within a sphere of equal radius from the injection. Moreover, in nearby portions of caudate nucleus there was a 30% loss of Som neurons and no loss of cholinergic neurons. Our findings signify the following: 1) BFS neurons are extremely sensitive to excitotoxin-induced degeneration; 2) An excitotoxic mechanism could account for loss of either BFS or BFC neurons in AD; 3) The learning/memory deficits associated with an NMA lesion (or with AD) could be due, in part, to loss of BFS neurons. Supported by NIH grants AG05681 (DFW & JWO) and RSA MH38894 (JWO).

421.12

RADIO-FREQUENCY LESIONS OF THE NUCLEUS BASALIS (nbM) INDUCE LONG-LASTING DEFICITS IN STIMULUS-PROCESSING ASPECTS OF DIFFERENTIAL CONDITIONING PERFORMANCE. J.E. Dencoff, A.E. Butt, & G.K. Hodge. Department of Psychology, University of New Mexico, Albuquerque, NM 87131.

Previously, we have reported that bilateral radio-frequency lesions of the nbM resulted in deficits in differential conditioning performance. As compared to control animals, rats with bilateral nbM lesions showed impaired discrimination (i.e., reduced successes, increased errors, and increased session time on a bar-press task). Lesioned and control animals did not differ with respect to overall rate of responding. Results suggested that nbM-lesioned animals had an impaired ability to attend to and use stimulus cues in accurately guiding behavior. In the current study, we now report that such deficits persist months after lesions are induced.

Twelve months post-lesion, ten months after their last exposure to the task, animals were retested. In the interim, animals received no experimental manipulations. Initially, both lesioned and control animals showed poor discrimination, but on successive days, control animals improved whereas lesioned animals showed consistently poor discrimination ability.

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421.13

INDUCTION OF THE BETA AMYLOID PRECURSOR PROTEIN IN THE SUBCORTICALLY LESIONED RAT CORTEX. V. Haroutunian, V. Bragin*, K. Davis, W. Wallace; Dept. Psychiatry and Center for Neurobiology, Mt. Sinai School of Medicine, New York.

We have reported that NMDA lesions of the rat nucleus basalis of Meynert result in the increased synthesis of beta-amyloid precursor protein (B-APP) in the cortex. In order to determine the normal physiological function of B-APP in the intact brain, we have further characterized this induction. This induction is common to other cortically projecting neurotransmitter systems. Lesions of the dorsal raphe nuclei, which targets serotonergic innervation and ascending noradrenergic bundle, which targets adrenergic innervation, similarly induce the cortical B-APP. Thus, the induction of B-APP appears to be a general response of the lesioned cortex. However, treatment of naive rats with the acetylcholinesterase inhibitor, physostigmine does not alter B-APP synthesis, showing that increased levels of acetylcholine do not result in this induction. The elevated B-APP synthesis is accompanied by increased B-APP mRNA indicating increased gene transcription. The induction occurs as early as one hour after lesion and persists for at least seven days. These observations suggest that B-APP may be interacting with other neurotrophic factors in the cortex in response to the loss of subcortical innervation.

421.14

ALTERATIONS IN THE CIRCADIAN RHYTHMS OF RATS RECEIVING SCN IMPLANTS OF PC12 CELLS OVER-EXPRESSING ALZHEIMER'S DISEASE ß-AMYLOID. B.Tate, K.S. Aboody, A.M. Morris, R.E. Majocha and C.A. Marotta. Mass. General Hospital and Harvard Medical School, Boston, MA 02114.

Patients suffering from Alzheimer's Disease (AD) display a number of abnormalities in circadian rhythms. In order to model this uniquely human disorder, permanently transfected PC12 cells containing the ß-amyloid to C terminal region of the amyloid precursor protein have been developed. The cells were stereotaxically placed in the suprachiasmatic nuclei (SCN) of adult female rats and circadian rhythms in body temperature and activity were monitored using indwelling radio transmitters (Mini-mitters). Controls included animals receiving vehicle, normal PC12 cells or PC12 cells transfected with vector only, or no surgery. On light/dark cycles of 12:12, animals receiving amyloidotic cells showed activity rhythms but not temperature rhythms that differed significantly from all control animals ($p<0.001$). The data support an association between a behavioral disruption and amyloid. Similar mechanisms may be relevant to the clinical manfestations of AD. Supported by NIH AG02126 and Milton Fund Grant.

421.15

ALZHEIMER DEFICITS DEVELOP IN AGED RATS AFTER CHRONIC CEREBROVASCULAR INSUFFICIENCY. J.C. de la Torre, G.A.S. Park*, T. Fortin*, J. Katnick*, J. Saunders*, K. Butler*, P. Kozlowski*, B. Pappas, M. Richard*. Univ.of Ottawa, Ontario Canada K1H 8M5.

Alzheimer's disease (AD) is initially characterized by reduced CBF, spatial memory dysfunction, increased formation of senile plaques (SP), neurofibrillary tangles (NT), glial fibrillary acidic protein (GFAP) and by hippocampal cell loss. ^{31}P- NMR spectroscopy of AD brains also shows a phosphomonoester (PME) elevation which indicates an increase in membrane phospholipid synthesis.

We subjected aged rats to cerebrovascular insufficiency (CVI) for 9 weeks as we have described (Brain Res. Bull. 26:365, 1991). At 3, 6, 9 weeks after CVI, we measured CBF and tested rats for spatial memory, ^{31}P- ^{1}H- NMR spectroscopy/imaging using ISIS localization for the hippocampus. Post-mortem tissue was examined for CA1 sector cell counts and GFAP reaction. Results show that CVI rats but not matched controls developed spatial memory impairment in the Morris Water Maze test and elevation of PME in the hippocampus following ^{31}P- NMR spectroscopy. In addition, aged CVI rats had significant cell damage to CA1 and increased GFAP immunoreaction throughout the hippocampus. Rats had cortical and hippocampal CBF reduction 3 weeks after chronic ischemia but only hippocampal blood flow remained low for 6-9 weeks after CVI (equivalent to 4-6 human years).

These results suggest that membrane phospholipid synthesis is increased in CVI rat hippocampus possibly due to proliferation of reactive GFAP-positive astrocytosis.

We conclude that chronic CVI in aged rats induces a syndrome that mimics the cognitive, metabolic and morphologic changes seen in AD. It is tempting to speculate that the pathologic deficits seen in the early course of AD could also be triggered by chronic CVI.

Funded by the Ontario Mental Health Foundation

421.16

ATROPHY OF BASAL FOREBRAIN CHOLINERGIC NEURONS IS MODELED BY MOUSE TRISOMY 16 TRANSPLANTS. D.M.Holtzman, Y.Li*, S.J.DeArmond*, F.H.Gage, C.J.Epstein*, W.C.Mobley. Depts. of Neurology, Pediatrics, and Biochemistry, UCSF, San Francisco, CA 94143 and Dept. of Neurosciences, UCSD, La Jolla, CA 92093.

Vulnerability of specific brain regions and neuronal populations is a characteristic feature of Alzheimer's disease (AD) and Down syndrome (DS). One of the vulnerable populations includes cholinergic neurons in the basal forebrain. We transplanted brain tissue from mouse trisomy 16 (Ts 16), an animal model of DS, in order to develop a model to study the neuronal degeneration which occurs in AD and DS. Suspensions of fetal Ts 16 or control septum were injected into hippocampus of young adult mice. At one month post-transplantation, there was good neuronal survival and neurite outgrowth from all grafts. However after six months, Ts 16 cholinergic neurons and their proximal neurites frequently appeared shrunken and atrophic. They were significantly smaller than controls (125.9 vs. 166.2 μ^2). The atrophy of cholinergic neurons was selective in that no significant difference was found in comparing the size of non-cholinergic neurons. Some mice were given bilateral lesions of the fimbria-fornix. Fornix lesions increased the size of all cholinergic neurons; controls remained larger than Ts 16. Our findings indicate that one can model a genetic disorder leading to cholinergic atrophy. It should allow for dissection of the chromosomal region(s) responsible for degeneration of these neurons. This model may also be used to understand early molecular events important to the pathogenesis of AD. In addition, it provides a means to study potential therapies to prevent genetically determined neurodegeneration.

421.17

CHRONIC NEUROFIBRILLARY DEGENERATION INDUCED BY ALUMINUM FLAVONOL, A UNIQUE LIPOPHILIC ALUMINUM CHELATE COMPLEX. R.N.Katz*, A.Hsu*, P.F.Good, D.E.Wolfe and D.P.Perl. Fishberg Research Center for Neurobiology, Mt. Sinai Medical Center, New York, NY 10029.

Orvig and coworkers (J.Amer.Chem.Soc., 108:5033, 1986) have shown that aluminum-related neurotoxicity can be increased by an order of magnitude by administering aluminum as aluminum maltol. Following this lead we have prepared a lipophilic complex, tris(3-hydroxyflavonato)-aluminum (aluminum flavonol, $AlFl_3$), and have demonstrated acute and chronic neurotoxic effects in rabbits when administered intraventricularly as a single injection. $AlFl_3$ is not water soluble but a stable suspension can be prepared using a Brinkman tissue homogenizer. Intraventricular injection of 0.1mL of a 75mM suspension in New Zealand albino rabbits produced seizures at 18 days, and SMI-31 (monoclonal antibody to phosphorylated medium and heavy neurofilament proteins) immunoreactivity (*ir*) revealed extensive neurofibrillary degeneration in cortical layers III and V. Additional animals remained asymptomatic after four weeks but had numerous SMI-31*ir* neurons in the neocortex and subiculum. Animals exposed to 0.1mL of 7.5mM $AlFl_3$ showed only subtle symptomatology after five months. These animals also demonstrated neocortical neurofilamentous tangles. An interesting property of $AlFl_3$ is its autofluorescence, and using fluorescence microscopy we have observed particles of the compound in macrophages within the ventricles. $AlFl_3$ and other novel aluminum chelate complexes represent important new tools for developing animal models of chronic aluminum neurotoxicity. Supported by the American Health Assistance Foundation.

ALZHEIMER'S DISEASE: CYTOSKELETON

422.1

EXPRESSION OF TAU AND ALZ-50 IN HUMAN CORTICAL CELL LINE HCN-1A AND NON-HUMAN NEUROBLASTOMA CELL LINES. S.A.Enam, J.T.Megerian, & W.L.Klein. Northwestern University Institute for Neuroscience, Evanston, IL 60208

The relationship between tau and Alz-50 was studied by immunofluorescence in HCN-1A, and N1E-115, B103, and the F3 variant of PC12 cells. Results were as follows:

	Neurites	Alz-50	Tau
HCN-1A (Differentiated)	Yes	Negative	+ all neurites & soma
HCN-1A (Un-differentiated)	No	Negative	Very weak
N1E-115	Yes	+ all cells	+ all neurites & soma
B103	Yes	+ all cells	+ all neurites & soma
F3	No	Weak	+ all cells

In some cell lines, Alz-50 was present in all the cells, suggesting that Alz-50 is not a marker of cell death. The difference between the expression of Alz-50 in B103 and F3 cell lines is interesting when considered with the fact that almost all of the B103 cells constitutively express neurites in vitro, whereas F3 cells rarely express neurites. This interesting contrast is consistent with the hypothesis that Alz-50 may be associated with the process of neuritogenesis. These cell lines may be a valuable source for further understanding the role of Alz-50 in Aging and normal development.

422.2

BRAIN TAU LEVELS ARE ELEVATED IN ALZHEIMER DISEASE. S. Khatoon*, I. Grundke-Iqbal* and K. Iqbal, NYS Institute for Basic Research, Staten Island, N.Y. 10314

Microtubule associated protein tau which stimulates the assembly of α-β tubulin heterodimers into microtubules is abnormally phosphorylated in Alzheimer disease (AD) brain, and is the major component of paired helical filaments. In the present study, the levels of tau, abnormally phosphorylated tau and of tubulin were determined in brain homogenates of AD and age-matched normal cases. A radioimmuno slot blot assay was developed using a primary monoclonal antibody (mAb), Tau-1 and anti-mouse ^{125}I-IgG as secondary antibody. For assaying the abnormally phosphorylated tau, the blots were treated with alkaline phosphatase prior to immunolabeling. Amounts of α and β tubulin were determined by an ELISA using mAb YL1/2 to α and mAb KMX-1 to β tubulin. The levels of total tau were about 6-fold higher in AD (5.9 ± 1.3 ng/μg protein) than in control (1.0 ± 0.2) cases. Most of the tau in AD brain was abnormally phosphorylated (4.3 ng/μg protein). In contrast there were no significant changes in the levels of tubulin in AD brain. These studies show (1) that there is a marked increase in brain levels of tau in AD, and (2) that this increase is due to the accumulation of abnormally phosphorylated tau in the affected tissue. (Support by NIH grants AG05892, AG08076, NS18105 and AG04220 and a grant from AHAF).

422.3

A TAU POLYMER MODEL OF THE PAIRED HELICAL FILAMENT. D.M. Wilson* and L.I. Binder. Dept. of Cell Bio., Univ. of Alabama at Birmingham, 35294

The paired helical filament (PHF) is the basis of the intracellular neurofibrillary pathology observed in Alzheimer's Disease. The microtubule-associated protein tau is widely accepted as contributing to the structure of the PHF. Recent advances in purifying populations of PHF amenable to standard solubilization tecniques, revealed that tau isoforms are the only proteins isolated following filament dissociation. Therefore, believing that tau is the only integral component of the PHF, we have constructed a tau polymer model for these filamentous structures. Our tau polymer has the appearance of paired filaments, with a total mass/unit length of 55-60 kDa/nm. Its dimensions and cross-sectional mass distribution are consistent with data reported for PHF. Our model also accommodates the demonstrated beta-pleated sheet content of the PHF. Three mechanisms of tau self-association are invoked to generate the tau polymer. One of these mechanisms is phosphorylation dependent, and the model predicts specific phosphorylation sites and appropriate kinases which would be involved in producing assembly competent tau.

422.4

FUNCTIONAL STUDIES OF ALZHEIMER'S DISEASE TAU PROTEIN. Q. Lu* and J.G. Wood. Dept.of Anatomy and Cell Biology, Emory Univ. Sch. of Med., Atlanta, GA 30322.

In vitro assays were used to monitor and compare the kinetic behavior of microtubule assembly induced by tau proteins isolated from Alzheimer's disease (AD) and non-demented age matched control brains. Tau from 4 of 5 AD cases induced slower assembly and a steady state assembly approximately 50% of that stimulated by tau from 3 control cases. Tau from the most severe cases was least effective at promoting assembly. Dark field microscopy of the control samples indicated abundant microtubule formation and many microtubule bundles. Fewer microtubules were observed in the AD samples and bundling could not be detected. There was no obvious difference in the ultrastructure of microtubules formed in both cases. SDS-PAGE analysis revealed lower amounts of tau in microtubule pellets from experimental compared to controls which supports the assembly data. AD tau is modified by phosphorylation (PNAS, 83:4040-4043; 4913-4917). These results indicate that tau modification compromises its function in helping provide a population of stable neuronal microtubules needed for critical functions such as axonal transport. AG 06383; NS 17731.

422.5

CASEIN KINASE II BINDS PAIRED HELICAL FILAMENTS. L. Baum and T. Saitoh. Department of Neurosciences, 0624, University of California, San Diego, La Jolla, CA 92093.

In Alzheimer's disease (AD), neurofibrillary tangles (NFT) and dystrophic neurites contain paired helical filaments (PHF) composed largely of abnormally phosphorylated tau, a microtubule-associated protein. The kinases that phosphorylate tau in vivo are not yet known. Casein kinase II (CK-II), a highly conserved serine/threonine kinase, phosphorylates tau in vitro. NFT exhibit CK-II immunoreactivity, and purified CK-II can bind NFT on AD brain sections. We explored the nature of this binding using purified CK-II and purified PHF. To detect any PHF-associated CK-II, PHF was purified by a relatively gentle method (Greenberg and Davies). Immunoblots of PHF showed no CK-II, indicating either that: the amount of CK-II present was too little to detect; CK-II does not bind PHF; or detergent in the PHF purification removes CK-II. To determine whether CK-II can bind PHF, CK-II was added to centrifuge tubes containing PHF, spun to pellet the PHF, and the amount of CK-II pelleting with the PHF was measured on immunoblots. Although some CK-II, which can form filaments in vitro, pellets in control tubes, more CK-II pellets in PHF tubes. Low salt enhances co-pelleting, suggesting an ionic interaction. Thus, CK-II binds PHF in vitro. In vivo, the normal functions of CK-II might be disrupted by its abnormal localization in AD. In addition, since the phosphorylation of a substrate by CK-II can be enhanced by previous phosphorylation of the substrate, CK-II might contribute to the overphosphorylation of tau in PHF.

422.6

CYTOSKELETAL mRNAs IN ALZHEIMER'S DISEASE TEMPORAL CORTEX AND CEREBELLUM. KM Chan, JR Goss & DG Morgan. Gerontol. Ctr. & Dept. of Biol. Sci., Univ. Southern Cal., Los Angeles CA 90089-0191

We have shown previously by northern hybridization that glial fibrillary acid protein (GFAP) RNA prevalence increased in Alzheimer's Disease (AD) cortex. In this study, we have examined three other cytoskeletal RNAs: vimentin, the low molecular weight neurofilament (NF-L), and β-tubulin. RNA prevalence in 8 AD and 8 control specimens from each region was measured by a solution hybridization method (RNase-protection assay), carried out in triplicate using six concentrations of total RNA per sample. The mass of each specific message per unit mass of total RNA was calculated (pg/μg).

Marker	TCX (C)	TCX (AD)	CBL (C)	CBL (AD)
GFAP	6.7±0.5	29.8±7.2	11.7±1.4	8.2±0.8
Vimentin	1.6±0.1	2.6±0.6	0.8±0.1	0.68±0.07
NF-L	2.0±0.04	1.9±0.03	0.8±0.1	0.56±0.05
β-Tubulin	4.3±0.5	3.5±0.4	3.8±0.4	4.1±0.4

The 4.5 fold increase in GFAP message between AD and control cortex was the only statistically significant change. Two cortical AD samples had 4 fold elevations of vimentin RNA suggesting microglial/macrophage proliferation, but other samples were within the control range. No change in NF-L or β-tubulin were detected, although northern analysis suggested a different size for the NF-L mRNAs in AD T-CX. As expected, no changes were observed in the relatively intact AD cerebellum. We thank SA Johnson for organizing the brain tissue samples and CA Zarow for modifying the NF-L clone. Supported by AG-07892, AG-00093, AHA-GIA 891079, and AHA-EIA 890173.

422.7

CHARACTERIZATION OF HMW TAU IN THE PERIPHERAL AND CENTRAL NERVOUS SYSTEM N.Taleghany* and M.M.Oblinger Dept. of Cell Biology & Anatomy, The Chicago Medical School, North Chicago IL 60064

Tau is a complex family of neuron-specific microtubule-associated proteins that are encoded by a single gene. The major tau proteins in brain are a complex of low molecular weight (LMW) proteins (50-70 kDa). However, a HMW tau isoform (110 kDa) has been found in peripheral nervous tissue as well as in PC12 cells. Little is known about the distribution of this HMW tau form in the nervous system. Is this isoform restricted to the PNS, and are the properties of HMW tau different from those of other isoforms? In order to address this issue immunoblotting analysis with the tau-1 antibody was employed and such studies revealed that while LMW tau was present throughout CNS samples, HMW tau was largely restricted to tissues that were obtained from the PNS. An intermediate-sized tau isoform (MMW tau, 90-110 kDa), was also identified in this study. This isoform was localized in the optic nerve (ON) as well as the cerebellum. To explore some of the molecular properties of HMW tau, we examined its behavior under a series of conditions, namely its gel mobility after treatment with phosphatase, its heat stability and its partitioning when exposed to cold-calcium and Triton extraction paradigms. Interestingly, HMW tau partitioned with the soluble fraction while LMW & MMW tau partitioned mainly with the stable microtubule pellets. Immunocytochemical studies revealed that DRG cell bodies stain with tau specific antibodies. This is in sharp contrast to previous findings that neuronal cell bodies in brain don't exhibit staining with tau-1 antibody. Since immunoblots show that the dorsal roots & DRG contain only the HMW tau form we can suggest that the DRG cell bodies contain HMW tau and that the tau-1 epitope is not the masked in these cell bodies. Northern blot analysis of total RNA from different regions of the adult nervous system revealed different sized tau mRNA's (6-8 kb). This suggests that alternative splicing of tau mRNA is a significant factor in generating tau heterogeneity. The HMW tau generated by such a mechanism has unique characteristics from other tau forms.

422.8

MAP-2 mRNA LEVELS ARE DECREASED IN PRIMARY MOTOR CORTEX IN ALZHEIMER'S DISEASE. K.E. Rogers*, H. A. SantaLucia*, and P. D. Coleman. Dept. of Neurobiol. & Anat., University of Rochester, Rochester, NY. 14642

Morphometric studies have suggested that dendritic plasticity is altered in Alzheimer's Disease(AD). MAP-2, a microtubule associated protein, has been shown to be a dendritic specific protein whose levels may parallel the volume of the dendritic compartment. Thus, measurement of MAP-2 mRNA levels could reflect the state of the dendritic compartment. Total RNA was extracted from AD and control tissue taken from Brodman's area 4. MAP-2 message prevalence was measured by solution hybridization to a MAP-2 cRNA. mRNA content was determined by oligo-dT slot blot hybridization. Data analysis demonstrated that there was a 57% decrease in MAP-2 messages in AD when compared to age-matched controls. Area 4, primary motor cortex, has not previously been thought to be significantly affected in AD. (Supported by R35 AG 09016, P01 AG 03644, R01 AG 01121, P30 AG 08665(PDC) AND ADRDA PRG-89-120(KER).

422.9

TIME-DEPENDENT EFFECTS OF INTRAHIPPOCAMPAL NMDA ON THE MICROTUBULE PROTEINS TAU AND MAP2. D.K. Rush[1], R.K. Lartius[2], S.H. Aschmies[1]* & E.Uemura[2]. [1]Dept. Biol. Res., Hoechst-Roussel Pharmaceuticals Inc., Somerville, NJ, 08876 and [2]Dept. of Anat., Iowa State Univ., Ames, IA 50011.

The plaques and tangles characteristic of Alzheimer's disease (AD) are composed in part of microtubule associated proteins (MAPs). Since excitatory amino acid neurotransmission may play a role in AD neurodegeneration, we examined the effects of intrahippocampal N-methyl-D-aspartic acid (NMDA) on the MAPs, tau and MAP2. NMDA (20 nmoles in 1 µl dH_2O) was unilaterally injected into the dorsal hippocampus of rats under chloral hydrate anesthesia. The animals were sacrificed 1, 3, or 7 days later and brain sections immunohistochemically stained for either MAP2 or tau with HM-2 or Tau-1 monoclonal antibodies. The pattern of MAP2 and tau staining differed in terms of both regional specificity and time course. One day post-lesion, tau staining was significantly increased in all three layers of the CA1 region (strata oriens, moleculare, and pyramidale) in the NMDA as compared to the vehicle injected hippocampus. MAP2 staining was unchanged. At 3 and 7 days post-lesion, tau staining had returned to normal but MAP2 staining was decreased in all three CA1 layers of the lesioned hippocampus. The cell necrosis and effects on microtubule associated proteins found in the present study differ from the pattern of neuropathological changes in AD; tau did not remain elevated as it does in the disease state. The pattern of effects does support previously reported findings of NMDA on MAP2 and tau staining in cortical culture (Bigot & Hunt 1990) and suggests a modulatory effect of NMDA on the neuronal cytoskeleton.

422.10

SUBPOPULATIONS OF MICROTUBULES REVEALED BY EXPRESSION OF INDIVIDUAL HUMAN TAU ISOFORMS AND PERTURBATION BY COLCHICINE, COLD, AND CALCIUM.
T.E. Norris, A.F. Fieles*, C.B. Caputo, C.W. Scott, M. Goedert*, and M.M.S. Lo. ICI Americas, Inc., Wilmington, DE 19897 and MRC Laboratory of Molecular Biology, Cambridge CB2 2HQ, England.

The neuronal cell contains subpopulations of microtubules (MT) as defined by their composition and function. Different MAPs, and their isoforms are associated with different MT populations. In addition, these distinct MT show selective sensitivities to different MT depolymerizing conditions.

In order to investigate the early events in MT degeneration, we have used 3T3 cells stably transfected with single isoforms of human tau. These cell lines have revealed that MT behavior depends on the specific tau isoform present. Expression of individual tau isoforms confers unique cellular morphologies as well as different MT stabilities to depolymerizing conditions. These morphology differences are further accentuated by the MT depolymerizing conditions of colchicine. Degradation of MTs resulting from various treatments shows that the MT subpopulations depolymerize via different mechanisms. Colchicine causes a loss of cell processes, however, an intracellular MT matrix remains intact in the cell body which contians concentrated "pools" of tubulin. In contrast, cold treatment causes a progressive degradation of MT into small fibers without altering the cellular morphology. Increased levels of intracellular calcium causes a redistribution of the MT networks into bundles yet does not change the cell's morphology. The different cytoskeletal changes caused by these MT depolymerizing conditions, as well as the morphological changes caused by expression of different tau isoforms, reveals that there are numerous subpopulations of MTs. We will be focusing on the effects of elevated intracellular levels of calcium as it relates to cell morphology, cytoskeleton and organelle redistribution, and the calcium regulated phosphorylation events of tau.

422.11

TAU PROTEIN INDUCES BUNDLING OF MICROTUBULES IN VITRO. C.W Scott, A.B. Klika, M.M.S. Lo, T. Norris and C.B. Caputo, Dept. of Pharmacology, ICI Americas, Inc. Wilmington, DE 19897.

Expression of tau protein in non-neuronal cells results in the redistribution of the microtubule (MT) cytoskeleton into thick bundles of tau-containing MTs (Lewis et al., (1989) Nature, 342:498). The molecular basis for tau-induced bundling of MTs is not understood, therefore experiments were performed to reconstitute MT bundling using purified bovine tubulin and recombinant human tau isoforms. Tubulin was assembled into MTs by incubating with taxol in assembly buffer. The MTs were isolated by centrifugation, mixed with various concentrations of T4 (tau isoform containing four MT binding domains), and examined by negative stain electron microscopy. In the absence of tau the MTs were randomly distributed. When MTs were incubated with T4 at tubulin:tau molar ratios of 1:0.9 the MTs appeared as loosely- and tightly-packed parallel arrays. At molar ratios of 1:1.5 the MTs condensed into thick bundles containing multiple layers of tightly packed parallel arrays. MT bundling was observed with porcine tau and other human tau isoforms, and was blocked by the addition of 0.5M NaCl or 0.3mM estramustine phosphate. A tau deletion construct that contained only the four MT binding domains plus 19 amino acids to the C-terminus was capable of bundling MTs. These data further refine the region of tau required for MT bundling in vitro and demonstrate that bundling can be reconstituted using only tubulin polymers and tau protein.

422.12

PHOSPHORYLATION AND METABOLISM OF TAU. G.V.W. Johnson and J.M. Litersky. Depts. of Psychiatry and Cell Biology, Univ. Alabama at Birmingham, Birmingham, AL 35294.

The phosphorylation state of tau has been postulated to modulate its interactions with other cytoskeletal components and also may regulate its susceptibility to proteolysis. We are examining these processes because an inappropriately phosphorylated tau is an important component of paired helical filaments (PHFs), which are found in Alzheimer's disease brain. In this study we examined the in vitro phosphorylation of bovine tau by cAMP-dependent protein kinase (cAMP-PK), Ca++/calmodulin-dependent protein kinase II (CamKII), and protein kinase C (PKC), and how phosphorylation by these kinases modulates its hydrolysis by calpain or binding to microtubules.

Phosphorylation of tau by each of the kinases produced distinct 2 dimensional phosphopeptide maps. cAMP-PK phosphorylated unique sites, as well as sites phosphorylated by CamKII. Little overlap existed between the sites phosphorylated by PKC and either CamKII or cAMP-PK.

Phosphorylation by CamKII or cAMP-PK, but not PKC, retarded the migration of the tau isoforms on SDS-polyacrylamide gels. Phosphorylation of tau by cAMP-PK significantly inhibited its hydrolysis by calpain. In contrast, phosphorylation of tau by CamKII did not alter its susceptibility to calpain. Phosphorylation of tau by these kinases also altered the binding of tau to microtubules. These results suggest a role for cAMP-PK phosphorylation in regulating the degradation of tau. Abnormal phosphorylation could result in a protease-resistant tau population which may contribute to the formation of PHFs in Alzheimer's disease.

Supported by NIH grants NS27538 and AG06569 and a grant from the American Health Assistance Foundation.

422.13

PHOSPHORYLATION MODULATES DEGRADATION AND FUNCTION OF INDIVIDUAL HUMAN TAU ISOFORMS. J.M. Litersky, C.W. Scott and G.V.W. Johnson. Depts. of Psychiatry and Cell Biology, Univ. of Alabama at Birmingham, Birmingham, AL. 35294 and ICI Americas Inc., Wilmington, DE 19897.

Individual human tau isoforms purified from an E. coli system expressing each specific clone (Goedert and Jakes, 1990) were used in this study. Of the six isoforms cloned, the three used in this study were tau with either three or four microtubule-binding repeats in the carboxy-terminal half of the molecule (T3R, T4R) and a four repeat tau with the 58 amino acid insert in the amino-terminal region of the molecule (T4RL). The isoforms were phosphorylated by cAMP-dependent protein kinase (cAMP-PK) and Ca++/ calmodulin-dependent protein kinase II (CamKII), and subjected to one-dimensional polyacrylamide gel electrophoresis (1D PAGE), two-dimensional nonequilibrium pH gradient electrophoresis (2D NEPHGE), 2D phosphopeptide mapping, calpain hydrolysis and a binding assay. Phosphorylation by either kinase retarded the migration of all three isoforms on 1D PAGE. cAMP-PK produced two slower migrating species, the slower of which aligned with the single band produced by phosphorylating with CamKII. 2D NEPHGE revealed that T4R existed as one isovariant, while T3R and T4RL appeared to exist as 5 and 7 isovariants, respectively. Phosphorylation also increased the microheterogenity of the isoforms on 2D NEPHGE. The 2D phosphopeptide maps demonstrated that the isoforms were all phosphorylated in a similar manner, with the two kinases producing distinct peptide maps. The three tau isoforms were all susceptible to calpain hydrolysis, however T3R was proteolyzed at a slower rate than either T4R or T4RL. The hydrolysis of the isoforms by calpain was significantly increased after phosphorylation by CamKII. Phosphorylation by cAMP-PK did not alter calpain-induced proteolysis. In addition, phosphorylation appears to modulate the binding of the tau isoforms to tubulin. Supported by NIH grants NS27538 and AG06569 and a grant from the American Health Assistance Foundation.

422.14

ASSOCIATION OF TAU(τ) WITH RIBOSOMES IN ALZHEIMER'S DISEASE. S.Ch. Papasozomenos and Y. Su*. Univ. of Texas Med. Sch., Houston, TX 77225.

We showed that in Alzheimer's disease (AD) τ immunoreactivity is associated not only with the abnormal filaments but also with ribosomes (Lab. Invest. 60:123, 1989 and 60:375, 1989). We also observed that in chromatolytic Pick's cells and in Pick's bodies τ immunoreactivity is associated with ribosomes. In this study, we have isolated ribosomes from six AD cases and controls and used four monoclonal anti-τ antibodies (Tau-1, Tau-2, Tau-5 and T46), immunoblot analysis and EM immunogold labeling to further investigate the association of τ with ribosomes. In immunoblots of SDS extracts, three τ polypeptides of 60-, 62- and 66-kDa were present but only in regions that in parallel histologic preparations contained τ immunoreactivity. Dephosphorylation was necessary to demonstrate these three τ polypeptides with Tau-1 and enhanced staining with Tau-5. Also, the ribosomal 62- and 66-kDa τ polypeptides coelectrophoresed with the most slowly moving polypeptides in SDS extracts of cortical homogenates from the same region. Controls were negative. In addition, all four antibodies decorated isolated ribosomes at the EM level. The purity of the preparations was verified by EM of the ribosomal pellet and negative staining with uranyl acetate of ribosomal solutions. These findings show that in AD τ is indeed associated with ribosomes and will further elucidate the etiopathogenesis of AD.

422.15

MICROTUBULE-ASSOCIATED PROTEIN TAU IS A COMPONENT OF NEUROFIBRILLARY TANGLES OF GERSTMANN-STRÄUSSLER-SCHEINKER DISEASE-INDIANA KINDRED. F. Tagliavini*, F. Prelli*, G. Giaccone, M. Porro*, J. Ghiso*, M.R. Farlow*, B. Ghetti, O. Bugiani* & B. Frangione. Istit. Neurol. Besta, 20133 Milan, Italy, Indiana Univ., Indianapolis, IN 46202 & NY Univ., NY, NY 10012.

In the Indiana kindred of Gerstmann-Sträussler-Scheinker disease (GSS-IK) amyloid deposits immunoreactive for prion protein coexist with neurofibrillary tangles (NFT). NFT are composed of paired helical filaments and are immunoreactive with antibodies to NFT isolated from Alzheimer's disease (AD) brain. Our aim was to immunochemically characterize proteins extracted from NFT isolated from cerebral cortex of two patients of GSS-IK. Amyloid-NFT enriched preparations were sonicated in formic acid and centrifuged. The supernatant was fractionated by gel filtration and the fractions were subjected to SDS-PAGE and immunoblot analysis using anti-tau antibodies, including Alz50. Protein bands of molecular weights of 68 kDa and lower were present, and were immunoreactive with anti-tau antibodies. Immunoreactivity was abolished by absorption of the antibodies with human fetal tau. These findings indicate that in GSS-IK, similarly to what has been shown in AD, tau protein or its fragments are a component of NFT, suggesting that comparable pathogenetic mechanisms of different origin may lead to cytoskeletal damage.

422.16

QUANTITATIVE SOLUBILIZATION AND ANALYSIS OF DETERGENT-INSOLUBLE PAIRED HELICAL FILAMENTS (PHF) OF ALZHEIMER DISEASE (AD). G. Perry, P. Mulvihill*, S.L. Siedlak*, and Peggy Richey*. Case Western Reserve University, Cleveland, OH 44106

Historically, detergent-insoluble PHF have been qualititively evaluated by morphological, immunochemical or amino acid sequencing methodologies. The limitation of these approaches has been that the dominant subunit of detergent-insoluble PHF has not been determined. In this study, we use protein release and specific volume decrease as *quantitative* measures of PHF solubilization. Detergent-insoluble PHF were isolated from AD cases by homogenization in 1% SDS followed by sucrose gradient centrifugation. Solubilization was sequentially performed for 10-50 min with the following agents 1) 1% SDS, 2) 1% SDS/1% βME, 3) 0.1M EDTA or 5M NaCl, 4) 8M urea, 6M guanidine-HCl, or 70% formic acid, 5) 0.2M NaOH-37°C, 6) 1M NaOH-90°C. Protein was quantitated by laser densitometry of SDS-PAGE stained with Coomassie blue or silver. Only SDS/βME, NaOH-37°C or NaOH-90°C were effective in protein extraction. Sequentially, the volume occupied by PHF was reduced *to* 96±1.9% by SDS/βME; 5.2±0.2% by NaOH-37°C; 0.27±0.28% by NaOH-90°. Immunoblots of these extracts showed τ, tubulin, P-component and ubiquitin in the SDS/βME extract, and only τ (60-64kDa) in the NaOH-37°C and NaOH-90°C extracts. Treating neurofilaments, τ, or β-protein with NaOH-37°C for 10-30 min had minimal effect on densitometry or epitopes. Therefore, τ is correlated with PHF dissolution for detergent-insoluble as well as has recently been reported for detergent-soluble PHF. Supported by NIH AG09287 and KO4-00415.

422.17

SPECTRIN (240/235E) AND DENDRITIC REORGANIZATION IN ALZHEIMER'S DISEASE. Angela Lee and Neil W. Kowall. Neurology Service, Massachusetts General Hospital, Boston MA 02114

Neuronal degeneration in Alzheimer's disease (AD) is associated with axonal and dendritic plasticity and reorganization. The axonal microtubule-associated protein tau is aberrantly located in the somatodendritic compartment of degenerating neurons in the form of neurofibrillary tangles (NFT) and dystrophic neurites. It is not known if a more generalized disruption of neuronal polarity occurs in AD. In normal neurons, subtypes of the major submembranous cytoskeletal protein spectrin are differentially distributed in axons and dendrites suggesting a possible role in the generation and maintenance of neuronal polarity. We examined the distribution of dendritic spectrin in normal and AD hippocampus using a polyclonal antibody that recognizes the dendritic isoform of spectrin (240/235E). We sought to determine whether specific alteration of the somatodendritic compartment occur in AD. In normal human hippocampus, red blood cells were prominently stained. A discrete band of immunoreactivity defined the dentate gyrus granule cells and the inner molecular layer. Neurons were prominent in CA1 with occasional intensely reactive granuolovacuolar degeneration (GVD). Rare astrocytes were immunoreactive. In AD, immunoreactivity in the dentate gyrus extended into the outer molecular layer and a band of decreased immunoreactivity was seen in the polymorphic layer. Occasional intensely reactive neurons containing NFT or GVD were found in CA1 and the subiculum. Spectrin (240/235E) retains its normal somatodendritic localization in the dentate gyrus in AD but its distribution shifts in conjunction with dendritic reorganization. GVD contain prominent dendritic spectrin immunoreactivity. A small subset of NFT-bearing neurons are intensely immunoreactive suggesting that the synthesis and/or proteolysis of dendritic spectrin may be altered in AD.

422.18

ABNORMAL PHOSPHORYLATION OF TAU IS ONE OF THE EARLIEST EVENTS IN ALZHEIMER NEUROFIBRILLARY PATHOLOGY. Köpke-Secundo, E., Grundke-Iqbal*,I and Iqbal, K. Institute for Basic Research in Developmental Disabilities, New York, NY 10314.

Previously it has been shown 1) that the major protein subunit of paired helical filaments (PHF) in Alzheimer disease (AD) is microtubule associated protein tau and 2) that tau in PHF is abnormally phosphorylated. In this study the cytosolic tau was isolated from the 27,000 x g to 200,000 x g pellet of AD cerebral cortex by extraction in 8 M urea, followed by acid precipitation, GFAP affinity and phosphocellulose (PC) chromatographies. The PC-chromatography revealed a wide range of abnormally phosphorylated tau species (AD P-tau) with different charges which were not detectable in control brains. AD P-tau species had no ubiquitin reactivity as determined with mAb 3-39 and 5-25. Furthermore, AD P-tau had increased immunoreactivity when treated with alkaline phosphatase prior to immunostaining with both mAb Tau-1 (amino acids 191-207 of tau) and antiserum 102c (amino acid 39-49 of tau). These studies suggest that tau in AD brain is abnormally phosphorylated at more than one site prior to its polymerization. (Supported in part by NIH fellowship 1 F32 AG0 5541-01 to Köpke-Secundo and grants NS 18105, AG0 4220, AG0 5892 and AG0 8076.

ALZHEIMER'S DISEASE: NEUROCHEMISTRY II

423.1

ALTERATIONS IN THE PHYSICAL PROPERTIES OF BRAIN MEMBRANES IN ALZHEIMER'S DISEASE. A. S. Bloom, P.G. Antuono and L. F. Vena*, Departments of Pharmacology and Neurology, Medical College of Wisconsin, Milwaukee, WI 53226.

Several studies have indicated that there are changes the fluidity of a variety of biological membranes during aging and in Alzheimer's Disease (AD). We have expanded upon these observations by examining differences between the fluidity of purified synaptic plasma membranes (SPM) prepared from the brains of persons dying with AD (n= 11)and aged-matched controls (n=6). Membrane fluidity was determined in 7 brain regions using fluorescence polarization. A temperature of 35° C was used. When diphenylhexatriene (DPH) was used as as a fluorescent probe, (It measures membrane phospholipid ordering.) decreases in polarization were observed in SPM prepared from temporal and parietal cortex of AD brains when compared to controls. Polarization values decreased from 0.2737 to 0.2638 in parietal cortex and from 0.2709 to 0.2620 in temporal cortex. Significant differences were not seen in the other regions studied. When TMA-DPH which localizes to the more superficial regions of the lipid bilayer was used as a probe, decreases in fluorescence polarization were observed in temporal cortex and hippocampus of AD brains. Polarization values decreased from 0.3487 to 0.3364 in the hippocampus and from 0.3441 to 0.3340 in temporal cortex. Again, significant changes were not seen in other regions. Lastly, the effects on the polarization of 12-anthroyloxystearic acid (12-AS) fluorescence, which reports on the mobility of phospholipid acyl chains, were examined. Significant differences were seen only in the cerebellum, where polarization was increased from 0.1308 to 0.1429 in AD brains. These data indicate that there are regionally specific changes in synaptic plasma membrane ordering in AD brains that correlate with areas where AD pathology is observed. It is yet to be determined if these alterations are due to changes in membrane lipid or protein composition.

423.2

INCREASED SATURATION OF FATTY ACIDS IN ALZHEIMER'S DISEASE. P. G. Antuono, G. D. Cherayil* and K. C. Ho*. Departments of Neurology and Pathology, Medical College of Wisconsin, Milwaukee, WI 53226.

Compositions of phosphatidylethanolamine (PE), lysophosphatidylethanolamine (lysoPE) and phosphatidylcholine (PC) fatty acids (FA) were determined in post-mortem brain tissue of 12 AD patients and 8 controls. The two groups were matched for several clinical and post-mortem variables. PC, lysoPE and PE were transmethylated and the resulting FA methylesters were analyzed by gas liquid chromatography. FA compositions from white and gray matter of the frontal and occipital lobes were similar in the two groups. The temporal cortex showed a trend towards a reduction in the ratio of monounsaturated to polyunsaturated FA, reaching a significant difference for arachidonic acid (20:4n6) in PC and lyso PE. Temporal lobe white matter showed a similar abnormality with a reduction of monounsaturated FA in PC and PE. Conversely polyunsaturated FA as decosahexaenoic acid (22:6n3) were significantly increased in PC, lysoPE and PE; arachidonic acid was increased in PC.

These results suggests a regional decrease in monounsaturated FA and increase in polyunsaturated FA in the temporal lobe of Alzheimer patients. Although this change may be secondary to the pathological changes of this disease, it may contribute to the alterations in membrane fluidity and neurotransmission reported in AD.

423.3

THE EFFECT OF MUSCLE INACTIVITY ON CALCIUM ACTIVATED NEUTRAL PROTEASES. F. Grynspan , R.A. Nixon, S. Bursztajn. McLean Hospital, Harvard Medical School, Belmont, MA 02178

Calcium activated neutral proteases (CANP) are non-lysosomal intracellular proteases which play a major role in turnover and in degradation of cytoskeletal and membrane proteins. A potent endogenous inhibitor protein named calpastatin coexists with calpain in tissues. In this investigation we measured the enzymatic activity of mCANP, (the form of the enzyme that is optimally activated in vitro by millimolar calcium levels) and the inhibitor in pre- and post-fusion, and in paralysed muscle cells. Cultured chick muscle were harvested 1, 3 and 7 days after plating, and the inhibitor and mCANP were separated by DEAE cellulose column chromatography. The CANP activity was measured using ^{14}C-azocasin as a substrate in the presence of 7 mM calcium. Activity of the heat stable inhibitor was measured after 100°C treatment of the active fraction for 10 min, and adding an external source of mCANP. No inhibitor activity was detected day 1 after plating. A significant increase in inhibitor activity occurred at day 3 (45%) and day 7 (87%) after plating. The mCANP activity was low in cells at all ages. Muscle cells treated with tetrodotoxin (10 mM) (TTX, a sodium channel blocker that paralyzes the muscle) and assayed 7 days after plating showed a 2-7 fold increase in mCANP activity as compared to untreated cells. Inhibitor activity was not altered by TTX treatment. On the other hand, TTX-treated cells show a 2-3 fold increase in the acetylcholine receptor (AChR) protein and mRNA levels but no change in secretory or intracellular acetylcholinesterase (AChE) activity or creatine phosphokinase (CPK) activities. If the CANP system is activated by TTX treatment, these results imply selective roles of CANP in protein modification and degradation. (Supported by NIH grants.)

423.4

MONITORING ACTIVITY OF THE MULTICATALYTIC PROTEASE IN LIVING NEURONS. R. Siman and S. Mistretta*, Cephalon, Inc., West Chester, PA. 19380.

The multicatalytic protease (proteasome) and the calpains are two cytoplasmic, non-lysosomal protein-degrading systems present in many cell types. We have begun to evaluate the involvement of these systems in protein turnover in neurons. Protease activity was monitored in cerebellar granule neurons grown in primary culture by use of membrane-permeable fluorogenic substrates, such as Suc-LLVY-AMC. Experiments with purified proteases confirmed that both calpain and the multicatalytic protease readily degrade the substrates. Granule neurons produced a time- and dose-dependent substrate hydrolysis. Protease activity occurred intraneuronally, and did not result from any enzymes secreted into the medium. Several observations argue that the activity is due to the multicatalytic protease. A protease with the size (700 kD) and properties of the multicatalytic protease could be extracted and purified from granule neuron cultures. Moreover, the purified enzyme degraded the same substrates that were degraded by the granule neurons, and was selectively inhibited by the same agents which blocked the activity within the living neurons. The substrates cannot be ubiquitinated, because of a blocked N-terminus, indicating that the multicatalytic protease of neurons can degrade substrates without prior ubiquitination. Activation of calpain, evoked by raising the intraneuronal calcium content, caused only a small increase in substrate hydrolysis. The results indicate that the multicatalytic protease is a major cytoplasmic proteolytic system within neurons. The ability to monitor the activity of this protease in living neurons should facilitate identification of the factors that regulate its activity.

423.5

"MULTICATALYTIC PROTEINASE COMPLEX, A NONLYSOSOMAL PROTEINASE, IS PRESENT IN SIGNIFICANT AMOUNTS IN A NEURONAL CELL LINE" M.E. Pereira[¶], K. Berg, S. Maayani and S. Wilk[¶], Departments of Pharmacology[¶] and Anesthesiology, Mount Sinai School of Medicine, C.U.N.Y., New York, N.Y. 10029

Recent findings suggest that Alzheimer's disease (AD) may result from abnormal processing of multiple proteins. The multicatalytic proteinase complex (MPC) constitutes a major nonlysosomal proteinase with unique catalytic properties, splitting peptide bonds on the carboxyl side of hydrophobic (chymotrypsinlike activity), basic (trypsinlike activity) and acidic (peptidyl glutamyl activity) amino acids. Furthermore, MPC has been suggested to be the "catalytic core" of the Ubiquitin/ATP-dependent protein degradation pathway. The neuropathological finding of ubiquitinated proteins in neurofibrillary tangles in AD and in other neuronal inclusion bodies may implicate MPC-malfunction in neurodegenerative disorders, resulting in abnormal accumulation of ubiquitin-protein conjugates. In addition, MPC has chymotrypsinlike activity, the proteolytic activity believed to produce β-protein, the most abundant insoluble protein of senile plaques in AD brains. It is thus important to find a model system to study the regulation of MPC-activity. We report that the cytosolic fraction of a neuronal cell line (HT_4) has chymotrypsinlike, trypsinlike and peptidyl glutamyl activities, as shown by hydrolysis of the chromogenic substrates Cbz-Gly-Gly-Leu-pNA, Cbz-D-Ala-Leu-Arg-2NA and Cbz-Leu-Leu-Glu-2NA respectively. Specific immunoprecipitation of MPC with polyclonal antibodies generated in our laboratory resulted in the inhibition of the above described peptidase activities, providing evidence for the involvement of MPC in these activities. Moreover we demonstrate that low concentrations of SDS (0.04%) stimulate the peptidyl glutamyl activity. The HT_4 cells have been shown to differentiate with neuronal morphology, express neuronal antigens, synthesize and secrete nerve growth factor (NGF), and have receptors for NGF. Thus HT_4 cells are an excellent tool for studying the regulation of MPC-activity. (Supported by the Alzheimer's Association/New York City Chapter Pilot Research Grant PRG-90-114 to M.E.P.; NIH grant NS-17392 and an ADAMHA Research Scientist Award MH-00350 to S.W.; USPHS grant GM-34852 to S.M. The HT_4 cells were kindly provided by R.D.G.McKay.)

423.6

SELECTIVE LOSSES OF SOMATOSTATIN SS1 BINDING SITES IN FRONTAL AND TEMPORAL CORTEX OF ALZHEIMER'S PATIENTS. S. Krantic, Y. Robitaille, and R. Quirion, Douglas Hospital Research Center, Dept. of Psychiatry, McGill University, Verdun, Quebec, Canada.

Somatostatin (SS) binding sites were found to be decreased in frontal and temporal cortices of Alzheimer's diseased (AD) patients (Beal et al. 85). However, the human brain cortex was shown to contain two sub-types of SS receptors, classified as SS1 and SS2 (Reubi et al. 87). Therefore we assessed if a sub-type is preferentially altered in AD. Direct saturations of the non-selective (^{125}I-Tyr^0,D-Trp^8-SS$_{14}$) and SS1-selective (^{125}I-SMS-204090) ligands were performed in homogenates of frontal and temporal cortices from AD (n=14) and control (n=8) patients. Apparent binding affinities (Kd) were not different between these two groups, neither in frontal nor in temporal cortices. However, maximal binding capacity of SS1 sites were significantly lower ($p<0.05$) in both regions of AD subjects vs. controls. In contrast, Bmax value of the non-selective radioligand (SS1+SS2) was decreased only in the temporal cortex in AD. These results indicated that cortical SS1 and SS2 binding sites are differentially affected in AD. Supported by the MRC.

423.7

PNMT mRNA LEVELS IN C-1 NEURONS IN ALZHEIMER'S DISEASE. W.J. Burke, C.A. Schmitt* and H.D. Chung*. St. Louis VAMC and St. Louis University Medical School, St. Louis, MO 63125

Phenylethanolamine N-methyltransferase (PNMT) activity and protein levels decrease in Alzheimer's disease (AD) brains without loss of C-1 neurons (Ann Neurol 22:278, 1987; 24:532, 1988). To determine whether the deficit is due to decreased levels of PNMT mRNA we measured PNMT mRNA using PCR amplification and densitometric quantitation of northern blots of PNMT mRNA extracted from human C-1 neurons. Ten AD cases (75.6 ± 26 yrs; PMI of 5.1 ± 1.3) were compared to 4 controls (71 ± 3.6 yrs; PMI of 5.7 ± 1.1). There was no difference in C-1 PNMT mRNA levels between the groups (AD:32.1 ± 8.8 units/ng RNA vs. controls: 40.6 ± 14.1). We conclude that decreased PNMT protein in AD is not due to decreased transcription of PNMT mRNA but may be due either to decreased translation of PNMT mRNA or increased degradation of PNMT protein.

423.8

OXYGEN-FREE RADICAL MEDIATED PROCESSES IN ALZHEIMER'S DISEASE. L.J. McIntosh, M.A. Trush* and J.C. Troncoso. Department of Environmental Health Sciences, The Johns Hopkins University School of Hygiene & Public Health, and Neuropathology Laboratory, The Johns Hopkins University School of Medicine, Balto., MD 21205.

Free radical damage has been proposed as a factor in aging and in several neurodegenerative disorders, including Alzheimer's disease (AD). Changes of nucleic acids, proteins, and membranes may result from reactions with oxygen species, including superoxide and hydroxyl and peroxyl radicals. To assess the role of free radicals in AD, we used chemiluminescence (CL), which measures bond breakage as it occurs, and the thiobarbituric acid test, which measures malondialdehyde (MDA) formed during lipid peroxidation. Tissue samples were obtained from the temporal cortices of six pairs of cases of AD and controls matched for age, postmortem delay, and accession date. In AD tissues, we found significant increases in CL (28%) and MDA formation (42%), as compared to controls. These were differences in basal levels from tissue supernatants that were not stimulated by iron, ascorbate, or hydrogen peroxide. Studies with biochemical probes to determine the predominant radical species in these cases are in progress. Our results support the hypothesis that oxidative stress may contribute to the damage and degeneration of neurons at risk in AD.

423.9

DIFFERENTIAL SCREENING OF A MONKEY FRONTAL POLE cDNA LIBRARY: IDENTIFICATION AND CHARACTERIZATION OF mRNAs WITH REGIONAL DISTRIBUTION. K.Chandrasekaran, J.Stoll, T.Giordano, J.R.Atack*, M.F.Matocha*, S.P.Wise and S.I.Rapoport. Lab of Neurosci, NIA, Bethesda, MD 20892 and Lab of Neurophysiology, NIMH, Poolesville, MD 20837.

Alzheimer's disease afflicts selectively association as compared to primary sensory and motor neocortices. To understand the molecular basis of this selective vulnerability, we screened a frontal pole cDNA library from the rhesus monkey (Macaca mulata) brain to identify mRNAs that are expressed in frontal pole as compared to primary visual cortex. Six cDNA clones, whose differential expression was confirmed by Northern blot analysis were identified. These cDNA clones showed higher levels of mRNA in frontal pole and dorsal lateral prefrontal regions as compared to primary visual cortex. Sequencing and homology search with GenBank showed that 3 of the cDNA clones were homologus to the mitochondrial DNA encoded genes, cytochrome oxidase subunits I, II and III (COX I,II,III). Cytochrome oxidase histochemistry showed highest level of enzyme activity in dendritic rich neuropil areas of cortex. *In situ* hybridization with COX cDNA probes showed laminar distribution in visual and somatosensory cortices, but was not apparent in the prefrontal cortex. These results suggest differences in the distribution of neuropil as compared with cell bodies among the brain regions . Comparison of COX gene expression in motor and temporal regions of normal and Alzheimer brain samples showed 2-5 fold lower levels in temporal region of Alzheimer brain. Abnormal synaptic and dendritic metabolism in the dementia in Alzheimer's disease is suggested.

423.10

TRANSFERRIN AND ALUMINUM: EFFECTS ON RAT HIPPOCAMPAL CULTURES. R.K.Lartius and E. Uemura. Neuroscience Program, Department of Anatomy, Iowa State University, Ames, IA 50011.

Aluminum (AL) has been found associated with very specific brain regions in Alzheimer's disease (AD). Higher than normal levels of AL can be detected in the hippocampus, basal forebrain and cortical regions. This regional specificity might be explained by a receptor mediated mechanism, where AL is transported to these select areas. In the present study, we have examined the metal chelating protein, transferrin (Tr), and its ability to enhance AL uptake in primary rat hippocampal cultures. Further, we examined the effects of the transferrin-AL complex on the microtubule-associated-proteins, MAP-2 and TAU. AL was complexed to TR as previously described by Roskams and Connor (1990) and at 4 days post-plating, the hippocampal neurons were exposed to either solvent (10 mM sodium bicarbonate in Tris Saline), 1.5 uM Tr, 1.5 uM AL+Tr or 3.0 uM AL. Twenty-four hours later the cultures were fixed and MAP-2 and Tau-like immunoreactivities were detected using the HM-2 antibody to MAP-2 and the Tau-1 antibody to Tau. The intracellular AL accumulation was also detected using Morin Stain at low pH. We found that cultures exposed to AL+Tr were the only cultures that had detectable levels of intracellular AL. Further, these neurons displayed extensive neuritic retraction and decreased Tau and MAP-2 immunoreactivities compared to control. No other cultures were affected. These results support the theory that transferrin or a similar chelator may play an integral role in the AL accumulation seen in Alzheimer's Disease. While it appears unlikely that AL is responsible for initiating the pathology, the ultimate presence of this bioactive metal warrants further investigation.

423.11

ALZHEIMER'S ANTIGEN: A RE-EXPRESSED PROTEIN OF IMMATURE CNS

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Anti-PHF-1 (CD1) is a novel monoclonal antibody that recognizes an epitope abundant in AD brain tissue but totally absent from age-matched controls. Species screening has detected the epitope in immature rat and chicken CNS; immunofluorescent studies have been done using chicken to compare CD1 antigen, tau and Alz-50 antigen. *Brain Distribution* Tau and CD1 antigen were both abundant, similarly occurring in immature axon tracts of the central nervous system, and in the cranial and peripheral nerves. Alz-50 immunoreactivity (IR) was present only in occasional cells of the developing neuroepithelium. *Retina Development* At embryonic day 6, tau occurred in the ganglion cell layer; post-hatch, it was detectable in perinuclear regions of most cells, but was abundant only in the optic fiber layer. CD1 antigen distribution resembled tau initially, but unlike tau, it underwent total down-regulation in optic nerve axons. Alz-50 was nearly negative throughout retina development. *Cell Culture* Tau in explant cultures maintained a normal distribution, but it was expressed aberrantly in dissociated cell culture, appearing in all neurites, not just axons. CD1 in dissociated cultures occurred less frequently than tau, with unlabeled neurites often observed. Alz-50 IR was absent from explant cultures, but in dissociated cell cultures, it was expressed in many neurites, particularly within cell clusters. Initially, Alz-50 IR was absent from all cells (tau was present), appearing only with time, suggesting its aberrant expression was induced by lack of proper cell-cell interactions. *Conclusion* Alzheimer's pathogenesis entails an aberrant re-expression of proteins from the immature CNS, evidenced by CD1 antigen, a growth-associated axonal protein. Possibly triggered by multiple stimuli, re-expression of developmentally-regulated proteins into an improper milieu may create dysfunctional complexes that disrupt and ultimately destroy the cell.

423.12

ELEVATED TEMPORAL CORTEX SUPEROXIDE DISMUTASE IN ALZHEIMER'S DISEASE.

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Recent analyses indicate that Alzheimer's disease (AD) is a heterogeneous disorder, certain types of which may involve a genetic component residing on chromosome 21. The gene encoding copper/zinc superoxide dismutase (Cu/ZnSOD; SOD-1) is also located on chromosome 21. To determine whether levels of Cu/ZnSOD might be altered in AD, SOD activity was measured in postmortem samples of temporal cortex from 12 patients with AD, 12 age- and sex-matched controls, and four patients with non-AD neuropathies (N-ADN). Cu/ZnSOD activity was significantly increased in samples from patients with AD; activity of Cu/ZnSOD was (mean $\pm$ SEM, in U SOD/mg protein): Controls, 56.2 $\pm$ 3.9; N-ADN, 52.1 $\pm$ 3.4; and AD, 67.5 $\pm$ 3.5 ($p < 0.05$ AD versus Controls and N-ADN). There were no differences between groups in mean postmortem time. The patients affected by AD were further subdivided by age into two groups, < 80 years of age (N = 9) and $\geq$ 80 years of age (N = 3) at autopsy. Cu/ZnSOD activity in samples from patients $\geq$ 80 years of age (33.5 $\pm$ 3.6) was not significantly different from levels detected in similarly age-stratified Control (33.3 $\pm$ 1.9, N = 4) or N-ADN (37.6, N = 1) groups. Cu/ZnSOD activity measured in samples from patients < 80 years of age at autopsy (47.4 $\pm$ 1.6, N = 9) was significantly greater ($p < 0.01$) than the activity in Control samples (32.2 $\pm$ 5.5, N = 8), N-ADN samples (30.8 $\pm$ 3.8, N = 3), and samples from patients $\geq$ 80 years of age (33.5 $\pm$ 3.6, N = 3). There were no differences between any of the groups, regardless of age stratification, in the activity of manganese SOD.

423.13

A PILOT CROSSOVER STUDY OF THE ANGIOTENSIN CONVERTING ENZYME INHIBITOR (ACEI) SQ29,852 IN ALZHEIMER'S DISEASE.

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Animal experiments as well as results from a study on the quality of life of hypertensive patients treated with captopril suggest that ACEI may possess cognitive enhancing properties. The present study was a 3-way crossover trial of two dose levels (5 mg and 40 mg b.i.d.) of the ACEI SQ29,852 and placebo b.i.d. in normotensive patients with Alzheimer's disease (n=30; mean age 67.5 years). Each patient participated in three consecutive periods of 5 weeks (1 week single blind placebo lead-in and 4 weeks double blind comparison treatment). Cognitive evaluation was performed using a computerized battery of tests examining attention, verbal learning, and short- and long-term memory. Analyses by chi square on the frequencies of "best score" on each drug condition for all tests showed no significant differences for individual tests. However, the visual memory tests approached significance (p=0.058) in favor of SQ29,852 and best scores were obtained by the greatest number of patients on 9 of the tests at the high-dose SQ29,852, on 7.5 at the low-dose, and only on 4.5 with placebo. Overall, patterns and relationships of results were consistent with a drug effect. A study of longer duration and/or higher dosages using a stratified design would be indicated to clarify these observations.

423.14

INTERLEUKIN-1 INCREASES THE EXPRESSION OF AMYLOID PRECURSOR PROTEIN mRNAs IN NEURONAL BUT NOT GLIAL CELLS.

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Increased levels of interleukin-1 (IL-1) have been observed in brain of patients with Alzheimer's disease (AD) and it has been demonstrated that IL-1 enhanced the expression of amyloid precursor protein (APP) in human endothelial cells. We compared the effects of IL-1 treatment on APP mRNAs expression in neuronal, glial and endothelial cells.The gene encoding the precursor of the APP can be expressed as APP695 and/or APP563/751/770 depending on the presence of a Kunitz family protease inhibitor sequence. We investigated the expression of APP gene by using three different probes recognizing the total APP mRNA, the APP563/751/770 mRNA or the APP695 mRNA. Total RNA was extracted from human and mouse endothelial cells, from rat cortical neurons dissociated at E17 and from rat forebrain astrocytes. The Northern blot analysis was performed using the APP ^{32}P-cDNA probe to detect the general APP sequence and two ^{32}P-labelled oligonucleotides (40 mer) complementary to the sequence of the protease inhibitor or to the APP695. In endothelial cells, we observed similar increase of APP695 mRNA or APP563/751/770 mRNA after treatment with human recombinant IL-1β. In neuronal cells, IL-1 (50-200ng/ml) induced a significant increase of APP mRNAs, detected with all three probes. In glial cells, the expression of APP mRNAs did not appear altered by IL-1. The present results demonstrate the potential role of IL-1 in the mechanisms related to the β-amyloid protein deposition in AD.

423.15

COMPARISON OF THE EXPRESSION AND TOXICITY OF THE COOH TERMINAL 100 AMINO ACIDS OF APP CONTAINING A SIGNAL SEQUENCE.

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Previously we and others (Yankner et al., Science 245: 417-420, 1989) have demonstrated that the carboxyl terminal 100 amino acids of APP yields a product that is toxic to differentiated PC12 cells. Recently, Maruyama et al., (Nature 347: 566-569, 1990) have reported that the carboxyl terminal 100 amino acid sequence of APP when attached to a signal peptide results in secretion of the full length APP fragment into the media. We have constructed an analogous signal sequence containing carboxyl terminal APP construct and compared it to the unmodified construct in a variety of cell lines. Western blot analysis of transient and stable lines reveals an approximately 14 kilodalton band using a carboxyl terminal APP antibody; while the signal sequence construct containing line shows an additional band of 15-16 Kd. (R1, N. Robakis, Mt. Sinai School of Medicine). Medium conditioned by each cell line was tested for toxicity in NGF differentiated PC12 cells. Preliminary data indicates that medium conditioned by cells transfected with the non signal sequence containing construct was toxic whereas the medium conditioned by the signal sequence containing lines was not toxic. These data suggest that the signal sequence containing construct may be subject to normal processing thereby eliminating the toxic amyloid fragments while the unmodified carboxyl terminal APP fragment may be abnormally processed to yield a toxic species.

423.16

DEXAMETHASONE AND DIBUTYRYL CYCLIC AMP INDUCE THE EXPRESSION OF ALPHA-1-ANTICHYMOTRYPSIN IN RAT ASTROCYTES: IMPLICATIONS FOR ALZHEIMER'S DISEASE.

S. Das*, R.B. Nelson, and H. Potter. Department of Neurobiology, Harvard Medical School, Boston, MA.

The acute phase protein α_1-antichymotrypsin (ACT) is a primary component of the senile plaques characteristic of Alzheimer's disease (AD), where it is found bound to β-protein, a proteolytic fragment of the amyloid precursor protein (APP). Immunocytochemistry and *in situ* hybridization indicates that astrocytes are a source of the increased amounts of ACT in the AD brain. Thus it appears that an acute phase response occurs in the CNS that may be instrumental in the etiology of Alzheimer plaque formation.

In light of the fact that the mediators of the acute phase response, dexamethasone and interleukin-1, can induce the expression of ACT in rat hepatocytes *in vitro*, we asked whether these mediators can also induce the expression of ACT in astrocytes. Astrocyte cultures were prepared from newborn CD rats and grown to confluence. Cells were then switched to serum-free N2 medium for two hours and then treated with the appropriate agents for four hours. Total RNA was isolated and subjected to Northern blot analysis.

We found that dexamethasone induced the expression of ACT message synergistically with dibutyryl-cAMP. The effect of dexamethasone was blocked by treatment of the cells with cyclohexamide, indicating that protein synthesis is necessary for the induction. Thus, there are multiple regulators of ACT expression in astrocytes of which we have found one; the intracellular messenger of another is cAMP. These results, indicating an acute phase expression of ACT in astrocytes, joins a growing body of evidence that an inflammatory response is involved in β-protein amyloid deposition. However, since not every inflammatory event leads to plaque formation, there must be a second prerequisite event: the abnormal processing of APP. We posit that the pathology observed in AD arises from the presence of β-protein (or a fragment of APP containing intact β-protein) in the extracellular compartment of the CNS, coupled with an acute phase response.

423.17

IS ALZHEIMER'S DISEASE A LATE-ONSET, MOSAIC FORM OF DOWN SYNDROME? H. Potter and L.N. Geller. Dept. Neurobiology, Harvard Medical School, Boston, MA 02115.

Perhaps the most interesting clue to the cause of Alzheimer's disease is the fact that Down syndrome patients who live beyond the age of 30 or 40 develop dementia and neuropathology essentially indistinguishable from classic Alzheimer's disease. The implication of this finding is that trisomy for chromosome 21—the pathogenetic cause of Down syndrome—is also capable of causing Alzheimer's disease, possibly through the overexpression or duplication of a gene residing on chromosome 21. A hypothesis has been presented (Potter, Am. J. Hum. Genet., June 1991) that explains both the familial and sporadic forms of Alzheimer's disease as arising from the accumulation of chromosome 21 trisomy cells during the life of the individual. That is, trisomy 21 cells, developing over time by unequal chromosome segregation during mitosis, may ultimately lead to many cases of Alzheimer's disease through the same (as-yet unknown, and perhaps multistep) mechanism by which Down syndrome patients get the disease, but at a later age due to the modulating effect of the mosaicism. The propensity to develop trisomy 21 cells could be genetic in origin (either due to an aberrant chromosome 21 centromere or to a mutation occurring elsewhere in the genome and affecting all chromosome segregation), or it could be caused by environmental factors. *In situ* hybridization studies are being carried out to determine the extent of trisomy 21 mosaicism in the skin and central nervous system of familial and sporadic Alzheimer's disease cases.

423.18

IDENTIFICATION OF CANDIDATE ALZHEIMER β-PROTEIN-GENERATING PROTEASES FROM RAT BRAIN USING THE POLYMERASE CHAIN REACTION. R.B. Nelson, S. Das*, and H. Potter. Department of Neurobiology, Harvard Medical School, Boston, MA.

A chymotrypsin-like protease (CLIP) is predicted to be able to directly generate the N-terminus of β-protein from the β-amyloid precursor protein (APP). Moreover, an inhibitor specific for certain CLIPs, α_1-antichymotrypsin (ACT), shows increased expression by reactive astrocytes in affected areas of Alzheimer brain, and is co-deposited with β-protein as an integral component of Alzheimer amyloid. We have previously characterized and purified a CLIP from 7-day-old rat brain which selectively cleaves a synthetic substrate containing the APP amino acid sequence immediately "upstream" from the N-terminal cleavage site of β-protein. The amino acid sequence of this protease matches that of rat mast cell protease I (RMCP I) and its distribution and ontogeny in rat brain are consistent with a mast cell origin.

We are currently searching for other ACT-inhibited CLIP species in brain by using PCR. The amino acid sequences of the serine active site present in all serine proteases and of an N-terminal domain found in all ACT-inhibited CLIPs were used to construct degenerate primers spanning a ~560 base pair coding region for CLIPs. We first prepared poly A+ RNA from 7-day-old rat brain meninges (a tissue enriched in RMCP I activity), generated cDNA by reverse transcription, and amplified this cDNA. We have successfully used this approach to sub-clone and partially sequence an amplification product coding for RMCP I. Restriction enzyme mapping indicates the presence of at least one other cDNA species in this amplified band, probably corresponding to a second mast cell CLIP. We have also used this approach to examine cDNA from cultured astrocytes expressing ACT (see Das et al., this meeting) because cells expressing a protease inhibitor will also commonly express the target protease. We have amplified from astrocyte cDNA a single PCR product of appropriate size which we are sub-cloning and sequencing, and have identified a corresponding activity by gel enzymography.

423.19

EFFECT OF AGING ON NA^+/CA^{2+} EXCHANGE ACTIVITY IN HUMAN FRONTAL CORTEX. R.A. COLVIN*, L. WINCE, A. WU* and N. DAVIS*. **Zoological and Biomedical Sciences, Ohio University College of Osteopathic Medicine, Athens, OH 45701.**

Cerebral plasma membrane vesicles (PMV) were purified by sucrose density gradient centrifugation from either rat brain or frozen human post-mortem frontal cortex tissue slices. Human specimens were obtained from the National Neurological Research Bank, Los Angeles, CA. An animal model was tested for the effect of short term post-mortem delay on Na^+/Ca^{2+} exchange activity. Rats were decapitated and their skulls were incubated at 0-4°C for various times up to 4 hrs. The brains were then removed and processed into PMV. No significant effect of post-mortem delay was seen on the purification, as judged by the activity of plasma memebrane markers. Proteolysis of memebrane associated proteins, as judged by SDS-PAGE, was not evident even after 4 hr of preincubation. No significant effect of post-mortem delay was seen on estimates of Na^+/Ca^{2+} exchange V_{max} or K_m, or Ca^{2+} passive permeability. PMV from human frontal cortex samples (ranging in age from 20 to 90 yrs.) were assayed for Na^+/Ca^{2+} exchange V_{max} and K_m, and passive Ca^{2+} permeability, At present, no age related effects have been observed in these parameters of Na^+/Ca^{2+} countertransport in human tissues. Mean values obtained thus far are: V_{max}=2.13±.86 nmol/mg/sec;K_m=.143±.06mM; and the rate constant of Ca^{2+} release = .032±.03 min^{-1}. The results indicate that cerebral plasma membrane Na^+/Ca^{2+} countertransport is extremely stable with respect to post-mortem delay. Although more human tissues must be assayed before final conclusions are made, preliminary studies indicate few, if any age related effects on plasma membrane Na^+/Ca^{2+} countertransport.

423.20

PRESENCE OF ABERRANT PROTEINS IN DORSAL ROOT GANGLIA PRODUCES INTRANEURONAL INCLUSIONS CONTAINING CYTOSKELETAL COMPONENTS AND UBIQUITIN. P.L. Di Patre, N. Wicker*, Y. Chen*, L. Autilio-Gambetti, and P. Gambetti. Division of Neuropathology, Institute of Pathology, Case Western Reserve University, Cleveland, Ohio, 44106, USA.

Formation of intraneuronal inclusions containing both normal and altered cytoskeletal proteins as well as ubiquitin is a feature of age-related neurodegenerative disorders. These inclusions might result from the presence of aberrant proteins. To test this hypothesis, we studied the effect of canavanine (Can), an arginine analogue, on cultured rat dorsal root ganglia (DRGs). In DRGs kept for 5 days in medium in which Can replaced Arginine, most neurons contained rounded cytoplasmic inclusions that were silver positive and immunoreacted with antibodies to phosphorylated and non-phosphorylated neurofilaments, tau and ubiquitin. These findings indicate that a condition resulting in the presence of a large amount of aberrant proteins is associated with the formation of intracellular inclusions. The antigenic characteristics of these intraneuronal inclusions are similar to those of the inclusions present in neurodegenerative diseases. Thus, we suggest that the presence of aberrant proteins secondary to the disease process might play a role in the formation of neuronal inclusions of neurodegenerative diseases such as the neurofibrillary tangles of Alzheimer disease.

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423.21

NEUROCHEMISTRY OF THE AMYGDALA IN ALZHEIMER'S DISEASE (AD), HYPERTENSION AND CRITICAL CORONARY ARTERY DISEASE (cCAD). D. Larry Sparks, John C. Hunsaker* & William R. Markesbery. Alzheimer's Disease Research Center/Sanders-Brown Center on Aging; U.K.M.C., Lexington, KY 40536

The importance of the amygdala in memory function has been established by lesion studies in animals. In man, bilateral lesions of the amygdala cause amnesia. Histologic investigation of the amygdala in Alzheimer's disease (AD) has revealed the presence of senile plaques (SP) and neurofibrillary tangles (NFT), as well as reduced volumn of most nuclei of the amygdala. Abundant SP are also found in the amygdala of non-demented individuals with cCAD and hypertension compared to non-demented non-hypertensive non-heart disease (non-HD) controls, often at levels diagnostic of AD, while NFT are rarely found in the amygdala of these individuals.

Utilizing established methods we have investigated markers of cholinergic, serotonergic and noradrenergic neurotransmission in whole amygdala from 19 age-matched individuals (5 AD, 3 hypertensive, 5 cCAD and 6 non-HD). Scatchard analysis of QMB and 5-HT binding, single point analysis of imipramine and DHA binding, monoamine oxidase A and B (MAO-A &B) and acetylcholinesterase (AChE), kinetics of choline acetyltransferase (ChAT), and HPLC quantification of NE, 5-HT and 5-HIAA were performed simultaneously. Postmortem interval and age at death was not significantly different among the groups.

The Vmax, but not the Km, of ChAT activity, AChE activity, NE and 5-HT levels, and Bmax, but not the Kd, of QMB binding were reduced ($p<0.05$) in AD compared to all three non-demented control groups. Binding of 5-HT and DHA were non-significantly decreased and increased, respectively, in AD compared to all three control groups. Imipramine binding and MAO-A activity were significantly ($p < 0.05$) increased in AD compared to non-HD and cCAD controls, only. The Vmax of ChAT activity was significantly ($p < 0.05$) increased in cCAD and non-significantly increased in hypertension compared to non-HD controls. The data indicate a possible over estimation of cholinergic decrements in AD by the inappropriate use of controls with cCAD and raise questions about the pathophysiologic importance of SP in AD.

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423.22

DIFFERENTIAL CELLULAR EXPRESSION OF MAO-A AND MAO-B IN CONTROL VS. ALZHEIMER BRAINS. J. Saura*,[1] J.C. Shih, M. Da Prada*,[2] V. Chan Palay,[3] J. Ulrich*, G. Huber, J. Löffler* and J.G. Richards, Pharma Division, Preclinical Research, F.Hoffmann-La Roche Ltd, CH-4002 Basel, [1]John Stauffer Pharmaceutical Scl. Center, USC, Los Angeles CA; [2]Neurology Clinic, Kantonsspital, Zürich, and [3]Pathology Institute, Kantonsspital, Basel, Switzerland

Monoamine oxidases (MAO) oxidatively deaminate neurotransmitter and xenobiotic amines in the CNS and peripheral organs. Two forms (MAO-A and MAO-B) have been identified by substrate selectivity, inhibitor sensitivity and primary structure. Quantitative enzyme radioauto-graphy and in situ hybridization have now revealed the distribution, abundance and cellular localization of the isoenzymes and their mRNAs in human postmortem brainstem. Whereas MAO-A (protein and mRNA) is expressed by noradrenergic neurons of the locus coeruleus, MAO-B is expressed by serotoninergic neurons of the raphé nuclei. In the substantia nigra, MAO-A is restricted to the zona compacta whereas MAO-B was found mainly in the zona reticulata. To date, mRNA for neither MAO-A nor MAO-B could be detected in the substantia nigra or in glial cells of the brainstem. In cerebral cortex and hippocampus but not cerebellum from Alzheimer individuals, MAO-B was markedly increased in astrocytes surrounding senile plaques (identified by ß-amyloid- and GFAP-antigenicity); MAO-A was unchanged. MAO-B mRNA could not be detected in plaques. The functional implications of the differential compartmentalization and regulation of these isoenzymes will be discussed.

423.23

MAPPING THE DEFECT IN M1 MUSCARINIC RECEPTOR - G PROTEIN COUPLING IN ALZHEIMER'S DISEASE: AN AUTORADIOGRAPHIC STUDY. D. C. Mash, M. J. Basile and D.D. Flynn, Departments of Neurology and Pharmacology, University of Miami School of Medicine, Miami, FL., 33101.

Cortical M1 receptors are the current target for the development of potent and selective cholinomimetic drugs to improve memory and cognitive impairments associated with Alzheimer's disease (AD). We have demonstrated a defect in the ability of the M1 receptor subtype to form high affinity agonist receptor-G protein complexes in AD frontal cortex (Flynn et al., 1991). Since the high affinity agonist state mediates signal transduction by the M1 receptor, we have speculated that this 'altered state' of the cortical M1 receptor for agonists may account for the relative lack of effectiveness of cholinergic replacement therapies attempted thus far. In this study, quantitative autoradiographic methods were used to map the defect in M1 receptor coupling in AD. To visualize the density and distribution of M1 receptors, brain sections were labeled with 3 nM [^{3}H]-pirenzepine (33% occupancy of M1 receptor subtype). Competition curves were generated by increasing carbachol concentrations (10^{-8} to 10^{-2}) with 3 nM [^{3}H]-pirenzepine. The effect of guanine nucleotides on [^{3}H]-pirenzepine and carbachol binding was measured in the presence of 0.2 mM GppNHp. In aged control brain sections, the full agonist carbachol (10^{-5} M) displaced 40 - 55 % of the specific binding of [^{3}H]-pirenzepine to frontal, temporal and occipital cortices. Treatment with GppNHp abolished high affinity agonist binding in brain sections co-incubated with carbachol and [^{3}H]-pirenzepine. In AD cases with severe neocortical pathology, carbachol failed to displace [^{3}H]-pirenzepine binding in frontal (Brodmann 9) and temporal (Brodmann 28) cortices. However, high affinity agonist binding to M1 muscarinic receptors was spared in the motor and occipital cortices and in the putamen. These results demonstrate a regional specificity of the pathological defect in M1 receptor coupling in AD. (Supported by NS25785, NS19065 and the AHAF.)

423.24

DIFFERENTIAL EXPRESSION OF CORTICAL NICOTINIC AND MUSCARINIC CHOLINOCEPTORS IN ALZHEIMER'S DISEASE H. Schröders#, E. Giacobini‡, R.G. Struble+, K. Zilles#, P.G.M. Luiten‡, A.D. Strosberg^ and A. Maelicke°. Depts. Pharmacology‡ and Psychiatry+ Southern Illinois Univ. Sch. Med., Springfield, IL 62794, #Dept. Anatomy, Univ. Köln, F.R.G., ‡Dept. Animal Physiology, Univ. Groningen, The Netherlands, ^Institut Cochin, Paris, France and °Dept. Physiological Chemistry, Univ. Mainz, F.R.G.

The cellular expression of nicotinic cholinoceptors (nAChR) is significantly reduced in the frontal cortex in Alzheimer's disease (AD) (Schröder et al., *Soc. Neurosci. Abstr.*, 16:612, 1990) whereas no data are available on the expression of muscarinic AChRs (mAChR). Using the monoclonal mAChR-antibody M35 (André et al., *J. Recept. Res.*, 7:89, 1987) autopsy samples of human frontal cortex were studied immunohistochemically in: (1) middle-aged controls [n=3; 55±5yrs], (2) age-matched controls [n=3; 73±6yrs] and (3) AD cases [n=6; 74±5yrs]. Densities of M35-labeled [(1) 4673±1364 neurons/mm^3, (2) 2393±499, (3) 5070± 1008)] and of cresylvioletstained neurons did not show statistically significant differences ($p>0.05$). By contrast to nAChR protein, mAChR expression appears to be virtually not affected in AD. Since nAChRs and mAChRs are colocalized in about 30% of cholinoceptive cortical neurons (Schröder et al., *Synapse*, 4:319, 1989) in AD cholinergic cortical transmission might be mediated in great part exclusively by mAChRs. Supported by the Deutsche Forschungsgemeinschaft (Schr 283/6-1), Southern Ill. Univ. Central Res. Committee award and R.J. Reynolds Tobacco Co..

423.25

THE EFFECTS OF TETRAHYDROAMINOACRIDINE ON ATTENTION AND MEMORY IN ALZHEIMER'S DISEASE B.J. Sahakian, S.A. Eagger* and R. Levy* Section of Old Age Psychiatry, Dept. of Psychiatry, Institute of Psychiatry, London, U.K.,SE5 8AF.

The efficacy of tetrahydroaminoacridine (THA) plus lecithin in the treatment of cognitive impairment in patients with probable Alzheimer's disease (AD) was compared to that of placebo. Eighty-nine patients entered the study, 24 were withdrawn, 19 due to side effects and 5 for other reasons. Patients received the maximum tolerated dose of up to 150 mg per day of THA and 10.8 gm/day of lecithin. They were randomly assigned to double-blind treatment of either active or placebo drugs crossing over after 13 weeks treatment and 4 weeks washout. An analysis of the 65 patients who completed the trial showed a statistically significant beneficial effect of THA over placebo on the Minimental State Examination with 45% showing an improvement of 3 or more points compared to 11% in the placebo phase. Similar changes occurred in the Abbreviated Mental Test Score. There was however a considerable inter-subject variability in response. It is concluded that THA produced a demonstrable improvement in key outcome measures roughly equivalent to the deterioration which might have occurred over 6 - 12 months. Significant improvements were also seen on tests of attention but not memory as measured by the CANTAB batteries. Results are discussed in terms of the role of the cholinergic system in the control of attention and information processing.

423.26

LOW FRONTAL CHOLINE ACETYLTRANSFERASE ACTIVITY IS ASSOCIATED WITH SLOWING OF THE EEG AND WIDENING OF THE THIRD VENTRICLE IN ALZHEIMER'S DISEASE. H.Soininen, K.Reinikainen*, J.Partanen*, M. Puranen* and P.J.Riekkinen. Dept. of Neurology, Neurophysiology and Radiology, Univ. Kuopio, 70211 Kuopio, Finland.

Alzheimer's disease (AD) is characterized by marked loss of neocortical choline acetyltransferase (ChAT), slowing of the EEG, and ventricular widening. In 20 autopsy confirmed AD cases, we studied relationship between ChAT depletion of the frontal cortex, EEG slowing and enlargement of the third ventricle on the brain CT scan. The ChAT activity for patients with the dominant occipital rhythm of 5-6 Hz (n=7), 7 Hz (n=8) and 8-9 Hz (n=5) was 36.6±7.8, 42.5±7.8 and 49.6±12.6 (mean±SEM, pmol/mg protein/min), respectively, the control values being 94.5±6.5; the groups with highest and slowest rhythm differed significantly (p=.011). The width of the third ventricle correlated with the frontal ChAT activity (r=-.65, p=.005). The finding supports the contribution of the cholinergic deficit to EEG slowing in AD. The width of the third ventricle may partly reflect shrinkage of the basal forebrain and thus be related to the neocortical ChAT depletion.

423.27

ADENOSINE (A1) AND NMDA RECEPTORS IN THE HIPPOCAMPUS AND PARAHIPPOCAMPAL GYRUS OF ALZHEIMER'S DISEASE BRAINS. J. Ulas, L.C. Brunner and C.W. Cotman, *Department of Psychobiology, University of California, Irvine, CA 92717, USA.*

Adenosine acting *via* presynaptically located A1-receptors can inhibit the release of several neurotransmitters including the potentially neurotoxic excitatory amino acid glutamate which has been implicated in the pathology of Alzheimer's disease (AD). Our previous studies have demonstrated a significant decrease of binding to the NMDA receptor channel-associated sites in the CA1 hippocampal region of AD brain. This region is selectively vulnerable to AD pathology. In the present study, we compared the distribution of adenosine A1-receptors with that of NMDA receptors in the hippocampus and parahippocampal gyrus of six normal elderly controls and five AD patients in order to determine if a loss of A1-receptors in AD might play a role in CA1 vulnerability. The control and AD group were matched according to the age and postmortem delay. Autoradiography of A1-receptors was performed with the use of an antagonist ([3H]DPCPX) and an agonist ([3H]PIA), and binding to the NMDA receptor channel was studied with the use of [3H]MK-801. In control brains, adenosine A1 and NMDA receptors exhibited quite similar distributions with high density of both receptors in the CA1 hippocampal region and the dentate gyrus molecular layer. In AD brains, there was a significant decrease in binding to A1-receptors in the dentate gyrus molecular layer which was accompanied by a smaller drop in binding to NMDA receptors. This seems to reflect the pathology in the entorhinal cortex and loss of the perforant path input to the hippocampus. On the other hand, a significant loss of [3H]MK-801 binding in the CA1 strata pyramidale and radiatum was not paralleled by changes in binding to A1-receptors. Since some loss of neurons was observed in the CA1 area of AD hippocampi, the data suggest that adenosine A1 and NMDA receptors are not located on the same neuronal elements and/or are differentially regulated in AD.

423.28

HUMAN BRAIN FIXATION AND STORAGE CONDITIONS FOR CHOLINESTERASES HISTOCHEMISTRY. M.A. Morán and P. Gómez-Ramos. Dept.Morfología, Fac.Medicina, Univ.Autónoma,Madrid,Spain.

Collection of human brains must be preceded by the analysis of fixation and storage conditions.We searched for the optimum preservation of both acetylcholinesterase and butyrylcholinesterase, which accumulate in senile plaques and neurofibrillary tangles. Three fixatives (A,B and C) were analyzed: A) 4% paraformaldehyde in 0.1 M phosphate buffer. B) 10% formaline. C) 2% glutaraldehyde + 2% paraformaldehyde in 0.1 M phosphate buffer. The best fixative for both cholinesterases was A), and the optimum fixation-time was 48 hours. With B) both enzymes were reduced after short fixation-times (24-48 h), and completely inactivated after longer fixation-times (weeks or months), invalidating the use of old formaline collections. With C) very short fixation-times (less than 24 h) only slightly reduced the activity of both enzymes, but after 42 h the enzymatic preservation (mainly of butyrylcholinesterase) was very low. Still, glutaraldehyde can be used for a few hours to improve the E.M. ultrastructure.

Fixed blocks were stored at 4°C in 0.1 M phosphate buffer + 40% sucrose for 2-3 months before a reduction of butyrylcholinesterase was observed. For longer periods (a year or more)blocks were kept unfrozen at -17°C in a 30% glicerol + 30% etilen glycol buffered solution, without further decrease of enzymatic activity. Tissue already sectioned was successfully stored unfrozen at -17°C for up to 4 months in the glycol solution. Supported by FIS 88/0922 and 90/0259.

424.1

A CELL MODEL FOR THE STUDY OF PARKINSONISM.

W.L. Yang, F.F. Oldfield and A.Y. Sun. Department of Pharmacology, University of Missouri, Columbia, MO 65212.

A loss of dopamine (DA) containing neurons appears to be the primary pathological changes in Parkinson's disease. The cause of DA cell death remains unclear. Since paraquat is chemically similar to MPTP and was implicated in causing Parkinsonism's symptom in humans. We have studied the action of paraquat on catecholamine containing cells. We have demonstrated that the rat pheochromocytoma cell line (PC12) can actively take up both dopamine and paraquat. Incubation of cells with paraquat leads to some cell death in a dose- and time-dependent manner. We have also demonstrated that pretreatment of cells with paraquat at various time intervals have led to progressive decrease in dopamine uptake activity. Since oxygen radicals may be involved in the toxicity of paraquat as demonstrated (Yang *et al.*, Free Radical Biol. Med. 9 Suppl. 1, 165, 1990). Our results correlated well with synaptosomal DA uptake and lipid peroxidation activity after mice treated with paraquat. It appears that the neurotoxins, such as MPTP and paraquat may cause Parkinson's disease by free radical attack on dopaminergic neurons. The possible protective effect by free radical scavengers and endogenous agents which might cause Parkinson's disease through free radical mechanisms are under extensive investigation in our laboratory using the cell model.

424.2

DIHYDREXIDINE, A FULL DOPAMINE D1 AGONIST, REDUCES MPTP-INDUCED PARKINSONISM IN THE AFRICAN GREEN MONKEY.

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The antiparkinsonian effects of dopamine agonists have largely been attributed to activation of D2 dopamine receptors. Reports in human and non-human primates using SKF38393, the prototypical D1 agonist, have failed to demonstrate significant antiparkinsonian effects, suggesting that D1 receptors were not involved. Based on stimulation of cAMP synthesis, SKF38393 is only a partial agonist. Recently, dihydrexidine has been reported to be a full D1 agonist, bioavailable in brain, and to have a ten fold D1:D2 selectivity. We have therefore administered dihydrexidine to MPTP-treated non-human primates. MPTP produces a parkinsonian syndrome in humans and in non-human primates. In Parkinson's disease and the MPTP-treated primate, motor deficits are correlated with regional dopamine losses and decreased eye blink rates. Dihydrexidine (0.3-0.9 mg/kg, i.m.) significantly reversed parkinsonism and increased eye blink rates in five parkinsonian MPTP-treated African green monkeys in a "blind", pseudorandom sequence, saline-controlled study. Behavioral measures of parkinsonism were markedly reduced for 30-45 minutes after the drug, while eye blink rates and healthy behaviors increased. After 45 minutes, the monkeys returned to their prior level of disability. Antiparkinsonian effects were correlated with changes in blink rate. Our pharmacological studies show that blink rate can be independently regulated by D1 and D2 receptors. Dihydrexidine's dramatic antiparkinsonian effects indicate that it and other full efficacy D1 receptor agonists may be of particular utility in the treatment of Parkinson's disease.

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424.3

BIOCHEMICAL AND PHARMACOLOGICAL STUDIES ON CABERGOLINE, A NEW PUTATIVE ANTIPARKINSONIAN DRUG.

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Cabergoline (CAB) is a new ergoline derivative. CAB in vitro showed high affinity binding for striatal dopamine (DA) receptors labeled by [^{3}H]-n-propylnorapomorphine (K_i=3nM) or [^{3}H]-spiroperidol (K_i=11nM) with lower affinity for other neuronal receptors such as D-1 dopamine, α-1, α-2 adrenoreceptors, S-1 and S-2 serotonin. CAB specificity for D-2 receptors was confirmed by its inhibiting effect on striatal DA-sensitive adenylate cyclase activity (IC_{50} in μM range). In vivo CAB (ED100=1 mg/kg s.c.) elicited contralateral rotation in 6-OHDA unilaterally lesioned rats in the nigra and completely reversed (0.5 and 1 mg/kg s.c.) the parkinsonism in MPTP-treated monkeys. These functional changes were blocked by sulpiride but not by SCH 23390 a D-1 receptor antagonist. CAB decreased DA turnover as indicated by the reduced DOPAC/DA and HVA/DA ratios in the striatum (-17% and -26% respectively) and n. accumbens (-26% and -34% respectively). The lowering effects on HVA and DOPAC peaked at about 9 hr after drug administration and vanished 24 hr later. The rate of the DA synthesis and utilization evaluated by GBL and α-MT was also significantly reduced in the striatum (-33% and -66% respectively) and n. accumbens (-21% and -70% respectively) suggesting an activation of both pre-and postsynaptic DA receptors. In contrast to other ergot derivatives e.g. bromocriptine, pergolide and lisuride, CAB had no significant effects on noradrenaline and serotonin turnover. The results indicate that CAB is a potent and selective DA D-2 receptor agonist with long duration of action.

424.4

BEHAVIORAL EFFECT OF CHRONIC TREATMENT WITH CY 208-243 A PARTIAL D-1 AGONIST IN DRUG NAIVE MPTP-TREATED MONKEYS.

B. Gomez-Mancilla and P.J. Bédard. Lab. of Neurobiology, Laval University, Fac. of Med., Ste-Foy, (Qc), CANADA G1K 7P4.

In order to study pharmacologically the action of CY 208-243 a partial D1 agonist we performed the following experiment. A group of 5 female cynomolgus monkeys (±3kg) was rendered parkinsonian by one or repeated doses of MPTP (0.3-10 mg/kg). At least two months after the last injections, a daily s.c. injection of CY 208-243 at 0.05, 0.1 and 0.5 mg/kg was started. All animal improved their parkinsonian symptoms, but 3 out 5 animals developed dyskinesias under treatment. After 36 days of treatment the daily dose of CY 208-243 was replaced by an experimental agent and the animal was monitored for dyskinesias and other effects. LY-171555 at 0.01 mg/kg reproduced the same dyskinesias produced by CY 208-243. The D1 antagonist SCH-23390 at 0.05 mg/kg as well as Sulpiride at 20 mg/kg were without effect by themselves. However dyskinesias induced by CY 208-243 could be antagonized by SCH-23390, partially antagonized by Sulpiride at 20 mg/kg, and prevented by previous treatment by alpha-methyl-paratyrosine. Our results show that chronic treatment with CY 208-243 can produce dyskinesias in MPTP-treated monkeys and D1 and D2 receptors activation appears necessary for their manifestation.

Supported by MRC of Canada and Parkinson Foundation of Canada.

424.5

MOTOR AND NON-MOTOR ASPECTS OF HEMI-SPATIAL NEGLECT IN HEMI-PARKINSONIAN MONKEYS.

W.W. McLaughlin, J.S. Schneider and D.P. Roeltgen*, Dept. of Neurology, Hahnemann Univ., Phila., PA. 19102.

Monkeys with unilateral damage to the nigrostriatal dopamine system display a hemi-parkinsonism characterized by a flexed posture, rigidity and disuse of the limbs (particularly the arm) contralateral to the dopamine denervation. The present study was conducted to assess the degree to which lateralized non-motor deficits such as hemi-neglect might be present in the primate lesion model. Three male rhesus macaque monkeys were trained to perform motor tasks which measured bar press rate and reaction and movement times and tasks such as response to double simultaneous stimuli, response to lateralized moving stimuli, and lateralized reward retrieval, designed to assess neglect phenomena. Normal data were obtained over several weeks from the left arm with the right arm restrained and vice versa. All animals then received unilateral (right side) intracarotid infusion of MPTP (performed by K. Bankiewicz). All monkeys developed a stable left-sided hemi-parkinsonism characterized by lack of performance of motor tasks with the left arm and normal performance with the right arm. Post-lesion, all monkeys showed significant deficits in the use of the left arm in both hemispaces and in the use of the intact right arm in response to stimuli or performance of tasks in the left or impaired hemispace. When the left arm performed tasks, it did so on the right and not on the left. With the intact right arm, monkeys showed a clear response bias to the right and an inattention to the left in response to double simultaneous stimulation and lateralized reward retrieval. In response to lateralized moving stimuli, there were deficits in directing attention into the impaired hemispace. Deficits on these tasks could be overcome after administration of N-0923 (D2 agonist) but not after SKF-38393 (D1 agonist). The response deficits of the right arm cannot be explained by motor dysfunction since this arm performed normally on motor tasks and on tasks centered in the intact hemispace. Instead, these results show that hemi-parkinsonism in the monkey is a complex syndrome characterized by overt motor disturbances and less overtly apparent defects in sensorimotor integration.

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424.6

DOPAMINE RECEPTORS IN THE GLOBUS PALLIDUS AND SUBSTANTIA NIGRA OF MPTP-MONKEYS TREATED CHRONICALLY WITH BROMOCRIPTINE OR BROMOCRIPTINE+CY 208-243,

C. Gagnon[1], B. Gomez-Mancilla[2], P.J. Bédard[2] and T. Di Paolo[1], [1]Sch. of Pharmacy, Laval Univ. and Dept of Mol. Endo., CHUL, Quebec, G1V 4G2; [2]Dept of Pharmacol., Fac. Med., Laval Univ., Quebec, G1K 7P4, Canada.

Dopamine (DA) receptor densities, as measured by the autoradiography of antagonist (^{3}H-SCH 23390 (D-1), ^{3}H-spiperone (D-2)) and agonist ligands (^{3}H-SKF 38393 (D-1) and ^{3}H-N-propylnorapomorphine (D-2)) binding, were compared in the globus pallidus (external (GPE) and internal (GPI) segments) and substantia nigra (SN) of MPTP-monkeys treated daily during one month with bromocriptine (BROMO) alone or in combination with CY 208-243 (CY). MPTP decreased by 67% or more DA levels in the GPE and GPI. Following MPTP, antagonist and agonist sites decreased in the GPE and GPI (except D-1 antagonist sites which remained unchanged in the GPI). Neither DA agonist treatments changed antagonist sites in the GPE and GPI compared to the untreated MPTP-animal. However, agonist sites increased after both dopaminergic treatments in GPI, whereas only agonist sites of D-2 receptors increased in the GPE after BROMO treatment. Antagonist sites of D-1 and D-2 receptors were, respectively, increased and not detectable in the SN of MPTP-monkeys, whereas agonist sites were, respectively, decreased and unchanged. Both dopaminergic treatments increased antagonist sites in the SN compared to the control animal. Agonist sites returned toward control values in the SN of BROMO+CY-treated monkeys, whereas agonist sites of D-2 receptors decreased in the BROMO-treated animals in this brain region. In summary, changes in DA receptors in the GP and SN may play a role in the changing response to DA agonists after chronic treatments.

Supported by the Parkinson Foundation of Canada.

424.7

INTRA-CEREBRO-VENTRICULAR APOMORPHINE INFUSION IN MPTP MONKEYS. A.L. Benabid, P. Pollak*, P. Limousin*, F. Serre-Debeauvais*, E. Seigneuret*, A. François-Joubert*, C. Feuerstein and M. Gavend*. Dept. of Clin. and Biol. Neurosciences and Lab. of Pharmacology, INSERM U-318, Joseph Fourier University of Grenoble, France.

Apomorphine, a dopaminergic agonist drug, administrated by continuous subcutaneous infusion is efficient in parkinsonian patients suffering from severe L-dopa-induced on-off effects. As the best temporal mode of infusion is not well established and external infusion methods are difficult to implement in such patients, a continuous intra-cerebro-ventricular (ICV) apomorphine administration was carried out in MPTP primates using an implanted programmable pump. A Medtronic DAD pump connected to an intra-ventricular catheter was placed intraperitoneally in 7 macaca fascicularis monkeys. An access system was implanted for cisternal CSF sampling. Permeability of catheters and pump system was checked by contrast radiography and ^{99m}Tc-DTPA scintigraphy. CSF and blood pharmacokinetic studies of apomorphine were carried out in 3 animals following ICV and intramuscular (IM) continuous infusions. MPTP was intravenously injected in 4 monkeys until a stable parkinsonian motor state was obtained.

The implanted system was well tolerated in all animals up to 12 months but recurrent surgery was sometimes needed to repermeate occluded catheters. Pharmacokinetic results have shown that ICV apomorphine infusion led to CSF apomorphine concentrations far higher than those obtained after IM infusion of the same apomorphine dose, whereas blood concentrations remained to low values compared to peripheral administration. ICV apomorphine infusion induced the recovery of a normal motricity in MPTP monkeys during the time of administration and 30 min after its arrest. Long term ICV apomorphine infusions are in progress to evaluate the motor response induced by temporal modalities of administration during the nycthemere and by various apomorphine flow rates.

424.8

THE ROLE OF DOPAMINERGIC NERVE TERMINALS IN THE TREATMENT OF PARKINSON'S DISEASE WITH L-DOPA. E.D. Abercrombie and S.R. Wachtel. *Center for Molecular & Behavioral Neuroscience, Rutgers University, Newark NJ, 07102 (USA).*

Previously, we reported that systemic doses of L-DOPA which increase tissue levels of dopamine (DA) in striatum produced either no significant change (50 mg/kg) or a relatively modest 54% increase (100 mg/kg) in extracellular (EC) DA level in that structure despite large increases in EC DOPAC at both doses (Abercrombie et al., 1990). In animals treated with 6-OHDA, sustaining virtually complete depletions of striatal DA, 100 mg/kg of L-DOPA produced a 4-fold greater absolute increase in EC DA relative to that observed in controls. A model is proposed in which DA terminals, by efficiently metabolizing DA formed from L-DOPA, may actually be a sink rather than a source of transmitter in L-DOPA therapy of Parkinson's disease. In order to examine more closely the role of DA terminals in L-DOPA-induced increases in EC DA, in vivo microdialysis was used to monitor the effect of pretreatment with 50 mg/kg L-DOPA on increases in EC DA produced by 0.5 mg/kg HAL (Ca^{++} dependent release) or by 0.5 mg/kg AMPH (Ca^{++} independent release). The vesicular release of DA produced by HAL was not affected by L-DOPA pretreatment whereas the translocation of cytoplasmic DA produced by AMPH was significantly enhanced. Furthermore, the increase in EC DA produced by 100 mg/kg L-DOPA was not abolished when 10^{-5}M TTX was infused via the dialysis probe, a manipulation that decreased basal EC DA to non-detectable levels. These results suggest that DA formed from exogenous L-DOPA in DA neurons is efficiently metabolized in the cytoplasm and that the efficacy of L-DOPA in the treatment of Parkinson's disease results from non-specific spillover of DA from non-DA striatal elements.

Abercrombie, E.D., Bonatz, A.E. and Zigmond, M.J. (1990) Brain Res. 525:36-44.

424.9

CONCURRENT DEVELOPMENT OF BEHAVIORAL SENSITIZATION AND BIOCHEMICAL TOLERANCE WITH CHRONIC L-DOPA TREATMENT. Robert J. Carey Brown University and VA Medical Center, Providence, RI 02908

In L-DOPA therapy for Parkinson's disease, overstimulation of the motor system often occurs with chronic treatment resulting in dyskinetic effects which are often followed by on-off and wearing-off effects. Unilateral 6-OHDA lesion animals were used to study this process in an animal model analogue. Following 30 L-DOPA treatments with subthreshold (10 mg/kg) and suprathreshold (20 mg/kg) doses, behavioral sensitization of contralateral rotation was observed in both groups. In conjunction with the behavioral sensitization, there was a substantial decline in dopamine metabolite levels from acute treatment levels, indicating a tolerance effect. Behavioral sensitization and biochemical tolerance effects with chronic L-DOPA treatment were interpreted by a two-factor model. Behavioral sensitization and overstimulation were assumed to reflect the combined effects of dopamine receptor priming and Pavlovian drug conditioning whereas the eventual wearing-off effect of L-DOPA could reflect the development of tolerance in the conversion of L-DOPA into dopamine.

424.10

NICOTINE METABOLITES ALTER DOPAMINE UPTAKE AND RELEASE IN RAT STRIATUM IN VITRO. L.L. Leibee, P.A. Crooks*, S.T. Buxton, A.L. Jewell and L.P. Dwoskin. College of Pharmacy, University of Kentucky, Lexington, K.Y. 40536-0082.

Although controversial, chronic nicotine and cigarette smoke exposure antagonize the MPTP-induced decrease in striatal DA stores and tyrosine hydroxylase activity (Janson et al., 1988; Carr and Rowell, 1990). Recently, nicotine has been reported to stereoselectively inhibit striatal dopamine (DA) uptake at lower concentrations (pM-nM) than induce DA release in vitro (Izenwasser et al., 1991). The aim of this study was to determine if metabolites of nicotine inhibit [3H]DA uptake or increase DA release from rat striatal slices in vitro. Significant inhibition (60%) of [3H]DA uptake occurred with S(-)N-methylpyridinium nicotine metabolites (10 uM), which are structurally similar to the neurotoxin MPP^+, while the S(-) N'-methylpyrrolidinium metabolites (10 uM) exhibited no inhibition of [3H]DA uptake. S(-)Nornicotine (100 uM) evoked a greater increase (3-fold) in the concentration of endogenous dihydroxyphenylacetic acid in superfusate than the same concentration of the parent compound, S(-) nicotine. Endogenous DA was not detected in striatal superfusate. Therefore, alterations in DA uptake and DA release by metabolites of nicotine may contribute to the neuropharmacology of nicotine. (Supported by the Tobacco and Health Research Institute, Lexington, KY and the R.J. Reynolds Tobacco Company.)

424.11

HUMAN AND RAT MESENCEPHALIC AND STRIATAL NEURONS STUDIED IN ORGANOTYPIC SLICE CULTURES. K. Østergaard, B.R. Finsen and J. Zimmer. PharmaBiotec, Institute of Neurobiology, University of Aarhus, DK-8000 Aarhus C, Denmark.

Tyrosine hydroxylase immunoreactive (TH-i), dopaminergic (DA) neurons and striatal neurons can survive and retain their in vivo morphological characteristics for 60 days in slice cultures of ventral mesencephalon (VM) and striatum prepared from newborn to seven day old rats. We now report long term survival for up to nine months of TH-i, DA neurons in slice cultures of rat VM.

We also report the presence of TH mRNA and cholecystokinin (CCK) mRNA in VM slice cultures prepared from newborn rat as visualized by *alkaline phosphatase labeled oligonucleotide probes. Likewise somatotatin* (SS) mRNA and enkephalin (ENK) mRNA were visualized in rat striatal neurons.

A second aim was to examine whether human, fetal mesencephalic and striatal neurons can survive and differentiate in slice cultures. Slices of VM and striatal tissue were obtained from two 7 week old (postconception), aborted fetuses according to the permission granted by the Regional and Central Ethical Committees. The VM and striatal slice cultures survived for 5 weeks before immunocytochemistry and in situ hybridization were performed. Human TH-i neurons were observed to have mature in vivo morphological characteristics. Further results of these studies will be presented.

Storage of human, fetal DA neurons in slice culture would allow treatment with trophic factors, pooling of neurons and examination for infectious agents before transplantation to patients with Parkinson's disease.

424.12

INTRACEREBRAL GRAFTING OF AADC-EXPRESSING PRIMARY FIBROBLASTS FOR SITE-SPECIFIC DELIVERY OF DOPAMINE. U.J. Kang, L.J. Fisher, H.A. Jinnah, M.B. Rosenberg, T.H. Joh, T. Friedmann, F.H. Gage. Departments of Neurosciences & Pediatrics, Univ. California San Diego, La Jolla, CA 92093 and Burke Medical Research Institute, Cornell University Medical College, White Plains, NY 10605.

Primary dermal fibroblasts from adult, female Fischer 344 rats have been infected with a retroviral vector containing the cDNA for bovine aromatic L-amino acid decarboxylase (AADC). This enzyme catalyzes the conversion of L-DOPA to dopamine. Transduced fibroblasts (FF2/AADC) showed positive immunoreactivity for AADC protein in vitro. The recombinant AADC in FF2/AADC cells has a Km of 29 μM for the substrate L-DOPA and Vmax of 26 nmoles dopamine/mg/20 mins with most optimal activity when the cofactor pyridoxal phosphate concentration ranges from 10 to 100 μM. The AADC activity remains stable in culture for up to at least 18 passages.

The FF2/AADC fibroblasts synthesize and release dopamine into the media (3.9 nmoles/mg/hour) when incubated in DMEM/10 % fetal calf serum with pyridoxal HCL and L-DOPA. FF2/AADC fibroblasts implanted in the striatum of Fischer 344 rats survived through 13 weeks post-implantation with relatively constant graft size from 3 weeks to 13 weeks. There appears to be a decrease in the number of fibroblasts within the graft during that period. Immunolabeling for AADC protein is strongly positive at 1 and 3 weeks, with decreased AADC-reactivity at later times. Further studies using these FF/AADC fibroblasts to reverse dopamine-deficient behaviors in a rat model of Parkinson's disease are in progress.

424.13

STRIATAL TRANSPLANTATION OF BOVINE CHROMAFFIN CELL-LOADED MICROCAPSULES REDUCES EXPERIMENTAL PARKINSONISM IN RATS S.R. Winn[1], S.A. Tan*[1], P.A. Tresco[1], J. Sagen[2], P. Aebischer[1]. Brown Univ., Providence, RI[1] and U. of IL, Chicago[2].

Striatal transplantation of catecholamine-releasing cells encapsulated within a selectively permeable membrane may provide a strategy for dopamine replacement in animal models of parkinsonism and prevent immune rejection in xenotransplants. Immobilizing adult bovine adrenal chromaffin (BAC) cells in a polyelectrolyte matrix prevents fusion of cellular aggregates which may develop necrotic centers due to inadequate nutrient support. Coseeding with an NGF-releasing cell line was attempted to transform BAC cells from an endocrine to a more neuronal phenotype. In vitro, cell-loaded microcapsules were characterized by analyzing basal, amphetamine and high K^+ evoked release of catecholamines and observing cell viability over time. Cells within the microcapsules continued to survive for at least 12 weeks in vitro. Short neurites were seen extending from the coseeded BAC cells after 4 weeks in vitro. This differentiation increased amphetamine and high K^+ stimulated dopamine output from 1 to 4 weeks that was maintained for up to 12 weeks in vitro. BAC-loaded microcapsules were implanted into 6-OHDA unilaterally lesioned striata of rats. From one to four weeks post-implantation, BAC cell-loaded recipients (n=6) showed a 50% reduction in rotation behavior under apomorphine challenge as compared to animals which received empty microcapsules (n=6). Intact microcapsules containing DBH and TH immunopositive BAC cells were observed after 4 weeks of implantation in animals exhibiting a reduction in turning behavior. Immobilization of discrete cell clusters in polyelectrolytes prevents reaggregation and may provide a method for enhancing long-term maintenance of chromaffin cells.

424.14

REVERSAL OF BEHAVIORAL RECOVERY FOLLOWING THE REMOVAL OF POLYMER ENCAPSULATED PC12 CELLS IMPLANTED IN THE LESIONED RAT STRIATUM. P. A. Tresco[1], S. R. Winn[1], B. Zielinski*[1], C. B. Jaeger[2], L. A. Greene[3], P. Aebischer[1]. Brown Univ.[1], Purdue Univ.[2], and Columbia Univ.[3]

The feasibility of delivering physiologically relevant concentrations of dopamine (DA) to the striatum with an implantable, selectively-permeable, polymer capsule containing DA-secreting cells was investigated. A significant reduction in the apomorphine-induced rotational response was observed when PC12 cell-containing capsules were implanted into the 6-OHDA lesioned striata of rats, whereas no reduction was observed following the implantation of empty polymer capsules. Polymer-encapsulated PC12 cells survived in the striatum, did not form tumors, and expressed tyrosine hydroxylase after 4 weeks in situ. Cellular viability was consistently better within implanted capsules when compared to those maintained in vitro. Microdialysis studies of denervated striata revealed the presence of DA near PC12 cell-loaded capsules which was comparable to extracellular DA levels of unlesioned controls. To further clarify if the observed reduction in rotation was mediated by chronic stimulation of DA receptors secondary to its diffuse release from the PC12 cell-containing capsules, a flushable PC12 cell-containing encapsulation system was implanted into the 6-OHDA lesioned striata of rats. Only cell-containing devices reduced the apomorphine-induced rotational response. Morever, one week after removing the PC12 cells by flushing the U-shaped encapsulation system the effect was reversed. Viable cells were confirmed by staining with a fluorescent vital dye. The reversibility suggests that release of a neurotransmitter, most likely DA, was responsible for the observed functional effect using this cell line.

424.15

EXPRESSION OF TYROSINE HYDROXYLASE IN STRIATAL CELLS WITH A HSV-1 VECTOR CAUSES STABLE PRODUCTION AND REGULATED RELEASE OF DOPA AND DOPAMINE: POTENTIAL GENE THERAPY FOR PARKINSON'S DISEASE. A. Freese, M.J. During, K. O'Malley, and A.I. Geller. Dept. of Neurosurg., Hosp. Univ. of PA., Phila. PA. 19104; Dept. Neurosurg., Yale Univ. Sch. of Med., New Haven CT. 06510; Dept. Anat. and Neurobiol., Washington Univ. Sch. of Med., St. Louis MO. 63110; Div. of Cell Growth and Regulation, Dana Farber Can. Inst., Boston MA. 02115.

We have developed defective Herpes Simplex Virus (HSV-1) vectors to deliver genes into neurons in culture and in the adult mammalian brain (Science 241, 1667, 1988, Proc. Natl. Acad. Sci, 87, 1149, 1990; Biochem. Pharm., 40, 2189, 1990). Thus, a new gene therapy approach to Parkinson's Disease is to use a HSV-1 vector expressing tyrosine hydroxylase (TH), the rate limiting enzyme in dopamine synthesis, to direct dopamine synthesis in striatal neurons and glia. Towards this goal we constructed a HSV-1 vector (pHSVth) which expresses the human TH gene from the HSV-1 IE 4/5 promoter, a constitutive promoter active in most neural cells. Fibroblasts infected with pHSVth contain TH DNA, TH RNA, and TH immunoreactivity (in approximately 10 % of the cells). pHSVth infected fibroblasts also contain a 90-fold increase in TH enzyme activity and a 10-fold increase in both L-dopa and dopamine levels. Cultured striatal cells were infected with pHSVth, one week later TH immunoreactivity was detected in approximately 70 % of the cells. Furthermore, both L-dopa and dopamine were released from these cells in a regulated fashion for at least one week after infection. The levels of TH enzyme and monoamines was comparable to cells which naturally synthesize monoamines, or to fibroblasts transfected with the TH gene; both of these cell types demonstrate behavioral improvement upon transplantation into the animal models of Parkinson's Disease. Therefore, pHSVth should be an effective therapeutic tool if it can be delivered into a sufficient number of cells in vivo; such studies are in progress.

424.16

QUANTIFYING IMPROVEMENT FOLLOWING CNS TRANSPLANTATION IN HEMIPARKINSONIAN MONKEYS USING AN OPERANT BEHAVIOR TASK. R.A.E. Bakay, L.D. Byrd* and J. Tigges. Yerkes Regional Primate Research Center, Emory Univ., Atlanta, GA 30322.

One of the most difficult problems in evaluating the success of CNS transplantation techniques is obtaining a quantitative measure of the disability produced by the parkinsonian model. Our experience with MPTP hemiparkinsonian monkeys suggests rotational behavior is not a satisfactory index of disability. In an attempt to obtain a more representative quantitative analysis, monkeys were trained to perform a discrete-trial operant task which required the simultaneous depression of two levers on a response panel. Once both levers were depressed, lights above the right or left lever were darkened to indicate which hand to use in touching an illuminated area on a touch-sensitive screen. Correct responses were rewarded with a food pellet. Highly stable and accurate performance with both hands was established prior to MPTP injection. Testing allowed the separation of hand functions into response time, movement time, precision and accuracy of the responses, and provided a quantitative behavioral measure of parkinsonism. Preliminary results with this technique suggest quantitative improvement after transplantation procedures which yielded viable grafts as opposed to those performed on a sham basis. The smaller the MPTP-induced deficit, the more likely the behavioral improvement. Supported by VAR&D, RO1 NS24340 and RR-00165.

424.17

QUANTIFYING BEHAVIORAL IMPROVEMENT IN HEMIPARKINSONIAN MONKEYS USING TWO DIFFERENT OPERANT TASKS. R.L. Watts, L.D. Byrd*, R.A.E. Bakay and A. Mandir*. Yerkes Regional Primate Research Center, Emory Univ., Atlanta, GA 30322.

One of the most difficult problems in evaluating the success of CNS transplantation techniques is obtaining a quantitative measure of the disability produced by the parkinsonian model. We have been investigating techniques for characterizing and quantifying behavioral performance related to parkinsonism in monkeys. One group of monkeys was trained to perform a discrete-trial operant task which required the simultaneous depression of two levers, release of the correct hand in response to a light cue, and touch-contact with a lighted CRT (see related presentation). Another group of monkeys was tested on a ballistic wrist flexion/extension task. A potentiometer connected to the manipulandum provided a signal of wrist angular position and velocity. Visual feedback of hand position and task performance was provided to the monkeys. Agonistic and antagonistic EMG potentials were recorded using surface electrodes. Highly stable and accurate baseline performances were established, and response time and movement time, as well as the precision and accuracy of the response, were measured. Subsequent manipulations with MPTP injection, surgery and drug therapy could be quantitated. Comparisons of the two techniques will be made to each other and to other behavioral measures. Supported by VAR&D, RO1 NS24340 and RR-00165.

425.1

GUANIDINOETHANE SULFATE ENHANCES THE SURVIVAL RATE OF MICE EXPOSED TO ANOXIA: BRAIN LACTIC ACIDOSIS PROTECTIVE AGENT? T. Nakada and I. L. Kwee. Neurochem Res Lab, VA Med Ctr, Martinez, CA 94553 and Dept of Neurology, Univ of California, Davis, CA 95616.

Several lines of evidence now support the concept that lactic acidosis plays a key role in determining the outcome of brain anoxia/ishemia. Our previous studies on brain of the fetus and the 1 and 10 day old neonate demonstrated that taurine increases the brain cytosol acid buffering ability and plays a fundamental role in the immature brain's high resistance against anoxia. In this study, we assessed the protective effects of guanidinoethane sulfate (GES), a taurine analogue, on brain lactic acidosis in adult mice. The data indicated that GES can serve to increase the brain acid buffering ability and effectively prevents severe acidosis from lactic acid generation in response to anoxia. GES greatly enhanced the survival rate of mice exposed to anoxia.

	Survival rate	
Duration of Anoxia	GES	Control
2.5 minutes	100%*	40-60%
2.75 minutes	80-90%	0%

*Each experiment consisted of 5 GES and 5 control animals and experiment was repeated three times.

425.2

DIETARY NONPROTEIN CALORIES AND CEREBRAL INFARCTION SIZE. C.S. Robertson,* J.C. Goodman, R.G. Grossman, Department of Neurosurgery, Baylor College of Medicine, Houston, TX 77030

The effect of reducing carbohydrates in the diet on cerebral infarction size was studied after a 45 minute reversible middle cerebral artery occlusion (MCAO). Rats were either fasted for 24 hours prior to MCAO or were fed a control diet containing 51.5% of the calories as carbohydrates, or one of 5 experimental diets in which 60% of the carbohydrate calories were replaced with one or more of the following substrates: 1,3-butanediol, triacetin, and tributyrin. The lowest plasma glucose concentration was achieved in the fasted group, 6.5±1.1 mmol/ml, and the highest was found in the control diet, 9.2±1.4 mmol/ml. The 1,3-butanediol diet resulted in a plasma glucose concentration of 7.8±1.3 mmol/ml. The smallest infarct volume was found in the fasted group, 53±43 mm^3, and the largest in the control diet group, 162±56 mm^3. Significantly smaller infarcts were found with the 1,3-butanediol diet, 98±41 mm^3, and with the triacetin/tributyrin diet, 105.4±52.6 mm^3. The size of the infarction was directly related to the preischemia blood glucose concentration (n=69, r=.47, p<.01). It may be possible to develop a diet using nonglycolytic calorie sources which would supply systemic caloric and protein requirements without the adverse effect of conventional diets.

425.3

INTRAVITREAL INJECTION OF GLUCOSE PROTECTS AGAINST ISCHEMIC DEGENERATION OF RETINAL NEURONS C.Romano, M.T.Price, H.Y.Bai, J.W.Olney, Depts. Ophthalmology and Psychiatry, Washington University School of Medicine, St. Louis, MO 63110.

We reported last year that Honghua, an extract of safflower used as an herbal medicine in China, has neuroprotective activity in experimental models of excitotoxicity. In the *in vivo* adult rat retina, ischemic damage induced by dye photothrombosis was greatly reduced by intravitreal injection of Honghua. In the isolated chick embryo retina (CER), Honghua protected against the excitotoxicity of glutamate receptor agonists, N-methyl-D-aspartate, kainic acid and quisqualic acid (QA), and against neuronal degeneration caused by simulated ischemia (30 min glucose/oxygen deprivation). Subsequently, we found that Honghua more potently protected against simulated ischemia than against the agonists. Biochemical fractionation procedures were used to purify an active component of Honghua. An approximately 100-fold purification of an active principle was acheived using ion-exchange, gel filtration, and reverse-phase chromatography. The purest fractions were determined to be rich in glucose, so the effects of glucose in the models of excitotoxicity were determined. Many of the neuroprotective effects of crude and highly purified Honghua were quantitatively mimicked by pure solutions of equivalent glucose concentration. Concentrations of glucose ≥ 3.2 mM in the CER-ischemia assay provided complete protection from neurotoxicity, despite the absence of oxygen. Intravitreal injection of 1.05 µmol of glucose provided highly significant neuroprotection (67% reduction in retinal damage determined histologically at 1 hr post ischemia; n=14, $p < 0.001$) in the adult rat retina dye-photothrombosis model. It should be noted that substances injected intravitreally diffuse freely into the ischemic retina. Thus, direct delivery of glucose to ischemic neurons in the immediate period following CNS vascular occlusion (stroke), delays progression of neuronal degeneration for at least 1 hr. These findings have relevance for the management of retinal vascular occlusion and possibly other neuro-ischemic disorders. This work was supported by grants from Fight-for-Sight (CR) and NIH (JWO).

425.4

PATHOPHYSIOLOGY OF ASPHYXIA-INDUCED LESION TO THE RAT NIGRO-STRIATAL DOPAMINE SYSTEM. EFFECTS OF NICOTINE TREATMENT. K.Andersson*, B.Bjelke, S.O. Ogren*, P.Bolme* and P.Bach-y-Rita. Karolinska Institutet, 104 01 Stockholm, Sweden.

Asphyxia to male Sprague-Dawley rat pups was induced by a delayed cesarean section (Bjelke et al.,1991). Immunohistochemical and functional analyses were carried out on the pups at an age of 3 weeks. It was demonstrated that asphyxia time-dependently produced an increase in the number of tyrosine hydroxylase immunoreactive (TH-IR) nerve cell bodies in the substantia nigra. Furthermore, asphyxia time-dependently reduced rearing behavior and increased locomotion (Bjelke et al.,1991). In the present study the pups received nicotine via the breast milk of dams implanted with an Alzet minipump (0.125 mg nicotine/kg/h for 3 weeks). It was found that nicotine treatment counteracted the asphyxia-induced increase in the number of TH-IR nerve cell bodies of the substantia nigra as well as the changes in rearing and locomotor behavior.

In conclusion, nicotine treatment of male rat pups can counteract asphyxia-induced changes in nerve cell body number in the substantia nigra, as well as changes in locomotor behavior.

Ref.: Bjelke et al., Brain Res. 543:1-9, 1991.

425.5

BMY-14802 AND CLOZAPINE ARE NEUROPROTECTIVE IN A GERBIL MODEL OF ISCHEMIA. P. C. Contreras, D. M. Ragan*, N. M. Gray*, T. S. Rao and T. H. Lanthorn, CNSDR, G. D. Searle & Co., Skokie, IL 60077.

Recently, BMY-14802, a putative sigma antagonist and antipsychotic agent, and clozapine, an atypical antipsychotic agent, were shown to antagonize harmaline and D-serine induced increases in cGMP in the cerebellum, a well characterized second messenger response mediated by the NMDA receptor-channel complex. The purpose this study was to assess whether clozapine and BMY-14802, like NMDA antagonists, could also be neuroprotective in an *in vivo* model of ischemia. Using the gerbil model of ischemia, there was significant neuroprotection when gerbils were pretreated with 50, 30 or 10 mg/kg of BMY-14802 or 10 or 20 mg/kg of clozapine. Unlike many of the NMDA competitive and noncompetitive antagonists, BMY-14802 did not induce stereotyped behavior or ataxia. These results suggest that clozapine and BMY-14802 may be useful in attenuating ischemia-induced injury in the CNS.

425.6

THE EFFECTS OF FOSPHENYTOIN ON INFARCT SIZE AND BEHAVIORAL DEFICITS IN RAT MODELS OF FOCAL ISCHEMIA. J.J. Cordon, P.A. Boxer and F.W. Marcoux, Department of Pharmacology, Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, Ann Arbor, MI, 48105

Fosphenytoin is a water soluble prodrug of phenytoin that is rapidly converted to phenytoin by non-specific phosphatases in plasma. Previous experiments with phenytoin have indicated that it has neuroprotective effects in models of both global and focal ischemia. These experiments were conducted to determine if fosphenytoin would decrease the size of ischemic damage in a distal model of middle cerebral artery (MCA) occlusion and decrease the neurological deficits caused by a proximal MCA occlusion. In halothane anesthetized Fisher-344 rats the common carotid and ipsilateral MCA were occluded. Drug or vehicle was administered IV, 30 minutes and 24 hours post occlusion and the infarct was measured using TTC staining 48 hrs post surgery. In the distal MCA occlusion model fosphenytoin was tested at 17, 30, 56 and 100 mg/kg (n=12 per group) and significantly decreased the infarct volume at each dose by 22%, 35%, 39% and 26% respectively as compared to vehicle treated rats. In the proximal MCA occlusion model fosphenytoin was tested at 30 mg/kg and decreased the infarct volume by 37%. These animals were tested for neurological deficits by measuring the duration they remained on a 50° inclined plane. Fosphenytoin reduced the neurological deficits to sham levels as compared to vehicle treated rats. These experiments suggest that fosphenytoin reduces ischemic damage and may be useful in the treatment of human stroke.

425.7

CYCLOHEXIMIDE OFFERS PROTECTION AGAINST THE DELETERIOUS INFLUENCES OF ANOXIA IN THE RAT.

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Forebrain ischemia in the rat results in decreased protein synthesis and delayed neuronal death (DND) in CA1 of the hippocampus. However, treatment of these rats with cycloheximide (CHX), a protein synthesis blocker, prevents DND in CA1 (Goto et al., 1990), implicating protein synthesis as a mediator of ischemic damage. We have now examined *in vivo* and *in vitro* effects of CHX on the influences of anoxia in the rat.

Adult male Wistar rats were subjected to forebrain ischemia (4-vessel occlusion model) for 30 min and monitored for 7 days. Rats were injected (i.p.) with saline or CHX (1.5 mg/kg) 1h before or after occlusion. CHX treatment prior to ischemia decreased ($p<0.05$, student' s t-test) weight loss (2.5 ± 11.0g vs. 28.5 ± 8.0g in controls, mean±SEM) and enhanced survival rate (73% vs. 48% in controls) of rats. Weight loss was similar to controls ($p>0.05$) and survival rate (25%) decreased in rats given CHX after occlusion. Hence, protein synthesis occurring during or within 1 h of insult may mediate *in vivo* effects of forebrain ischemia.

Effects of anoxia on CA1 field EPSPs in the presence of CHX were also examined. EPSPs in adult hippocampal slices incubated in Krebs (controls) or 60 uM CHX were subjected to various periods (3min 15s to 4min 30s) of anoxia (glucose-free Krebs,95%N_2/5%CO_2 perfusion) and their percent recuperation determined 20 min later. EPSP recuperation in control and CHX slices was similar for all ranges of anoxia, except 3min 45s. After this period, CHX treated slices exhibited a greater ($p<0.05$) recuperation ($57.5\pm17.0\%$) than controls ($11.0\pm11.0\%$). CHX may thus enhance CA1 resistance to anoxia, and some of its protective effects may occur in the hippocampus.

425.8

BMY 14802 IMPROVES BEHAVIORAL OUTCOME AFTER BILATERAL CAROTID OCCLUSION IN THE GERBIL.

S. L. Moon, K. E. Timko*, J. A. Stanley*, M.N. Duquette*. CNS Neuropharmacology, Bristol-Myers Squibb Co., Wallingford, CT 06492.

We have reported that BMY 14802 (Bristol-Myers Squibb) is effective in attenuating hippocampal histopathology when assessed by means of a rating scale to denote the severity of cell loss after a 15-min bilateral carotid occlusion followed by a 96hr survival period in the Mongolian gerbil. To determine whether significant histological protection translated into significant behavioral protection, preservation of function after occlusion was evaluated by a passive avoidance step-through task.

Animals received BMY 14802 (10, 25, 50, 100 mg/kg) or vehicle 1hr pre- and 1 hr postsurgery, and once/day for the next 3 days. On day 2 after surgery, animals were trained in the passive avoidance task: each animal was placed on a brightly lit runway leading into a darkbox containing an electrified floorgrid. Training consisted of two trials, 10min apart. Animals that failed to enter the box during training were disqualified. Behavioral performance was assessed 24hrs after training as the number of animals/dose group to reach a 2-min. latency criterion.

A statistically significant difference between sham-operated and occluded controls indicated that this test is a behaviorally sensitive measure of impairment induced by forebrain ischemia. The results with BMY 14802 yielded $r=-0.95$ for group mean hippocampal damage vs. group passive avoidance score. BMY 14802 showed histological protection at all doses tested (10-100 mg/kg); however, only the 50mg/kg group performed significantly better than occluded controls while also not differing statistically from sham-operated controls on the passive avoidance task. These results are compared to some other putative neuroprotective agents.

425.9

DIAZEPAM FOLLOWING CEREBRAL ISCHEMIA PRESERVES CA1 PYRAMIDAL CELLS AND $GABA_A$ RECEPTORS OF THE HIPPOCAMPUS.

Robin A. Huff* and Rochelle D. Schwartz, Dept. of Pharmacology, Duke University Medical Center, Durham, NC 27710.

Cerebral ischemia-induced neuronal injury in selectively vulnerable areas is associated with excessive excitatory neurotransmission. We investigated whether enhancement of inhibitory neurotransmission could protect hippocampal neurons from ischemic damage in gerbils subjected to bilateral carotid occlusion (BCO) for 5 minutes. Diazepam (DZ), a drug that enhances GABA neurotransmission, was injected into sham-operated and occluded gerbils during the reperfusion phase. Post-ischemic hyperactivity was measured as a predictor of CA1 hippocampal damage 1 day following the occlusion. Seven days after the occlusion, CA1 damage was assessed by histologic procedures, and the distribution of the $GABA_A$ receptor-gated chloride channel was assessed by [^{35}S]TBPS autoradiography. One day following BCO, gerbils showed a robust increase in locomotor activity, while BCO gerbils that received DZ failed to show this hyperactivity. Histologic analysis revealed that DZ treatment also significantly protected CA1 pyramidal neurons from ischemia-induced degeneration. Autoradiography studies showed that [^{35}S]TBPS binding in the CA1 stratum oriens was significantly decreased following ischemia. DZ completely protected BCO gerbils from this loss of [^{35}S]TBPS sites. These results indicate that 1) DZ given after an ischemic insult is neuroprotective of CA1 pyramidal neurons, and 2) the neuroprotective effect of DZ prevents the loss of $GABA_A$ receptors residing on CA1 pyramidal cell dendrites. We conclude that enhancement of GABA neurotransmission following an ischemic event may limit excitatory neurotransmission and thereby avert neuronal death. Supported by the American Heart Association (R.D.S.). RDS is an Established Investigator of the American Heart Association.

425.10

ω-CONOPEPTIDES PREVENT DAMAGE TO CA1 NEURONS CAUSED BY GLOBAL ISCHEMIA.

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Synthetic ω-conopeptides (see abstract by Miljanich et al. for description) bind specifically to N-type calcium channels and block release of neurotransmitters. We have tested several peptides of this class, including some with novel structures, to determine if they are neuroprotective in the unanesthetized rat 4 VO model of global ischemia. Damage in the CA1 region of the hippocampus was determined semi-quantitatively in hematoxylin and eosin stained frozen sections 5 days following the 15 minute ischemic insult. Conopeptides were effective when administered into the lateral ventricle immediately following ischemia, by IV bolus injection up to 1 hour post ischemia, and via a 90 minute IV infusion post occlusion. Damage was decreased significantly from vehicle control values, and also compared to the non-competitive NMDA antagonist, MK-801. This work demonstrates the efficacy of the ω-conopeptides in global models of ischemia and suggests that presynaptic blockade of neurotransmitter release could be an important mechanism in preventing cell death.

425.11

DYNORPHIN A(1-13) IMPROVES OUTCOME FOLLOWING THREE VESSEL OCCLUSION IN THE CAT.

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Treatment of experimental stroke with opiate agonists has provided variable data. Previous models of focal ischemia involved occlusion of only the middle cerebral artery (MCA). In the current study, 20 cats underwent occlusion of 2mm of the right MCA, anterior cerebral and intracranial internal carotid arteries. Six hours post-occlusion half of the cats received an injection of 2mg/kg dynorphin A(1-13) (DYN) and a subcutaneous, 50ug/hr DYN osmotic pump, and half received saline (SAL). Cats were sacrificed on the eighth post-operative day and their brains stained with TTC.

Survival was 10% for SAL and 50% for DYN. Abnormally stained tissue was categorized into infarct (colorless) and ischemia (lightly stained). There was less infarcted tissue present in the DYN treated group. For example, at the level of the optic chiasm, the brain consisted of 67% infarct and 7% ischemia for SAL cats, and 43% infarct and 20% ischemia for DYN cats. Brainweight (edema) was higher in the SAL than in the DYN cats (20.8g vs 17.8g) The data suggests that tissue that would have been infarcted without treatment, recovered following DYN treatment. Our data is consistent with the theory that kappa opioid receptor agonists have a therapeutic anti-edema effect in cerebral ischemia. The three vessel occlusion model shows promise as a model for experimental stroke.

425.12

ANOXIC DEPRESSION OF NEOCORTICAL SYNAPTIC POTENTIALS.

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During the depolarization (AD) which brief anoxia (5 min) evokes in pyramidal neurons of layers II-III of rat neocortical slices (*Rosen & Morris 1991, Neurosci. Lett. 124:169*) both EPSPs and IPSPs are depressed. The early (*e*) EPSP is decreased by 50-75% at 5 min, while the late (*l*) EPSP is abolished within the first 2 min; persistence of these changes during temporary V_m restoration indicates a mechanism distinct from their post-synaptic occlusion by AD. EPSP amplitude fully recovers at 6-8 min of reoxygenation. Evoked IPSPs disappear even sooner than the *l* EPSP and their recovery is more delayed.

When temperature is changed from a control level of 33.5° C to 30° or 37° warming depolarizes the membrane slightly and reduces the amplitude of both EPSPs and IPSPs. Cooling reduces and delays anoxic-evoked depression of post-synaptic potentials and hastens recovery; warming has opposite effects. The peak amplitude of AD is not significantly altered, although the rising phase is slower at 30°. These findings show that in the neocortex anoxia produces a consistent depression of synaptic transmission, which can be opposed by hypothermia.

(Supported by the Medical Research Council of Canada).

425.13

PROTECTIVE EFFECTS OF BRAIN HYPOTHERMIA ON BEHAVIOR FOLLOWING GLOBAL CEREBRAL ISCHEMIA IN RATS. F. vanDijk*, E. J. Green, W.D. Dietrich, R. Busto, P.M. McCabe, C. G. Markgraf, M. Y-T. Globus, O. Alonso, M. D. Ginsberg, and N. Schneiderman. Dept's of Psychology and Neurology, University of Miami, Coral Gables, FL 33124.

Histopathological studies indicate that mild brain hypothermia can attenuate the neuronal and microvascular injury following transient global ischemia in rodents (e.g., Busto et al.,1987). Although histopathological measures can provide a general index of the ultimate neurological status of an animal, they do not reveal the functional integrity of an affected brain region. The present study evaluated whether mild brain hypothermia can influence behavioral outcome following ischemia induced by transient 4 vessel occlusion (4VO).

Wistar rats were subjected to one of three surgical procedures: a) 4VO with hypotension (80 mm Hg) for 12 1/2 minutes at a brain temperature of 36° C (4VO-36); b) 4VO with hypotension for 12 1/2 minutes at 30° C (4VO-30); and c) SHAM surgical procedures. Reflexes and sensorimotor function were evaluated between post-operative weeks 2 and 4. Beginning in week 5, the rats were trained in two tasks using a water maze: the simple place task, in which the hidden platform was kept in a fixed location, and the "learning set" paradigm, in which the platform was moved daily (e.g., Auer et al., 1989).

Histopathological examination of the tissue 7-8 weeks post-surgery revealed that 4VO-36 rats had substantial pyramidal cell death in subfield CA1 of the septal 2/3 of the hippocampus, and sparse damage in the dorsolateral neostriatum and neocortex. 4VO-30 rats showed only minimal necrosis in CA1, neostriatum and cortex. There were no group differences in any of the sensorimotor measures or in performance on the simple place task. In contrast, during acquisition of the more complex learning set task, the performance of 4VO-36 rats was significantly impaired relative to either of the other groups. The performance of 4VO-30 rats did not differ significantly from the SHAM rats. These data confirm the finding that global cerebral ischemia impairs behavioral performance in the water maze, and suggest that mild brain hypothermia can provide protection from behavioral deficits induced by global ischemia. Supported by NS05820.

425.14

POSTISCHEMIC HYPOTHERMIA PRODUCED BY MK-801 ACCOUNTS FOR SOME, BUT NOT ALL, OF ITS NEUROPROTECTIVE ACTION. R.E. Minahan*, K.H. Neill*, B.J. Crain and J.V. Nadler. Depts. Pharmacology, Pathology and Neurobiology, Duke Univ. Med. Ctr., Durham, NC 27710.

The ability of the NMDA receptor antagonist MK-801 to protect brain neurons against ischemic damage has been attributed by some investigators to a reduction in postischemic brain temperature. We reported previously that MK-801 (10 mg/kg) initially reduces postischemic brain temperature and then usually causes brain temperature to oscillate between hypothermic and normothermic-to-hyperthermic. To determine the extent to which this postischemic temperature pattern accounts for the neuroprotection obtained with the drug, we utilized a novel system that permits brain temperature to be monitored and regulated in a conscious gerbil. We selected representative postischemic brain temperature patterns that were associated with either complete protection of CA1 hippocampal pyramidal cells by MK-801, average protection or no protection and simulated each pattern in the absence of drug. Intraischemic brain temperature was maintained at 35.8 °C and intraischemic rectal temperature at 36.5 °C. The postischemic temperature patterns associated with average or complete protection partially prevented the degeneration of CA1 pyramidal cells, but not to the extent that pretreatment with MK-801 did. In addition, when MK-801 was administered before simulating a temperature pattern associated with either complete or no protection, additional CA1 pyramidal cells were spared.

These results support the view that MK-801 protects neurons from ischemic damage partly through reducing postischemic brain temperature. However, the drug also exerts a robust neuroprotective action that appears to be independent of postischemic brain temperature. (Supported by NIH grant NS 06233.)

425.15

INTRAISCHEMIC BRAIN TEMPERATURE, POSTISCHEMIC BRAIN TEMPERATURE AND NEUROPROTECTION BY MK-801. K.H. Neill*, R.E. Minahan*, B.J. Crain and J.V. Nadler. Depts. Pharmacology, Pathology and Neurobiology, Duke Univ. Med. Ctr., Durham, NC 27710.

Intraischemic and postischemic brain temperatures have been shown to influence the extent of ischemic brain damage and the neuroprotective action of MK-801. We have investigated this relationship in gerbils subjected to a 5-min bilateral carotid occlusion. MK-801 (10 mg/kg) or saline was administered either 1 h before or 30 min after ischemia. During the occlusion, rectal temperature was maintained at 36.5 °C and the brain was maintained at either 35.8 or 36.5 °C. When the intraischemic brain temperature was 36.5 °C, degeneration of CA1 hippocampal pyramidal cells correlated only with the difference between the preischemic baseline and the mean brain temperature for the first 30 min after ischemia. Lowering intraischemic brain temperature to 35.8 °C produced the following changes. (1) MK-801 more effectively protected CA1 and neocortical pyramidal cells. (2) The value of brain temperature 3-6 h after reperfusion became at least as important as brain temperature soon after reperfusion. (3) If the mean postischemic brain temperature was ≤ 36 °C, there was minimal CA1 damage. However, MK-801 achieved this degree of hypothermia in only a minority of animals. (4) Correlations between neocortical damage and postischemic brain temperature could also be demonstrated.

These results demonstrate that factors which influence ischemic brain damage - intraischemic brain temperature, postischemic brain temperature and treatment with MK-801 - are highly interactive. Maintaining intraischemic brain temperature at a nearly physiological value diminishes both the influence of postischemic brain temperature on ischemic damage and the neuroprotective action of MK-801. (Supported by NIH grant NS 06233.)

425.16

ISCHEMIA-INDUCED NEUROTRANSMITTER RELEASE: EFFECTS OF MILD INTRAISCHEMIC HYPERTHERMIA. L.L. Sternau, M.Y.-T. Globus, E. Martinez, W.D. Dietrich, R. Busto, and M.D. Ginsberg. Cerebrovascular Disease Research Center, Univ. of Miami School of Medicine, Miami, FL, 33101.

Elevated brain temperature may adversely influence the outcome of an ischemic insult. Using microdialysis, we examined neurotransmitter release in rats subjected to 20 min of forebrain ischemia by 2-vessel occlusion plus hypotension (45 mmHg). In Group 1 (n=5), intraischemic brain temperature (BT) was maintained at 37°C . In Group 2 rats (n=6), BT was raised to 39°C by a warming lamp over the head. Striatal extracellular fluid was sampled by microdialysis 30 min prior to and during ischemia, and for 4 hr following recirculation. Dialysate levels of GABA, glycine and glutamate were measured by HPLC. During ischemia, there was a 41-fold rise in GABA at 37°C and a 50-fold rise at 39°C . Glycine levels increased 3-fold in Group 1 and 7-fold in Group 2 during ischemia and remained high during recirculation. Glutamate levels rose 21-fold in Group 1, but in Group 2 there was a 37-fold rise over baseline. The excitotoxic index, reflecting the net influence of excitatory and inhibitory neurotransmitters (glycine x glutamate/GABA), was elevated only 2-fold during recirculation in Group 1, but in Group 2 there was a 20-fold increase over baseline. We conclude that mild cerebral hyperthermia imposed during transient cerebral ischemia increases extracellular levels of excitatory amino acids. This is consistent with the detrimental effect of hyperthermia on ischemic neuropathology and microvascular function.

425.17

ROLE OF HYPOTHERMIA IN MULTIPLE CEREBRAL ISCHEMIC INSULTS. B. Lin, W.D. Dietrich, M.Y.-T. Globus, R. Busto, and M.D. Ginsberg. Cerebral Vasc. Dis. Research Center, Univ. of Miami School of Medicine, Miami, FL, 33101.

To evaluate the cerebroprotective effect of hypothermia for repeated ischemic insults, we subjected male Wistar rats to three 5-min periods of global forebrain ischemia (by bilateral carotid artery occlusions plus hypotension to 50 mmHg), separated by 60-min periods of normotensive recirculation. Rectal temperature was held at 37.0-37.5°C . In Group A (n=5), *brain* temperature (BT), measured by a thermistor in frontal cortex, was thermostated at 36.5-37.0°C throughout. In Group B (n=6), BT was held at 36.5-37.0°C during ischemia but was reduced to 30°C for 30 min following the first insult, and for 60 min following the third insult. Ischemic cell change was graded blindly, on a 0-3 scale following 7-day survival. In area CA1 of hippocampus, Groups A and B showed moderate-to-severe ischemic changes in 4/5 and 5/6 brains, respectively. The range of injury-grades in somatosensory neocortex and thalamus was also similar for Groups A and B. In dorsolateral striatum, there was a trend toward lesser injury-grade in Group B rats (median 0.5-1) than in Group A (median 1.5). In 2 and 3 rats of Group B, respectively, the striatum and thalamus showed marked side-to-side differences in injury-grade. Although early postischemic cerebral hypothermia (30°C) reduces neuronal damage following a *single* 10-min period of ischemia (Busto et al, Neurosci Lett 101:299,1989), the present data suggest that multiple normothermic insults may be more resistant to protection.

425.18

PRE- BUT NOT POSTEXPOSURE MILD HYPOTHERMIA PROTECTS AGAINST HYPERGLYCEMIC ANOXIC BRAIN INJURY

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Hyperglycemic animals with normal core (38°C) and brain (36°C) temperatures at systemic anoxia develop neurologic signs including seizures and show diffuse brain injury with marked neuronal loss and glial and capillary proliferation upon long-term survival. In contrast, identically exposed hyperglycemic animals which are mildly hypothermic (core and brain temperatures = 33 and 32°C, respectively) are remarkably brain protected, remaining neurologically and neuropathologically intact. The cerebral cortex in both groups shows similar marked elevations of lactic acid (>25 μmoles/g) and reductions in high energy phosphates at the end of anoxia. Rapid reduction of brain temperature to 32°C following normothermic hyperglycemic anoxia fails to prevent postanoxic neurologic signs and brain injury. In conclusion, mechanisms underlying the development of diffuse brain injury from hyperglycemic anoxia are activated at anoxic exposure and can be prevented by mild hypothermia prior to but not following exposure. Supported by DVA Medical Research Service funds.

425.19

HYPOTHERMIA PROTECTS SOMATOSTATIN NEURONS IN RAT DENTATE HILUS FROM ZINC ACCUMULATION AND FAST ISCHEMIC CELL DEATH. F.F. Johansen, N. Tønder[1], J. Zimmer[1], N.H. Diemer. PharmaBiotec Research Center: Institute of Neuropathology, University of Copenhagen, and [1]Institute of Neurobiology, University of Aarhus, Denmark.

We have previously shown that somatostatin containing neurons in the rat dentate hilus are highly susceptible to ischemic brain damage. Within 48 hours after ischemia they demonstrate fast ischemic cell death. Simultaneously, we have shown that necrotic cells in dentate hilus accumulate free zinc in their cytosol. We have now demonstrated directly that somatostatin containing cells in dentate hilus also accumulate zinc during maturation of fast ischemic cell death. Since it is known that hypothermia ameliorates ischemic brain damage, we studied whether hypothermia (29° Celsius) protected the highly susceptible somatostatin containing neurons in dentate hilus from ischemic cell death and zinc accumulation. It was demonstrated that hypothermia during cerebral ischemia prevented cell necrosis and neuronal zinc accumulation in dentate hilus. Hypothermia possibly prevents excitotoxic glutamate release and associated translocation of zinc from the glutamatergic mossy fiber terminals to the vulnerable somatostatin containing cells during ischemia. We find it less likely that zinc is released from intracellular binding sites during ischemic cell death, because neurons located outside the mossy fiber layer do not show zinc accumulation during necrosis.

425.20

LIMITS OF HYPOTHERMIC PROTECTION OF HIPPOCAMPAL NEURONS DURING CARDIAC ARREST IN THE NEONATAL PIG D.T. Ross,[1] L.N. Sutton,[1*] E. Woodford,[2*] C. Norwood,[2*] and W.I. Norwood,[2*] B.J. Clark[2*] [1]Division of Neurosurgery, University of Pennsylvania, and [2]Dept. of Cardiothoracic Surgery, Children's Hospital of Philadelphia, Philadelphia, PA 19104

Hypothermia has a profound neuroprotective effect on the prevention of neuronal loss following ischemia. Results from a rodent global ischemia model (Brain Res 512:169-174, 1990) suggest that this protection is only relative; once a critical threshold duration is exceeded, selectively vulnerable structures such as the hippocampal CA1 are no longer protected. Since prolonged periods of hypothermic cardiac arrest are integral parts of pediatric open heart surgery, we sought to establish whether these procedures result in neuronal loss from vulnerable brain structures.

Neonatal (21 day old) pigs were anesthetized with Nembutal, placed on cardiopulmonary bypass, cooled to body temperatures of 15-20°C, and the bypass pump then turned off. The pump was restarted 45 minutes to an hour later, the animal taken off bypass, and its incisions closed. Animals survived for 7 days prior to sacrifice by nembutal overdose and transcardial perfusion with 4% paraformaldehyde. Brain sections were cut at 40μm and stained with cresyl violet.

Extensive neuronal loss was seen in the CA1 region in 2/6 hippocampi from cases which sustained 45 minutes of cardiac arrest at 20°C. Longer durations of cardiac arrest (1 hour) at colder temperatures (15-18°C) produced scattered patches of selective neuronal loss in the CA3 region in 2/6 hippocampi. All cases had evidence of partial loss of neurons from the regions of CA4 proximal to the granule cell layer. A partial loss of neurons from the rostral portion of the thalamic reticular nucleus was also evident in some cases. These results suggest that hypothermic neuroprotection alone may not be sufficient to prevent the loss of neurons from selectively vulnerable structures during open heart surgery. Augmentation with the prophylactic administration of pharmacological agents may be necessary for insuring complete neuroprotection during intra-operative ischemia.

ISCHEMIA III

426.1

COGNITIVE DEFICIT AS A RESULT OF CEREBRAL ISCHAEMIA. S.Hogg*J.M.Barnes*,B.Costall*,P.L.Irving*,M.E.Kelly* and J.R.McCurrie*. (SPON: European Neuroscience Association) Studies in Pharmacology, School of Pharmacy, University of Bradford, Bradford, BD7 1DP, UK.

We have reported a cognitive deficit in a spatial learning task as the result of carotid occlusion (Barnes et al, 1990). Here we assess the effects of an ischaemic insult on brain biochemical and histological parameters.

Lister-hooded rats underwent 45 min bilateral common carotid artery occlusion under ketamine anaesthesia (100mg/kg). 7 days later they were either tested in a swim maze task or sacrificed for choline acetyltransferase (ChAT) analysis (Fonnum, 1975) or haematoxylin and eosin (H & E) staining.

Carotid occlusion resulted in a loss in learning ability (escape latencies for sham and occluded 14.5±2.18 and 54.2±4.3s respectively, P<0.001, one way ANOVA). ChAT activity (sham and occluded) was only reduced in striatum and tuberculum olfactorium (779.0±64.8 to 465.5±50.5 and 504.9±52.3 to 352.2±20.0 nmol/min/mg protein, P<0.05,t-test) whilst H&E staining showed no change in density of hippocampal neurones.

Therefore the loss of learning ability in the rat following an ischaemic insult is not necessarily correlated with changes in ChAT activity or cell loss.

Barnes JM et al (1990) Br J Pharmacol 101(Suppl), 566P
Fonnum F (1975) J Neurochem 24, 407-409

426.2

IMPAIRED WATER MAZE ACQUISITION FOLLOWING CA1 ISCHEMIC DAMAGE IN THE GERBIL. D. Corbett, S. Nurse*, S. Evans* and D. W. McKay. Fac. of Medicine, Memorial Univ., St. John's, NF, CANADA A1B 3V6.

The Morris water maze is a sensitive test of spatial learning that has been used to document deficits in rats that have extensive loss of CA1 neurons induced by forebrain ischemia (Auer et al., 1989). Similarly, we have found this test to be effective in gerbils subjected to carotid artery occlusion.

Gerbils were given 5 days (4 trials / day) of swim training (to a visible platform) in a circular pool filled with warm water. The next day, the gerbils were exposed to 5 min carotid occlusions under Halothane anesthesia (N =12) ; sham surgery (N = 6) or left intact (N =6). Water maze acquisition took place 2 days later in a different testing room using a hidden platform and several conspicuous visual stimuli that were attached to the walls of the maze. Ischemic gerbils were slower to acquire the water maze task ($F = 6.00$, $p < .025$) than sham/normal gerbils although they eventually (7-10 days) learned to locate the platform. The Morris water maze has potential as a functional test in studies of cerebral ischemia in gerbils.

Supported by the MRC of Canada.

426.3

REMACEMIDE EFFECTS ON MEMORY AND HIPPOCAMPAL CA1 NEURONAL DAMAGE IN THE RAT FOUR-VESSEL OCCLUSION (4-VO) MODEL OF GLOBAL CEREBRAL ISCHEMIA. J.M. Ordy, G.J. Thomas, P. Bialobok*, T.M. Wengenack, R.J. Murray*, and W.P. Dunlap*. Fisons Pharmaceuticals, Rochester, NY 14623, Univ. of Rochester, Rochester, NY 14642, Tulane Univ., New Orleans, LA 70118.

Clinical studies have reported memory impairment and selective loss of hippocampal CA1 neurons after global ischemia. Significant memory impairment with selective CA1 cell damage have also been observed in the rat 4-VO model of global ischemia. The concordant pathophysiology of memory impairment and CA1 cell damage in man and in the rat 4-VO model have suggested that the 4-VO model could be useful for evaluation of "neuroprotective" efficacy of novel compounds after global ischemia. Remacemide, (±) 2-amino-N-(1-methyl-1,2-diphenylethyl)-acetamide, is an anticonvulsant currently in Phase II clinical trials. The desglycinated metabolite of remacemide is a non-competitive NMDA antagonist. The effects of remacemide were evaluated functionally in terms of neuroprotective effects on memory of 4-VO memory-impaired rats in a T-maze, and morphometrically, by evaluation of cell damage in stereotaxically comparable CA1 regions. Remacemide, at 20 mg/kg, or saline was administered i.p. at 1 hour post-4-VO, and then once daily for either 14 or 21 days, depending on the study design. Statistical comparisons of remacemide or saline-treated 4-VO rats indicated significant improvement of 4-VO memory impairment and reduction of CA1 cell loss in the remacemide-treated animals. Comparisons of remacemide with the non-competitive NMDA receptor antagonist, MK-801, were made on CNS safety profile studies in non-ischemic rats. Remacemide produced no significant effects on memory, psychotomimetic signs, sensory-motor functions, body temperature, blood glucose, or corticosterone levels. In contrast, MK-801 produced adverse effects on these same variables which would exacerbate ischemic CA1 neuronal damage.

426.4

GLOBAL ISCHAEMIA PRODUCES DEFICITS IN SPATIAL LEARNING AND MEMORY IN THE WATER MAZE BUT NOT IN THE RADIAL-MAZE. JA Nunn, E Le Peillet,* CA Netto,* P Sowinski,* H Hodges,* BS Meldrum and JA Gray*, Departments of Psychology and Neurology, Institute of Psychiatry, London SE5 8AF, UK

We have previously shown that both 15 and 30 min 4-vessel-occlusion ischaemia (4VO ISC) in the rat produce equivalent deficits in learning and memory in a water maze place task and are accompanied by >90% hippocampal CA1 cell loss. The present study compared the effects of 15 and 30 min 4VO ISC on water maze (WM) and radial-maze (RM) performance. The first test (WM: 7 wks post-op) confirmed that comparable spatial deficits are seen after both 15 and 30 min ISC. In ISC rats latency to find the platform was increased relative to controls during acquisition ($p<0.01$), whilst time spent in the quadrant containing the platform was reduced ($p<0.01$). Both ISC groups showed an equivalent deficit in retention of the platform location in a probe trial. All rats were tested on place and cue tasks in the radial-maze (10-25 wks post-op); no behavioural deficits were seen. Rats were retested on reversal learning in the WM (26 wks post-op). ISC spatial deficits were similar to those seen in the first WM test. These data indicate that ISC can impair WM performance but spare RM performance. The requirement for more accurate use of allocentric spatial cues and the higher motivational demands of the WM may explain the difference between the two tasks. Since WM learning was still impaired after RM testing, recovery as a function of time or training cannot explain the lack of RM deficits.(Supported by the Wellcome Trust and the British Heart Foundation)

426.5

4-VO INDUCED ISCHEMIA RESULTS IN CA1 CELL LOSS AND IMPAIRED 14-UNIT T-MAZE PERFORMANCE. E. Spangler[1], J. Ordy[2], P. Bialobok[2], G. Thomas[2], P. Garofalo[1], T. Wengenack[2], J. Hengemihle[1], M. Jucker[1], M. Talan[1], D. Ingram[1]. [1]Gerontol. Res. Ctr., NIA, NIH, Baltimore, MD 21224 and [2]Fisons Pharmaceut. Rochester, NY 14623

The four vessel occlusion (4-VO) model (Pulsinelli & Brierly, *Stroke*, 10: 267, 1979) of transient, global ischemia produces neuronal loss in hippocampus (HC), particularly CA1, and memory impairment in a variety of tasks. We used this model to assess the effect of CA1 damage in young rats on learning in a 14-unit T-maze that has provided robust evidence of age-related memory impairment (Ingram, *Neurobiol. Aging*, 9:475, 1988). Rats received either 15-min of 4VO ischemia, temperature maintained at 37.5°C during reperfusion, or were in an operated control (CON) group. Rats were trained 2 wk later in one-way active avoidance in a runway (0.8mA; criteria = 13/15 correct avoidances). Rats were given 15 trials in the complex maze the next day. With the maze divided into 5 segments, the rat had to move through each segment within 10 sec to avoid footshock (0.8 mA) while negotiating a series of 14 left-right turns enroute to the goal box. 4-VO rats had significantly higher scores than CON rats in 3 measures of maze performance: errors, alternation errors, and runtime (p's < 0.05) but were not markedly affected in shock frequency or duration measures. Six wk after maze training, rats were sacrificed to assess HC cell death. Estimation of relative cell density (thionin Nissl stain) measured by automated image analysis revealed significant cell loss in CA1 in 4-VO rats compared to CON, $p<0.05$. No difference in acetylcholinesterase staining density in CA1 between groups was observed, $p>0.05$. Mean error score/trial in 4-VO rats was correlated with relative CA1 cell density, $r=-0.62$. Subsequent ratings of CA1 neuropathology yielded a correlation of 0.67 with maze errors. The utility of this model in evaluating age-related impairments in tasks such as the 14-unit T-maze are suggested by the present findings.

426.6

CHANGES IN SYNAPTOPHYSIN LEVELS FOLLOWING ISCHEMIC INSULT. R.P. Stroemer, T.A. Kent and C.E. Hulsebosch. Depts. of Anat. and Neurosci., Marine Biomed. Inst., Neurol., Univ. of Texas Med. Br., Galveston, TX 77550.

Synaptophysin, a calcium binding protein, is present in presynaptic endings. Changes in the density of synaptic numbers and density are believed to be reflected in the amount of synaptophysin present. Using immunohistochemistry, The amount of synaptophysin is measured in the cortex in hypertensive and normotensive rats following ischemia. The insult is produced by permanent occlusion of the middle cerebral artery and the ipsilateral common carotid artery. Changes in synaptophysin are examined at time points of 3 days to 2 months following ischemia. We hypothesize that the differences in synaptophysin levels reflect the plasticity of the cortex following ischemia. This work is supported by NS 11255, NS 25400, RR 03779, NS 01217, and Bristol Myers-Squibb.

426.7

SPATIAL RESOLUTION OF K^+o AND CBF DERANGEMENT DURING MCA OCCLUSION IN RAT NEOCORTEX. Z.-C. Feng, T.J. Sick and M. Rosenthal. Dept Neurology, University of Miami School of Medicine, Miami, Fl. 33010.

Focal cerebral ischemia may produce a penumbral area outside of the ischemic zone with suppressed electrical activity but preserved ion homeostasis. To better understand focal ischemia-induced pathophysiology of brain, present studies sought to spacially resolve extracellular potassium ion activity (K^+o) and cerebral blood flow (CBF) during middle cerebral artery occlusion (MCAo) in exposed neocortex of rats anesthetized with pentobarbital. K^+o and CBF were monitored at multiple sites along the cortical surface from the MCA clamp toward the midline of the brain by ion selective and polarographic (hydrogen clearance) microelectrodes implanted approx 500 μ within the cortical gray mantle. At 1-3 mm medial from the MCA clamp, blood flow was reduced approx 75% and K^+o was elevated to the 50-80 mM levels characteristic of anoxic depolarization (AD) This situation persisted for 3 hours after MCAo. Approx 7-8 mm medial from the clamp, blood flow and K^+o were unchanged by MCAo during this period. Between these zones, intermediate CBF and K^+o levels (the latter in the range of 20-35 mM) were recorded at 30 and 90 min following MCAo. At 180 minutes, however, these intermediate zones were less evident as K^+o often increased in areas closer to the ischemic core to levels characteristic of AD while K^+o sometimes decreased in loci adjacent to normal sites. The presence of intermediate K^+o levels is unusual since regenerative spreading depression-like like depolarization often accompanies elevation of K^+o beyond approximately 10-15 mM. Identifying factors limiting K^+o in intermediate zones during focal ischemia may provide new insights toward salvaging tissues after such insults.

426.8

Near-term foetal rats resist hypoxic neuronal injury. L. Kendall*, S.P. Butcher* & J.S. Kelly, (SPON: Brain Research Association), Department of Pharmacology, University of Edinburgh, U.K.

Hypoxic and ischaemic insults during the perinatal period are known to be a major cause of non-progressive neurological deficits in humans. We have developed a model of perinatal hypoxia in anaesthetized rats which involves occlusion of the uteroplacental vessels of one uterine horn. After an occlusion period of 10, 20 or 30 minutes, which may or may not be followed by a reperfusion period, the pups are delivered by Caesarian section. The pup brains are extracted at 0, 1, 60 or 180 minutes for analysis of metabolites. The remaining pups are resuscitated then cross-fostered onto lactating dams and perfusion fixed at a later date for histological analysis. Following the most severe insult, a 30 minute occlusion, brain tissue levels of lactate are elevated from 6.1 $\pm$ 0.5 nmols/mg to 22.2 $\pm$ 1.5 nmols/mg tissue, ATP and phosphocreatine levels are reduced from 2.4 $\pm$ 0.3 nmols/mg to 0.9 $\pm$ 0.3 nmols/mg and from 1.0 $\pm$ 0.2 nmols/mg to 0.3 $\pm$ 0.2 nmols/mg tissue respectively. Histological investigations showed little or no damage 4 hours after the hypoxic insult however four to seven days post-insult discrete damage can be identified in some areas in the hind brain region.

426.9

REPRODUCIBLE ISCHEMIC CORTICAL LESIONS IN RATS BY PHOTOTHROMBOSIS OF THE DISTAL MIDDLE CEREBRAL ARTERY. C.G. Markgraf, S. Kraydieh*, R. Prado*, B.D. Watson*, W.D. Dietrich and M.D. Ginsberg. Cerebral Vascular Disease Research Center, Univ. of Miami Sch. of Med., Miami, FL 33101.

To model accurately the clinical disorder of human thrombotic stroke, the present study used a photochemical reaction to induce focal thrombosis of the distal middle cerebral artery (dMCA) in two strains of rats. Male Wistar (n=9) and Sprague-Dawley(n=12) rats were anesthetized and prepared with arterial and venous catheters. The right dMCA was exposed above the rhinal fissure. After i.v. injection of rose bengal, a potent photosensitizing dye, thrombosis was induced by irradiating the vessel with 20 mW from an argon laser-activated dye laser tuned to 562 nm. The right common carotid artery (CCA) was then permanently occluded and the left CCA was temporarily occluded for 60 min. Animals were perfusion-fixed 3 days later and infarct volume was determined. Infarcts of the right cortex varied in size by strain. Sprague-Dawley rats had significantly larger cortical infarct volumes ($\bar{x}$ $\pm$S.E.=116.29 $\pm$ 17.35 mm^3) than did Wistars ($\bar{x}$ $\pm$ S.E.=47.88 $\pm$ 8.86 mm^3). Blood pressure (BP) and pCO_2 did not differ between groups either before dMCA occlusion, during or after CCA occlusion. BP and infarct volume were significantly correlated, and showed that differences in BP accounted for 15.4% of the variability in size of infarcts. Mortality was 9%, similar to other models of distal MCA occlusion. The advantages of the present model are the reproducibility and consistency of cortical infarcts, the relative ease of the surgical procedure, and that the photothrombosis is less invasive than mechanical occlusion while it more realistically simulates the pathophysiological events of a thrombotic stroke in humans. Supported by NS-05820, NS-22603 and NS-23244.

426.10

EARLY CHANGES IN HIPPOCAMPUS FOLLOWING TRANSIENT GLOBAL CEREBRAL ISCHEMIA IN MONGOLIAN GERBILS. E. Fadda, S. Romanello*, M. Santi, R. M.M. Zanellato*, R. Arban*, Dal Toso, and S. Mazzari. Fidia Research Labs, 35031 Abano Terme, Italy.

A selective neuronal death (SND) in the CA_1 hippocampal region occurs within 3-4 days after a transient episode of global cerebral ischemia. We monitored, in Mongolian gerbils, hippocampal MK-801 binding sites and GFAP mRNA following 1 and 5 min of bilateral carotid artery occlusion, at both 24 and 72 hr of reperfusion. MK-801 binding sites were significantly reduced (-30%) within 24hr after 5 min of ischemia. GFAP mRNA levels dramatically increased, reaching a plateau (10-fold increase) at 24hr of reperfusion following 5 min of ischemia. No significant modifications of these indexes were seen following 1 min of ischemia, nor were there any evident histopathological changes (i.e. Nissl staining) in the hippocampal CA_1 area. These findings indicate that SND is associated with relatively rapid biochemical changes in the hippocampus in both neurons and glial cells, thus facilitating SND detection and quantification.

426.11

NEUROPATHOLOGICAL CORRELATES OF MAGNETIC RESONANCE IMAGES OF ISCHEMIC DAMAGE IN A BABOON MODEL OF CEREBRAL ISCHEMIA. C.A. Pardo, L.H. Monsein*, V.P. Mathews*, P.B. Barker*, S.J. Blackband* and R.N. Bryan. Neuropathology Laboratory, Department of Pathology, and Division of Neuroradiology, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

A baboon model of regional ischemia of the middle cerebral artery was developed to evaluate cerebral metabolism in the early postischemic period with magnetic resonance imaging (MRI), proton magnetic resonance spectroscopy (MRS), and neuropathology. Histological and immunocytochemical analysis (i.e., phosphorylated and nonphosphorylated neurofilaments, MAP2, calbindin, and parvalbumin) were used to evaluate morphological changes. Abnormal signal intensity was seen as early as 30 minutes with MRS, 3.12 hours with T2 weighted, 3.32 hours with T1 weighted, and 4.92 hours with spin density images. Neuropathological evaluation 18 hours after occlusion demonstrated regional dysruption of myelin and neuronal necrosis that correlated with MRI signal changes. However, additional neuronal changes, such as immunoreactivity for calbindin and phosphorylated neurofilaments, and loss of immunoreactivity for MAP2 were detected in a greater area than shown by MRI. In conclusion, MRI appeared to detect tissue necrosis but was less successful in detecting earlier changes of cell damage.

426.12

TEMPORAL RELATIONSHIP BETWEEN REACTIVE GLIOSIS AND NEURONAL NECROSIS IN POST-ISCHEMIC RAT HIPPOCAMPUS. C.K.Petito and I.A. Halaby. Dept. of Pathology and Neurology, Cornell University Medical College, New York, NY, 10021.

Since astrocytes may protect the brain from ischemia, this study tests the hypothesis that reactive astrocytosis develops prior to neuronal necrosis in CA1 neurons of post-ischemic hippocampus where cell death is characteristically delayed between 3 and 7 days. Cerebral ischemia for 2 or 10 min was produced by the 4-vessel occlusion rat model with reperfusion of 1 to 14 days, producing mild (2 min) or moderate-severe (10 min) CA1 necrosis. Astrocyte reactivity was evaluated by changes in glial fibrillary acidic protein (GFAP) and vimentin (Vim) and by incorporation of bromodeoxyuridine (BRDU) given 1 and 2 hours prior to sacrifice. Normal control(n=4) astrocytes were GFAP+, Vim-, and BRDU-. Increased GFAP preceded neuronal necrosis in 5 of 10 rats between 1 and 3 days after 10 min ischemia and in 5 of 6 rats between 3 and 7 days after 2 min ischemia. Vim+ astrocytes and BRDU + cells were present following 10 minutes of ischemia but rare after 2 minutes of ischemia. These results show that glial reactivity is proportional to the severity of neuronal necrosis. Furthermore, they indicate that reactive astrocytosis may precedes the development of neuronal necrosis in vulnerable CA1 neurons, especially when the ischemia is mild and the development of cell death is prolonged. Supported by NSO3346-30,NINCDS.

426.13

EARLY NEURONAL CHANGES IN THE GLOBAL CEREBRAL ISCHEMIA. K. Kawai, L. Nitecka, C. Ruetzler, J. Lohr, N. Saito, T.S. Nowak Jr. and I. Klatzo, Lab. of Neuropathology and Neuroanatomical Sciences, NIH, NINDS, Bethesda, MD 20892

In elucidation of the pathophysiology of global cerebal ischemia the early neuronal changes were studied in rats subjected to 10 minute cardiac arrest by compression of major cardiac vessels. The early neuronal changes were manifest in animals sacrificed 15 minutes after resuscitation and consisted in appearance of a clear peripheral zones in the cytoplasm of predominantly GABAergic neurons. After one hour these peripheral zones appeared to be compartmentalized into individual clear vacuoles and they were especially conspicuous in the n. reticularis thalami (NRT). The other neuronal populations showing similar changes were: n. dorsalis medialis thalami, n. centralis amygdalae, pars reticulata substantiae nigra, and hippocampal interneurons. Preliminary E.M. observations on the NRT neurons revealed peripheral vacuoles surrounded frequently by membranes, compatible with endoplasmic reticulum origin and the presence of well preserved mitochondria. The immunostaining for parvalbumin showed disappearance of specific staining of middle portion of the NRT in animals sacrificed 3 hours after resuscitation. Our observations suggest a possibility that early changes in GABAergic neurons may provide a period of disinhibition and thus contribute to an excitatory damage in regions connected by GABAergic circuitry.

426.14

Increased expression of mRNA encoding calbindin-D28K, the glucose-regulated proteins, or the 72 kDa heat-shock protein in three models of CNS injury. R.P. Gwinn*, M.S. Seren , R.P. Simon , T.K. McIntosh , M.J. Thomas*and D.H. Lowenstein , Epilepsy Res. Lab., Dept. of Neurology, UCSF, FIDIA Res.Labs, Italy, and Dept. of Surgery, U.CT. Health Ctr.

Changes at the level of gene expression are becoming an increasingly recognized component of the neuronal response to injury. We used Northern analysis and 3 *in vivo* models of CNS trauma in the rat to determine whether injury alters the expression of gene products potentially related to cellular homeostasis. The 3 models included: 1) seizures induced by 12 mg/kg IP kainic acid (KA), 2) 10 minutes of global ischemia following four vessel occlusion, and 3) focal fluid percussion injury to the cerebral cortex. Animals were sacrificed at various times after injury, and total RNA was isolated from specific CNS regions. Northern blots were hybridized with probes for calbindin-D28K, the 78 and 94 kDa glucose-regulated proteins (grp78 or grp94), the inducible 72 kDa heat-shock protein (hsp72), and a control probe for the 18S ribosomal subunit. Results show that mRNA for calbindin-D28K, grp78, grp94, and hsp72 increased in the hippocampus following seizures. Peak expression occurred 6-12hrs after administration of KA, and returned to baseline by 24hrs. Similar changes in all 4 transcripts were seen in the hippocampus and cortex following global ischemia, although the return to baseline tended to exceed 24hrs for the grps. In the focal trauma model, mRNA for hsp72 and grp78 was increased in the cortex and hippocampus ipsilateral to the impact 12 hours after injury.

These results expand the repertoire of known changes in mRNA expression following CNS injury. The increases in hsp72 and grps indicate the occurrence of a generalized stress response. Furthermore, given the evidence that grp78 and grp94 are induced by calcium ionophores *in vitro*, and the potential role of calbindin-D28K in buffering cytoplasmic calcium, the changes observed in this study may represent a cellular response to the perturbed calcium homeostasis that is common to all 3 forms of injury.

426.15

HEAT SHOCK AND TUBULIN mRNA IN POSTISCHEMIC BRAIN. K. Kumar, B.V. Madhukar. Departments of Pathology, and Pediatrics, Michigan State Univ, E. Lansing, MI 48824.

Recent evidence suggests that while total protein synthesis is reduced following brain ischemia, the expression of a family of heat shock proteins (HSP) may actually be enhanced in the postischemic (PI) brain during reperfusion. To examine the expression of HSP-70 in PI brain, and to compare it with that of tubulin, a protein abundant in neural tissue, ischemia was produced in gerbils by bilateral common carotid artery occlusion for 10 min and was followed by reperfusion for 6 and 24 h. The forebrains of PI and nonischemic controls (NIC) were processed for RNA extraction. The RNA from all 7 animals was analyzed for inducible HSP-70 sequences and for alpha-tubulin by Northern blot and hybridization by utilizing specific synthetic oligonuceotide probes. With the probe for HSP-70, no distinct hybridization bands were observed in the NIC, whereas a striking induction was evident in the 6 h PI group. Continued induction was present at 24 h. RNA for tubulin was expressed at high levels in the NIC as well as the 6 h and 24 h PI groups, with the trend of expression in PI brains toward an increase in comparison with the NIC. However, on quantitation, the difference was not statistically significant. This time course contrasts with the expression of inducible HSP-70 that is not detectable in the NIC but is significantly elevated in the PI brain.

426.16

HEAT-SHOCK PROTEIN (HSP70) EXPRESSION IN FOCAL BRAIN DAMAGE P.J.Lindsberg, T.S.Nowak, Jr.†, A.-L.Sirén, J.M.Hallenbeck*. Dept. of Neurol., USUHS and †NINDS, NIH, Bethesda, MD 20814.

Global ischemic or hyperthermic brain damage alters gene expression inducing production of mammalian heat shock proteins. We hypothesized that HSP70 expression indicates an area at risk in focal brain injury. Progressive cortical damage was produced by focal laser pulse in rats (n=14). Double-labelling fluorescence technique (Evans-Blue albumin, FITC-dextran, i.v.) was used to map and measure BBB-damage and microvascular perfusion from fresh-frozen brain sections. HSP70 mRNA was localized by in situ hybridization. While absent at 30 min after injury, striking HSP70 expression took place by 2 hrs in a ~300 μm wide rim surrounding an area of expanding BBB-damage. HSP70 mRNA was observed only in regions of preserved microcirculation. Subcortically migrated edema fluid apparently did not induce further HSP70 expression, which persisted at 8 hrs. We conclude that progressive focal brain damage rapidly induces localized HSP70 expression, which is dissociated from increasingly ischemic, edematous areas and may identify cells with the capacity to adapt to the metabolic challenge.

426.17

ZINC INDUCTION OF HSP72 AND CELL DEATH IN PRIMARY ASTROCYTE CULTURE. R.A. Swanson and F.R. Sharp Dept. of Neurology, Univ. of California San Francisco and Veterans Affairs Medical Center, San Francisco, CA 94121

Zinc is a potent inducer of the 72kD heat shock protein (HSP72), and extracellular zinc concentrations may be greatly elevated in brain during pathologic conditions. The present study examines the effect of zinc on HSP72 expression in primary rat cortical astrocyte culture. Astrocytes were grown to confluency and exposed to zinc chloride in Earle's buffered salt solution. HSP72 expression was induced by incubation with 40uM Zinc for 4 hours or 1mM for 15 minutes. Expression occurred in a patchy distribution with HSP72 positive astrocytes frequently forming the margin between dead and viable cells, similar to the pattern seen after cerebral ischemia in vivo. Exposure to zinc at higher levels, 100uM for 4 hours or 500uM for 1 hour, caused greater than 90% cell death. Elevated extracellular zinc concentrations may contribute to HSP72 induction and astrocyte death under pathologic conditions in brain.

426.18

RELATIONSHIP BETWEEN EXPRESSION OF HEAT SHOCK PROTEIN-70 mRNA AND HISTOLOGIC INJURY FOLLOWING FOCAL ISCHEMIA IN RAT BRAIN. F.A.Welsh, D.J.Moyer and V.A.Harris*. Div of Neurosurgery, Univ of Penn Sch Med, Philadelphia, PA 19104.

Cerebral ischemia induces the expression of stress proteins, which may limit the extent of cellular injury. The objective of this study was to compare the regional expression of heat shock protein-70 (HSP-70) mRNA with regional injury measured 4 hr and 24 hr after permanent occlusion of the distal middle cerebral artery in Wistar rats. HSP-70 mRNA was measured with a 30-mer oligonucleotide probe using in situ hybridization. Adjacent sections were stained with acid fuchsin and thionin to locate injured neurons. The regional relationship between HSP-70 mRNA and neuronal injury was similar at 4 hr and 24 hr. In all animals, the expression of HSP-70 mRNA encompassed a wider area of cortex than that undergoing infarction. Frequently, highest levels of HSP-70 mRNA occurred peripheral to the area of infarction. Further, the core of infarction occasionally showed background levels of HSP-70 mRNA. Thus, the expression of HSP-70 mRNA in regions bordering tissue injury is consistent with the hypothesis that induction of stress proteins may limit the regional extent of injury.

426.19

THRESHOLD DURATION OF FOCAL ISCHEMIA REQUIRED TO TRIGGER EXPRESSION OF HEAT SHOCK PROTEIN-70 mRNA IN RAT BRAIN. D.J.Moyer, F.A.Welsh, V.A.Harris.* Div of Neurosurg, Univ of Penn, Phila, PA 19104.

Reperfusion following 60 min of focal ischemia induces strong expression of heat shock protein-70 (HSP-70) mRNA in neocortex, striatum, and hippocampus (Neurosci Abstr 16:938, 1990). The objective of the present study was to determine regional expression of HSP-70 mRNA following focal ischemia of varying duration. Focal ischemia was produced in Wistar rats by transient occlusion the distal middle cerebral artery (MCA) and both carotid arteries for 5, 10, or 20 min, followed by reperfusion for 2 hr. Regional levels of HSP-70 mRNA were measured using in situ hybridization, employing a 30-mer oligonucleotide probe labeled with S-35. Following 5 min of focal ischemia, mild expression of HSP-70 mRNA was detectable in the outer layers of the MCA zone of neocortex. A 10-min insult triggered moderate expression in the outer and inner layers of the MCA neocortex. A 20-min insult produced stronger expression throughout the MCA neocortex and also in adjacent cortex and lateral striatum. Thus, lengthening the duration of focal ischemia not only increased the intensity of expression, but also increased the number of regions expressing HSP-70 mRNA.

426.20

72 KD HEAT SHOCK PROTEIN AND ISCHEMIC TOLERANCE I. Halaby, T. Mima*, C. Petito[1] and W. Pulsinelli. Dept. of Neurology and Neuroscience, Dept. of Pathology[1] Cornell University Medical College, New York, NY.

To test whether the 72kd heat-shock protein (HSP72) protects CA1 hippocampal neurons against severe ischemia, we induced HSP72 synthesis in rats with mild ischemia and later to ischemia that is normally lethal to most CA1 neurons. Adult rats were exposed to 2 min of 4-vessel occlusion (4-VO) ischemia (Pulsinelli and Duffy, J Neurochem, 40:1500, 1983) and then at 6 hr or 48 hr they were made ischemic for 10 min. Separate groups were exposed to 2 or 10 min of 4-VO alone. The animals were killed 5 days later by perfusion-fixation. Stained coronal (7 μm) sections were examined with the light microscope. CA1 damage was graded by a blinded observer on a scale of 0 (normal) to 3 (maximal). Separate rats were subjected to 2 min of 4-VO ischemia, killed by perfusion-fixation at 6 hr (n=3) or 48 hr (n=3), and HSP72 in the CA1 zone was identified with monoclonal antibody and the avidin-biotin-peroxidase method.

MEAN GRADE CA1 HIPPOCAMPAL DAMAGE

2 min Isch.	2 min + 10 min 10 min Isch.	2 min + 10 min Isch. (6 hr)	Isch. (48 hr)
0.6 ± 0.5 (n=9)	2.7 ± 0.5 (n=11)	2.5 ± 0.6 (n=9)	1.4 ± 0.8[a] (n=10)

[a]Kruskal-Wallis plus Mann Whitney U Test $0.025 < p < 0.05$

Immunoreactivity to HSP72 after 2 min of ischemia was barely detectable at 6 hr but prominent at 48 hr. Two min of ischemia followed by 10 min of ischemia at 48 hr, but not at 6 hr, significantly decreased CA1 damage compared to 10 min of ischemia alone. The ischemic tolerance induced by 2 min of sublethal ischemia may result from the induction of HSP72.

ISCHEMIA IV

427.1

EXTRACELLULAR AMINO ACID(AA) AND PURINE METABOLITE LEVELS IN A CORTICAL ISCHEMIA-REPERFUSION MODEL IN THE RAT. T-N. Lin, Y.Y. He, G. Wu, W. Liu, K.E. Peek, J.C. Goodman, and C.Y. Hsu Div. Resto. Neurol. BCM, Houston, TX 77030.

Excessive release of glutamate contributes to ischemic brain injury. Adenosine is thought to be an endogenous neuroprotective modulator. The aim of this study is to measure the extracellular changes of glutamate and adenosine in cerebral cortex following ischemia-reperfusion using a stroke model with irreversible ischemic injury confined to the right MCA cortex. The extracellular amino acids and purine metabolites were recovered from the extracellular fluid before, during ischemia and after reperfusion using microdialysis technique and analyzed by high performance liquid chromatography. Ischemia caused a 30-40 fold (peak level:12.8±1.8uM, mean±SEM, n=16) increase in extracellular levels of glutamate in the right MCA cortex. Aspartate, taurine, gamma-aminobutyric acid, phosphoethanolamine and glycine also increased but to a lesser extent. No significant changes occurred with other AAs. There was no changes in the level of AAs in the left MCA cortex which sustained only mild ischemia (blood reduction of 20-40%). The increase in extracellular adenosine levels was moderate and transient (4-5 fold; peak level:0.6±0.3uM, n=6). Extracellular inosine (peak level:2.2±0.5uM) and hypoxanthine (peak level:3.7±0.8uM) levels rose markedly (8-10 fold) during ischemia and sustained during early reperfusion. The transient elevation in extracellular adenosine concentration followed by a more pronounced increase of its metabolites, inosine and hypoxanthine, suggests rapid turnover of extracellular adenosine. The moderate rise in extracellular adenosine level in comparison to the more substantial and delayed increase in glutamate level during ischemia raise the possibility that this model maybe suitable for studying adenosine modulation of ischemia-induced glutamate release.

427.2

REGULATION OF ENDOGENOUS ADENOSINE DURING HYPOXIA IN THE RAT HIPPOCAMPAL SLICE. J.C. Fowler. Dept. of Physiology, Texas Tech Hlth. Sci.Ctr., Lubbock, TX 79430.

Adenosine levels were measured, using absorbance HPLC, in aliquots taken at 30 min intervals from static chambers of 2 ml volume each containing 4 hippocampal slices. Slices were kept on a net at an interface between the physiologic medium and the humidified atmosphere at a temp of 33-34°C. After a recovery period of 1 hr, fresh medium was placed in the chambers. In 95%O_2/5%CO_2 basal adenosine levels remained around .25 μM for at least 2 1/2hours. Typical population spikes could be evoked. During hypoxic conditions (95%N_2) adenosine levels rose to a new steady-state value of 1.35 μM by 60 min. Removing glucose from the medium during normoxic or hypoxic conditions did not significantly change adenosine levels from their control over 2 1/2 hr.

The adenosine agonist CHA (10 μM) and the antagonist 8-CPT (10μM) both slightly but significantly increased adenosine levels at 60 min in hypoxia. The adenosine transport blocker, dipyridamole (200μM), had no effect on basal adenosine but doubled extracellular adenosine levels above hypoxia alone. Inclusion of the sugar transport inhibitor, phloretin (1 mM), increased adenosine six-fold compared to normoxia alone and quadrupled adenosine levels from hypoxia alone.

Experiments attempting to demonstrate a negative feedback exerted by adenosine on its own levels were equivocal. Glucose-free medium may be ineffective because of glucose remaining in the slice as phloretin greatly increased adenosine. Blocking adenosine transport appears to increase extracellular adenosine but dipyridamole, like phloretin, can reduce sugar transport.

427.3

ADENOSINE ANALOGS MODULATE TRANSMITTER AMINO ACID RELEASE FROM THE ISCHEMIC RAT CEREBRAL CORTEX. R.E. Simpson, M.H. O'Regan, L.M. Perkins and J.W. Phillis. Dept. Physiology, Wayne State Univ., Detroit, MI 48201.

Ischemia is a powerful stimulus for excitotoxic amino acid release from the brain. Adenosine levels are also elevated during cerebral ischemia and purines are cerebroprotective against ischemic and excitotoxic cellular damage. Since adenosine analogs are able to reduce glutamate and aspartate release from nerve terminals, we examined the ability of two such analogs, N^6-cyclopentyl adenosine (CPA) and N-ethylcarboxamido adenosine (NECA) to depress amino acid release from the ischemic rat cerebral cortex. Using the cortical cup technique and HPLC analysis, superfusates of artificial CSF were obtained prior to, during, and following a 20 min period of four vessel occlusion. CPA (10^{-8} to 10^{-12}M) and NECA (10^{-9}M) significantly reduced the release of aspartate, glutamate and GABA, especially during the reperfusion period, in comparison to no drug controls. CPA was most effective at 10^{-10}M. However, at higher doses CPA (10^{-4} to 10^{-6}M) and NECA (10^{-5}M) had no significant effect on release. The specific A_2 receptor antagonist CGS 15943A (0.1 mg/kg i.p.) also reduced ischemia-evoked release of these amino acids. Adenosine depresses transmitter amino acid release via high affinity A_1 receptors and coactivation of lower affinity A_2 receptors may block the A_1 response.

427.4

IN VIVO RECOVERY BY MICRODIALYSIS PROBE DETERMINED BY USE OF MANNITOL. A.J. Hansen, M.J. Sheardown, M.E. Judge, and A. Fink-Jensen. Novo Nordisk A/S, CNS Division, DK-2860 Soeborg, Denmark.

The microdialysis probe collects substances from the interstitial space, but the fraction removed is unknown precluding determination of the interstitial concentration. We have used the extracellular marker, mannitol, to assess the change in recovery between saline and the tissue. Firstly, we showed that recovery of mannitol is similar to loss from the probe in vitro. Secondly, ^{3}H-mannitol was added to the perfusion medium of the microdialysis probe and the loss determined when the probe was immersed in saline +1/2% agar at 37°C and in the rat brain. The loss was reduced by a factor of 3-3.5 in brain, demonstrating the increased diffusion resistance of the tissue. Thirdly, we measured the recovery of calcium in the same experimental set-up. (Calcium was chosen because the interstitial concentration can be accurately measured by ion-selective microelectrodes). In this case the difference between agar and brain was a factor of approx. 5. We suggest that in vivo recovery of most solutes may be assessed by incorporation of mannitol in the perfusion medium.

427.5

ISCHEMIA-INDUCED EXTRACELLULAR RELEASE OF SEROTONIN PLAYS A ROLE IN CA1 NEURONAL CELL DEATH. M.Y.-T. Globus, P. Wester*, R. Busto, E. Martinez*, I. Valdés*, W.D. Dietrich and M.D. Ginsberg. CVD Research Center, University of Miami, School of Medicine, Miami, FL, 33101.

Serotonin (5HT), via $5HT_2$ receptors, exerts an excitatory effect on CA1 neurons and may play a role in ischemia-induced excitotoxic damage. To evaluate the role of 5HT in ischemia, rats were subjected to 12.5 or 20 min of normothermic ischemia by 2-vessel occlusion plus hypotension and extracellular 5HT levels were measured in the hippocampus (12.5 min ischemia, n=5) or striatum (20 min ischemia, n=5) by microdialysis. 12.5 min of ischemia induced a 7-fold increase in 5HT in the hippocampus (mean±SD, baseline=2.11±1.07, ischemia=15.6±7.7 pmol/ml perfusate; $p<0.05$ by ANOVA). Twenty min of ischemia induced a 41-fold increase in 5HT in the dorsolateral striatum (0.76±0.19 and 31.2±9.12, respectively; $p<0.001$). The effects of ritanserin ($5HT_2$ antagonist, 8 mg/kg), administered continuously from 30 min prior to ischemia until 1h of recirculation, was evaluated in animals subjected to 10 min of ischemia (n=5). After 3 days, quantitation of normal-appearing neurons in the CA1 region was performed. Non-treated animals demonstrated severe ischemic damage in all CA1 regions (medial=34±16, middle=52.2±22.9, and lateral=56.6±21.8). Treatment with ritanserin significantly attenuated ischemic damage (117.6±6.5, 131.4±4.9, and 130±7.5, respectively; $p<0.01$). Results suggest that 5HT plays a detrimental role, mediated by $5HT_2$ receptors, in the development of hippocampal ischemic damage.

427.6

DOPAMINE RELEASE AND CYTOSOLIC FREE CALCIUM DURING HYPOXIA IN PC12 CELLS. G.E. Gibson, L. Toral-Barza* and B. Tofel-Grehl*. Burke Med. Res. Inst. Cornell Univ. Med. Coll. White Plains, NY 10605

Calcium and neurotransmitter release have been implicated in hypoxia/ischemia-induced damage. To define the molecular basis of hypoxia-induced alterations in a cellular system, viability, ATP, cytosolic free calcium ($[Ca^{2+}]_i$) and dopamine release were examined during early stages (5 minutes) of chemical hypoxia (i.e. KCN) in PC12 cells. High concentrations of KCN (0.5 mM) significantly altered these variables only when glucose was omitted from the media. The response of these same factors to a range of KCN concentrations (0.005, 0.05 or 0.5 mM) was then examined in the absence of glucose. ATP levels did not change with 0.005 mM-KCN, but declined by 57% and 84% of control at the two higher concentrations. K^+ depolarization did not alter ATP further in any group. Similarly, $[Ca^{2+}]_i$ was unchanged at the 0.005 mM-KCN, but increased 2- and 5-fold of the control at the two higher concentrations. Depolarization increased $[Ca^{2+}]_i$ and the values in the hypoxic cells after depolarization were elevated. The increasing KCN concentrations elevated dopamine in the media under resting conditions (43, 134 and 296% of control) and after K^+-stimulation (5, 52 and 81 % of the K^+ stimulated control). PC12 cells are a convenient cellular system in which to establish the mechanism by which hypoxia alters dopamine release.

427.7

PERSISTENT DECREASE IN HIPPOCAMPAL NOREPINEPHRINE RELEASE ASSOCIATED WITH TRANSIENT FOREBRAIN ISCHEMIA. K.U. Frerichs, L. Näveri*, K. Xu*, J.M. Hallenbeck*, G. Feuerstein, J.N. Davis, and A.L. Sirén. Dept. Neurology, USUHS, Bethesda; Neurology Res. Lab., Durham VA, Duke University, Durham; SK&B Labs., King of Prussia.

Central noradrenergic neurons may modulate neuronal death in cerebral ischemia. Others have shown an increased release of norepinephrine (NE) during ischemia, but little is known about chronic postischemic changes. We measured extracellular NE in the hippocampus using microdialysis and HPLC for up to 48hours after 20min of forebrain ischemia (FI). FI was induced by four vessel occlusion in halothane-anesthetized rats. Baseline NE was 3.6±1.7pg/20µl (flow rate 1µl/min;n=12). During ischemia, there was a massive release of NE (90.8±10.5pg/20µl; n=6,$p<0.01$) followed by a gradual decline of NE to preischemic levels over 40min of reperfusion. Thereafter (8,24, and 48hours), NE was undetectable in the dialysate (limit of detection 1.0pg/20µl). In sham-operated rats, dialysate-NE was detected at baseline concentrations at all time points (2.3±0.4pg/20µl;n=6). These findings indicate an early and long-lasting depression of the noradrenergic system in the hippocampus, which coincides with the development of delayed neuronal death in this brain region. Therapeutic interventions aimed at maintaining sufficient extracellular NE levels may constitute an approach to protection from delayed neuronal death following global cerebral ischemia.

427.8

AMELIORATION OF TEMPORARY FOCAL CEREBRAL ISCHEMIC BRAIN INJURY IN TRANSGENEC MICE OVEREXPRESSING HUMAN SUPEROXIDE DISMUTASE Yang GY*, Epstein CJ, Chen SF, Weinstein PR, Chan PH. Depts. of Neurosurgery, Neurology, and Pediatrics. UC San Francisco, CA 94143

Oxygen free radicals have been implicated in the pathogenesis of brain edema and neuronal cell death in cerebral ischemia. Our previous studies have demonstrated that liposome-entrapped SOD significantly reduced the infarct size after middle cerebral artery occlusion(MCAO) in rats. We have confirmed that infarction volume in transgenic mice, which are genetically modified to overexpress human CuZn-SOD (h-SOD-1), was significantly reduced following permanent MCAO($P<0.05$). The purpose of present study was to determine the effect of temporary MCAO and reperfusion on transgenic mice as compared to normal diploid mice. Transgenic mice carrying the h-SOD-1 gene were produced as described by Epstein et al(1987). SOD-1 activity was identified by native gel electrophoresis followed by staining with nitroblue tetrazolium. All mice weighing 30±5 grams were anesthetized with chloral hydrate (350 mg/kg IP). The MCA was occluded by introducing a 5-0 rounded tip nylon suture into the left internal carotid artery 11 mm past the site of the common carotid bifurcation. Reperfusion was performed by withdrawal of the suture. All mice received 3 hrs MCAO followed by 3 hrs reperfusion. Infarction area, measured by image analysis after 2,3,5-triphenyltetrazolium chloride staining, was smaller in transgenic mice: transgenic/nontransgenic, (mean% of whole brain area at 3, 5, 7, and 9 mm distal to the frontal pole) 13.8/21.9, 23.1/28.6, 19.0/23.4, and 6.9/17.4, respectively. The neurological deficit scale (0-4) in transgenic mice was lower than that in nontransgenic mice($p<0.05$). Our results suggest that superoxide radicals play an important role in the pathogenesis of ischemic infarction in temporary MCAO and reperfusion. (Supported by AG08938, NS-14543, NS-25372, and NS-22022).

427.9

NEUROPROTECTIVE EFFECTS OF GM1 TREATMENT FOLLOWING CNS ISCHEMIA INVOLVE RESTORATION OF CELLULAR MEMBRANE LIPID METABOLISM AND DEFENSE AGAINST OXY-RADICAL TOXICITY.

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Several studies using both *in vivo* & *in vitro* models of CNS ischemia have shown that GM1 treatment reduces intracellular ionic imbalances & eventual cell death thereby indicating a protective & restorative effect on cellular plasma membrane structure and function (1,2). The precise mechanism of GM1's efficacy is unclear. Ischemia initiates membrane fatty acid release. Following reperfusion, the metabolism of released fatty acids together with increased cellular metabolism generate oxy-radicals which cause additional membrane damage by lipid peroxidation, inactivate vital cellular enzymes & the expression of certain vital genes. Using a model of focal cortical ischemia in rat (1) we find that *acute* GM1 ganglioside treatment (10 mg/kg, at 1,24&48hrs after ischemia) restored membrane lipid metabolism (increased fatty acid acylation) in peri-ischemic areas & partially restored metabolism in the primary ischemic area *at two weeks after the ischemic episode*. There was also an increase in the *capacity* of the oxy-radical defense system evidenced by an *additional* increase in the already elevated levels of superoxide dismutase, glutathione peroxidase & catalase. This increase by GM1 was sustained for up to 6wks. We hypothesize that the neuroprotective effects of GM1 are a result of its effect on those processes which restore and maintain plasma membrane fatty acid composition and distribution.

This work was supported in part by a grant from NINDS NS-25856 and a research grant from the FIDIA Research Foundation.

1. Karpiak et al. Temporal changes in edema, Na^+, K^+ & Ca^{++} in focal cortical stroke: GM1 ganglioside reduces ischemic injury. J Neurosci Res in press.

2. Mahadik et al. GM1 protects plasma membrane structure and function following global ischemia: changes in membrane fatty acids and ATPase. J Neurosci Res 24:402-412 (1989).

427.10

POST-ISCHEMIC DETECTION OF HYDROXYL RADICALS IN GERBIL BRAIN: ATTENUATION BY THE 21-AMINOSTEROID TIRILAZAD MESYLATE (U-74006F).

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During ischemia and particularly upon reperfusion, oxygen free radicals are formed. An important free radical species is the hydroxyl radical OH•. Salicylate can be used as a marker for the production of OH• as salicylate reacts with it to form 2,3- and 2,5-dihydroxy benzoic acid (DHBA). To investigate whether pretreatment with the 21-aminosteroid antioxidant U-74006F can reduce brain post-ischemic formation of OH•, as measured by DHBA formation, gerbils were given i.p. doses of either vehicle or U-74006F (10 mg/kg) ten min prior to bilateral carotid occlusion (BCO). Following the 10 min BCO, gerbils were allowed reperfusion times of 1, 5 and 15 min. Salicylate was administered i.p. 10 min before sacrificing the animals. Brain hemisections, hippocampus or cortex were removed and immediately frozen. Tissues were homogenized in ethanol (1% ascorbate) and analyzed by HPLC. Data were expressed as the ratio of DHBA's to salicylate measured. Results indicated the 2,5-DHBA/salicylate ratio was indeed increased at 1 min reperfusion; pretreatment with U-74006F attenuated DHBA production at all time points.

427.11

CHANGES IN PROTEIN PHOSPHORYLATION INDUCED BY SPINAL CORD ISCHEMIA.

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Protein phosphorylation, an important ATP-dependent process that may be altered during ischemia, is a molecular mechanism by which extracellular signals regulate many intracellular functions. Previously the activities of protein kinase C and the Ca^{2+}/calmodulin-dependent kinase were demonstrated to be reduced in the rabbit spinal cord ischemia model (RSCIM). To expand these studies we have taken two approaches to assay changes in (1) protein tyrosine kinases or (2) serine/threonine kinases. For both studies, cytosolic and particultate fractions of spinal cord homogenates from animals subjected to 0-60 min of ischemic insult were separated by SDS-PAGE and transferred to Immobilon filters. To analyze changes in protein tyrosine phosphorylation , which is one of the earliest metabolic changes observed after perturbation of cells with various agents resulting in either cell growth or death, filters were immunoblotted with affinity-purified rabbit anti-phosphotyrosine antibodies. This type of analysis revealed changes in tyrosine phosphorylation of at least 6 proteins in the M_r range of 20-120K detectable after 10-60 min of ischemia. To assay total serine/threonine kinase activities, the proteins bound to filters were denatured with guanidine-HCl and then allowed to renature slowly. The filters were incubated with [^{32}P] gamma-ATP to reveal kinase activities. In the RSCIM, this type of analysis revealed two interesting changes. First, a kinase of M_r= 80K was transiently detected after 10 or 25 min of ischemia but was absent after 30-60 min of insult. Second, a kinase of M_r= 55K was detected in the 0-25 min samples, but was not detected at later times. The time course of these changes suggests that these kinases could play a role in the ischemic-induced cell death.

427.12

THE RELATIONSHIP BETWEEN EXTRACELLULAR GLUTAMATE AND GLUCOSE UTILIZATION IN FOCAL CEREBRAL ISCHEMIA.

SH Graham, J Chen*, and RA Swanson. Department of Neurology, University of California, San Francisco, 94121.

The phenomena of increased glucose utilization in the border zone of infarcts in rat focal cerebral ischemia has been attributed to the effects of tissue hypoxia. Anaerobic metabolism is less efficient than aerobic metabolism and thus requires more glucose to meet cellular energy requirements. An alternative mechanism is that the release of the excitatory amino acids may contribute to increased glucose utilization in focal cerebral ischemia. We first studied the effect of glutamate upon glucose metabolism *in vitro* using primary neuronal-glial cocultures. Glucose utilization was determined by 2-deoxy-glucose (2DG) method. Addition of glutamate to the medium significantly increased glucose utilization in a dose dependent fashion at 100 and 500μM. The relationship between glucose utilization and glutamate release was also studied *in vivo* in the rat. Glucose utilization in the parietal cortex was measured at 5 minutes, 1.5 hrs., and 4 hrs. after middle cerebral artery (MCA) occlusion. Extracellular glutamate changes in this border zone region were monitored by use of microdialysis. 2DG uptake and microdialysate glutamate both peaked at 1.5 hrs. after MCA occlusion. These results suggest that glutamate release may contribute to increased glucose utilization in focal cerebral ischemia.

427.13

FRUCTOSE-1,6-BISPHOSPHATE (FBP) INCREASES CO_2 PRODUCTION BY THE PENTOSE PHOSPHATE PATHWAY (PPP).

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Fructose-1,6-bisphosphate (FBP) decreases CNS injury following ischemia/hypoxia. Similar protection occurs in cultured astrocytes. To determine whether this protection is the result of the metabolism of FBP itself or is the result of an FBP-induced increase in glucose (GLC) metabolism, CO_2 production was measured in normoxic, cultured astrocytes with and without 3.5 mM FBP in the culture medium. CO_2 production from [U-^{14}C]-FBP and from [6-^{14}C]-GLC (1.51±0.39 and 1.34±0.16 nmol/mg protein/hr, respectively) was small. However, CO_2 production from [1-^{14}C]-GLC increased from 30.12 ±3.4 to 48.26±5.11 nmol/mg protein/hr when FBP was present in the media. In separate studies, lactate production was shown not to be increase. These studies indicate that the protection afforded by FBP is not the result of FBP being utilized as a substrate. A small amount of FBP is taken up by the cells, which may be sufficient to increase metabolism of GLC through activation of one or more enzymes of the PPP. Alternatively, FBP may act as a Ca^{++} chelator which may protect hypoxic astrocytes from injury.

427.14

EARLY REDUCTIONS IN CYTOCHROME OXIDASE (CO) ACTIVITY PREDICT THE DISTRIBUTION OF ISCHEMIC INJURY IN PERINATAL RODENT BRAIN.

Carrie Nelson*, Faye S. Silverstein. Depts. of Pediatrics and Neurology, University of Michigan, Ann Arbor, MI.

In a model of perinatal stroke, induced by right carotid ligation and 8 % O_2 exposure (2.5-3 h) in 7 day old rats, diffuse hippocampal (HIP) injury, often including dentate gyrus (DG), evolves. We hypothesized that regionally selective disruption of energy metabolism might predispose HIP neurons to irreversible ischemic injury. CO histochemistry accurately estimates mitochondrial respiratory function and neuronal metabolic activity. Using computerized densitometry, we quantitated CO activity in frozen brain sections prepared from lesioned rats killed 1 (n=8) or 24 (n=6) h post-hypoxia. Adjacent sections were processed for CO and for Nissl substance to assess cellular integrity. At 1 h, Nissl staining was normal in 4/8 while 4/8 had very subtle reductions in HIP staining. In contrast, 6/8 showed prominent diffuse ipsilateral suppression of CO activity most marked in HIP (-9 % in striatum, -26 % in CA1, -23% in CA3, -18 % in CA4, -29 % in DG, p=0.0001, 2 way ANOVA). At 24 h, there was a close regional correspondence between loss of Nissl-staining in lesioned tissue and loss of CO activity. In perinatal rodent brain, after an acute hypoxic-ischemic insult, early disruption of CO activity predicts the distribution of irreversible injury. The prominent loss of CO activity in DG parallels the enhanced susceptibility to ischemic injury of this cell population at this developmental stage.

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427.15

EXPRESSION OF MAP2 mRNAs IN RAT AND GERBIL BRAIN AFTER TRANSIENT ISCHEMIA. N. Saito*, K. Kawai*, I. Klatzo and T. S. Nowak, Jr. Lab. of Neuropathol. and Neuroanat. Sci., NINDS, NIH, Bethesda, MD 20892

Levels of mRNAs encoding microtubule-associated proteins MAP2b and MAP2c were evaluated in rat and gerbil brain by in situ hybridization with oligonucleotide probes based on known rat sequences. Rats were subjected to 10 min cardiac arrest produced by compression of major thoracic vessels, under anesthesia with 2% halothane in 70% N_20, 30% O_2. Gerbils were subjected to 5 min bilateral carotid artery occlusion under the same anesthesia. Hybridization was carried out on 15 μm frozen sections using ^{35}S-labeled probes, and detected by film autoradiography. In the gerbil, significant reductions in MAP2b hybridization were detected in hippocampal CA1 neurons by 12 h recirculation, falling by approximately 50% at 2 days. The fall in MAP2b mRNA thus preceeded both the fall in immunoreactive MAP2b and the eventual cell loss in this region. A similar depletion of MAP2b mRNA in CA1 neurons was observed in the rat model. Preliminary results demonstrate a reciprocal change in MAP2c hybridization in rat hippocampus, with a striking increase in CA1 neurons at 24 h. MAP2c hybridization was not detected in the gerbil with the available probe. Induction of mRNA encoding MAP2c, a truncated form of the protein normally expressed during periods of neuronal plasticity, thus appears to occur selectively in vulnerable neuron populations after ischemia. It remains to be determined whether this increase occurs in dying or surviving cells, and whether functional MAP2c protein can be expressed under conditions of translational impairment known to occur following ischemia.

427.16

ACTIVATION OF PROTEIN KINASE C BY H_20_2 IN UCIIMG ASTROCYTES. W.J. Chiou* and K.L. Leach. Cell Biology, The Upjohn Co., Kalamazoo, MI 49001

Oxidative damage, which involves free radical formation and subsequent lipid peroxidation, plays a significant role in the pathogenesis of tissue injury, including the brain injury induced by hypoxia or trauma, and cardiac injury arising from ischemia and reperfusion. However, the mechanism(s) by which oxidative insult eventually leads to cell death are still unknown. In these studies we have examined the role of the calcium and phospholipid-dependent protein kinase C (PKC), which plays a major role in cellular signal transduction pathways, in oxidative injury. Treatment of UC11MG cells, a human astrocyte cell line, with 0.5 mM H_2O_2 resulted in a 2-3 fold increase in malondialdehyde levels within 30 min. PKC activation following oxidative injury was assessed by examining the phosphorylation of an endogenous protein, the 80 kD protein, which is a specific substrate for PKC. Incubation of UC11MG cells with 0.5 mM H_2O_2 for 30 min resulted in a 4-6 fold increase in the phosphorylation of the 80 kD protein. The increase in phosphorylation was apparent within 10 min following treatment, and was maintained for up to 60 min. The H_2O_2-stimulated increase in 80 kD phosphorylation was inhibited by treatment of the cells with either 1 μM staurosporine or 100 μM H-7, two PKC inhibitors. In addition, in cells in which PKC was down-modulated by pretreatment with 200 nM phorbol 12-myristate 13-acetate for 48-72 hrs, H_2O_2-induced 80 kD phosphorylation was markedly reduced. These results suggest that protein kinase C may be one of the early events activated in the cellular response to oxidative injury.

427.17

TRANSLOCATION OF Ca^{2+}/CALMODULIN-DEPENDENT PROTEIN KINASE II (CaM-KII) AFTER FOREBRAIN ISCHEMIA. J.ARONOWSKI*, J.C.GROTTA* AND N.WAXHAM. (SPON: N. WAXHAM). Depts.of Neurobiology and Anatomy and Neurology, Univ. of Texas Medical School, Houston, TX 77225

Transient forebrain ischemia induced by four vessel occlusion in rats, resulted in depressed activity of both CaM-KII and Ca^{2+}/phospholipid-dependent kinase while no changes were detected in the activity of cAMP-dependent kinase, as measured in both cerebral cortex and hippocampus. The decrease in CaM-KII activity, measured by phosphorylation of synthetic peptide substrate, coincided with translocation of the enzyme from the cytosol to the particulate fraction, determined using a CaM-KII specific monoclonal antibody followed by an ^{125}I-labeled secondary antibody. The inactivation and translocation of CaM-KII was fully reversible following 2 hr of reperfusion of animals exposed to 5 min of ischemia. In contrast, the translocation and inactivation of CaM-KII following 20 min of ischemia was not completely reversible with reperfusion. The mechanism(s) responsible for down regulation and/or translocation of CaM-KII is unknown. Phosphorylation due to increasing intracellular Ca^{2+} following ischemia is one possible step in this pathway. In preliminary experiments to test this hypothesis, we phosphorylated crude homogenates of rat cerebral cortex and examined changes in the distribution of CaM-KII between cytosolic and particulate fraction. A time dependent decrease occured in the amount of CaM-KII enzyme in the cytosol after the addition of Ca^{2+} and calmodulin. This process was partially blocked by specific inhibitors of CaM-KII and the phosphatase inhibitor okadaic acid.

427.18

DISAPPEARANCE OF BRAIN Ca^{2+}/CALMODULIN-DEPENDENT PROTEIN KINASE II AFTER BRIEF ISCHEMIA.

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Several studies have suggested that ischemia produces neuronal cell death by the elevation of intracellular calcium which activates calpain, resulting in extensive proteolysis. In this paper, we examined the effects of forebrain ischemia in gerbils in Ca^{2+}/calmodulin-dependent protein kinase II (CaM-kinase II) by both kinase activity assays and Western blot analysis. In cortex and hippocampus, cytosolic CaM-kinase II was completely lost within 2-5 min of ischemia. Particulate CaM-kinase II was more stable and decreased about 40% after 10 min of ischemia followed by 2 hrs reperfusion. CaM-kinase II in cerebellum, which does not become ischemic, was not affected. The rapid loss of forebrain CaM-kinase II was quite specific since hippocampal cytosolic cAMP-kinase and protein kinase C were not affected. These data strongly suggest that very brief ischemia causes immediate proteolysis of soluble CaM-kinase II which would be deleterious since CaM-kinase II modulates numerous neuronal cell functions.

427.19

The effect of nerve growth factor on the delayed neuronal death after ischemia. K. Tanaka, Y. Yonekawa, T. Tsukahara, N. Ogata, Y. Kaku, T. Kimiura[1], T. Taniguchi[1], O. Ohara[2]. Department of Neurosurgery, National Cardiovascular Center, Suita, Osaka, Japan. Department of Neurobiology, Kyoto Pharmaceutical University[1], Shionogi Research Laboratories[2].

Protective action of nerve growth factor (NGF) against delayed neuronal death (DND) and the involvement of neurofilament (NF) and microtubulin associated protein 2 (MAP2) in its protective mechanism of DND were investigated. Wister rats were divided into following three groups; Group 1; 8 min transient forebrain ischemia, Group 2; intraventricular administration of artificial cerebrospinal fluid (CSF) and the ischemia. Group 3; intraventricular administration of 2 μg of 2.5S NGF and the ischemia. Hippocampal neuronal death was examined on 1st and 7th day after the ischemia. The histology was confirmed by HE staining and by immunohistochemical staining with anti-NF200 and anti-MAP2 antibody. Quantitative measurement of NF and MAP2 in the hippocampus was also analyzed by western blotting method. Mean cell survive, NF and MAP2 content in the rat hippocampus were as follows.

	Cell survive		NF		MAP2	
	day 1	day7	day 1	day 7	day 1	day 7
Group 1	100%	15%	59%	47%	63%	55%
Group 2	100%	40%	63%	60%	78%	104%
Group 3	100%	67%	81%	63%	90%	110%

These results suggest that NGF has a protective effect on rat hippocampal DND and that its effect may be mediated by the restoration of the neuronal cytoskeletal proteins.

427.20

NEURONAL EDEMA MAY BE A CAUSE OF SPREADING DEPRESSION. M. Balestrino*, S. Mazzari**, A. Leon**
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In vitro rat hippocampal slices were equilibrated with artificial cerebrospinal fluid (ACSF) containing 100 mM mannitol. Ouabain (100 uM) was then added to the mannitol-containing ACSF to provoke a spreading-depression (SD) - like depolarization (Neurosci. Abs. 16:277, 1990). Ouabain was perfused for as long as 30 minutes, and no SD-like event was observed. Moreover, population spike (PS) in the CA1 region was still present at the end of this time. By contrast, in separate experiments neither APV (50 uM) nor kynurenic acid (100 uM) prevented ouabain-induced SD-like depolarization and the consequent disappearance of PS.

In other experiments, perfusion with ACSF that was made severely hyposmotic by decreasing NaCl concentration to either 50% or 25% of normal caused, by itself, an SD-like depolarization in 1 out of 3 (33%) and in 8 out of 11 (73%) slices, respectively. If the same, low-NaCl ACSF was made iso-osmotic with addition of the impermeant sugar saccarose, SD-like depolarization was prevented.

Thus, neuronal swelling may be a determinant of SD.

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SYMPOSIUM: HUMAN EYE SACCADES - SENSORY AND CORTICAL FACTORS. P.E. Hallett, Univ. of Toronto (Chairperson); H. Deubel*, Max Plank Institut Fur Psych.; L.M. Optican, NIH; M.E. Goldberg, NIH; R.A. Andersen, MIT.

Humans explore their world with saccadic eye movements. Saccades are used in orienting, looking, searching, tracking, and perhaps dreaming. The processing for saccadic eye movements includes analysis of what is on the retina, where those targets are in space, the cognitive decisions of choosing when and where to make a saccade, and the motor programming to move the eyes effectively. The oculomotor system is accessible to psychophysical, physiological, and theoretical study, and we will discuss the concepts and controversies that these different approaches have yielded. This symposium will not provide answers, but it will illuminate the problems in appreciating the synergy and interactions of the neural mechanism of this, the best understood sensorimotor system in man. **Hallett** will give an introduction. **Deubel** will talk about "The sensory stimulus". **Optican** will discuss "The superior colliculus and sensorimotor transformation". **Goldberg** will speak about "Cortical mechanisms for spatial accuracy" **Andersen** will conclude with "Coordinate transformations in posterior parietal cortex".

430

SYMPOSIUM: ACTIVITY-DEPENDENT PLASTICITY; ANALYSIS AT THE CELL AND MOLECULAR LEVEL. P.G. Nelson, NICHD, NIH (Chairperson); U. Rutishauser, Case Western Reserve Univ.; R. Zigmond, Case Western Reserve Univ.; and M.E. Greenberg*, Harvard Medical School.

The nervous system can alter its biochemistry and architecture in response to levels and types of synaptic activity. This remarkable interplay between function and structure, often referred to as plasticity, has long been recognized as fundamental for neural development, physiology and repair. For the most part, plasticity has been appreciated at the tissue level, but in recent years the cellular and molecular basis of plasticity has become increasingly defined. It is now clear that activity can influence all levels of cellular function from the genome to the plasma membrane. This symposium, which features four very different manifestations of activity-dependent plasticity within or on the cell, is designed both to illustrate the rapid progress being made in the field and to make neural plasticity a more palpable concept to the cell and molecular biologist. The first speaker, Phillip Nelson, will describe the effects of electrical stimulation on neurite outgrowth and synaptic efficacy, using spinal and sensory ganglion neurons grown in culture. The theme of neurite behavior will be further explored by Urs Rutishauser, in studies showing that activity-dependent sprouting of motor axons *in vivo* involves regulation of adhesion molecules via enhanced expression of an unusual cell surface carbohydrate. Richard Zigmond will discuss the mechanism by which changes in nerve activity alter catecholamine biosynthesis and neuropeptide levels in adult sympathetic neurons. To close, Michael Greenberg will illustrate the ability of neurotransmitters and depolarization to influence the expression of immediate early genes such as *c-fos*.

VISUAL CORTEX: ARCHITECTURE AND INTERACTIONS

431.1

The Organization of Cytochrome-Oxidase Blobs in Cat Visual Cortex. K.M. Murphy[1], R.C. Van Sluyters[2] & D.G. Jones[3]*. Departments of Psychology[1] and Electrical Engineering[3], McGill University, Montreal PQ H3A 1B1, School of Optometry[2], University of California, Berkeley, CA 94720.

The primary visual cortex is parcellated into modules that have been defined using both physiological and anatomical techniques. Quite possibly the most striking demonstration of cortical modularity is that of the blobs of higher cytochrome-oxidase activity in the supragranular layers of the visual cortex. Previously, cytochrome blobs had only been demonstrated in primate visual cortex which led to the supposition that they were a unique feature of the organization of the visual system in this species. However, recently we demonstrated that cytochrome blobs are also present in the supragranular layers of cat primary visual cortex. Using the technique of unfolding and flattening the entire cat visual cortex, patterns arrayed tangential to the cortical surface, such as the cytochrome blobs, are most easily appreciated. Examination of such sections taken through supragranular layers of cat visual cortex and stained for cytochrome-oxidase reveals marked periodicity throughout the entire extent of area 17. The blobs of higher cytochrome-oxidase activity in cat visual cortex appear qualitatively similar to those observed in the primate.

Cytochrome blobs in primate cortex have been related to the organization of ocular dominance, and we have examined this relationship in cat primary visual cortex. As in the monkey, cytochrome blobs in cat visual cortex overlie both right- and left-eye ocular dominance columns. However, whereas cytochrome blobs in the monkey are aligned with the centers of ocular dominance columns, in the cat this relationship is less clear-cut. Analysis of the relationship between cytochrome blobs and ocular dominance columns is complicated by two factors in the cat. The ocular dominance columns in layer IV of the cat are not completely segregated, and their overall pattern consists of a series of irregularly branching, beaded bands. In an effort to overcome these problems, we have begun to study the arrangement of cytochrome blobs and transneuronally labelled ocular dominance columns in monocularly enucleated cats.

431.2

ROLE OF GABAergic ACTIVITY IN GENERATION OF UPPER LAYER OCULAR DOMINANCE COLUMNS IN MONKEY STRIATE CORTEX. Michael M. Haglund and Gary G. Blasdel, Dept. Neurobiology, Harvard Medical Sch., Boston, MA 02115

Using video imaging of voltage-sensitive dyes, we have shown previously that low doses of the $GABA_A$ blocker, bicuculline (BMI), disrupt images of ocular dominance columns in the upper layers (Neurosci. Abstr. 15:799, 1989). Even though the doses of BMI were below that which triggered seizure activity and the ocular dominance columns were clearly gone, independent confirmation that the cortex is still functional was lacking.

We have now shown that during video imaging of monkey striate cortex in low doses of BMI (10um) when ocular dominance columns are gone that retinotopic maps are still present. Using optical techniques (Blasdel and Salama, 1986), images of ocular dominance columns and retinotopic maps were obtained in 3 monkeys. BMI (10um) was then superfused onto the cortex and alternating ocular dominance columns and retinotopic maps were obtained. During the time when the ocular dominance columns were gone, the retinotopic maps were still present.

Microelectrode recordings were also performed in the presence of BMI to determine the single neuronal correlate of the ocular dominance column disruption. Vertical penetrations were made in the upper 500um of striate cortex. After identifying a region with primarily monocular cells, firing frequency histograms were obtained comparing the right and left eye. With low doses of BMI (10um), monocular cells consistently shifted toward binocular. In the presence of BMI, single IV boluses of thiopental (2.5-5.0 mg/kg) (potentiates GABA activity) shifted binocular cells back to monocular.

These findings suggest that $GABA_A$ activity is important in the generation of upper layer ocular dominance columns and that these inhibitory pathways are involved in sharpening individual cell ocular dominance preferences.

(Suppt. by Klingenstein, Grass, and McKnight Foundations).

431.3

ORIENTATION PREFERENCE, CONTINUITY AND DENSITY IN MONKEY STRIATE CORTEX. Gary G. Blasdel, Department of Neurobiology, Harvard Medical School, Boston, MA 02115.

Previous work (Blasdel and Salama, 1986) has demonstrated the usefulness of differential video imaging in obtaining maps of ocular dominance and orientation in monkey striate cortex. It has also shown that orientation preferences change continuously and linearly in most areas of cortex, but only in patches 0.5-1.0 mm across that are bounded semi-periodically by *fractures* and *singularities* where preferences change discontinuously. Recent measurements show that these discontinuous changes are 55% more abundant in the centers of ocular dominance columns than they are at the edges, and 46% more abundant in regions of intense cytochrome oxidase activity.

Fractures, which occur one dimensionally (as lines), are themselves disrupted by Singularities, which occur zero dimensionally (as points). The latter arise in the centers of two dimensional vortices, where orientation preferences cycle continuously through ±180° in one revolution and thereby ensure two things: 1) that preferences for all orientations are represented compactly, within the smallest possible areas, and 2) that perpendicular orientations are always preferred on opposite sides. Since dendritic arborizations appear relatively constant, extending in most cases 250-350 μm across (Lund and Yoshioka, 1991), the radically different densities of orientation preference (in the continuous and discontinuous zones), suggest large variations in the range available to different cells. Those in the continuous zones should receive precise information about a few orientations while those near singularities should receive imprecise information about all orientations - differences that may underlie separate perceptual tasks.

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431.4

SPATIAL INTER-RELATIONSHIPS OF FUNCTIONAL DOMAINS IN PRIMARY VISUAL CORTEX. Eyal Bartfeld, Dov Malonek, D. T'so and Amiram Grinvald. Dept. of Neurobiology, Weizmann Institute, Rehovot Israel, and Laboratory of Neurobiology, The Rockefeller University.

Detailed mapping of the visual cortex has revealed that cells with similar receptive field properties are clustered together and arranged in columnar organization (Hubel and Wiesel). We investigated the spatial relationships of ocular dominance, orientation columns and blobs by optical imaging in monkey V1. The optically derived information was correlated with cytochrome oxidase histology of the same area.

Recent improvements in intrinsic signal imaging has facilitated high resolution functional mapping, providing additional details. We studied from the same portion of visual cortex, activity maps evoked by several different visual stimuli. First we computed ocular dominance map, and then orientation specif'city map. The most prominent feature was a precise radial pin-wheel like architecture of orientation selectivity (Swindle et al.). This arrangement closely resembled the organization of orientation selective regions, around orientation centers, in cat area 18 (Bonhoeffer & Grinvald) and 17. The orientation centers were often found in the middle of ocular dominance columns. We found that in a patch of 4.5 by 3 mm of cortex there were 72 orientation centers and 62 blobs.

We are currently investigating the relationship between the orientation centers and the blobs.

Supported by IBM and The Riklis Foundation.

431.5

LINKING SENSORIAL MAPS IN THE VISUAL CORTEX

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Orientation specificity in the visual cortex is organized in an ordered manner and changes gradually so that adjacent cells have (on average) similar preferred orientations. The preferred orientations form an interlaced and curved structure and at several points (singularities) all orientations meet. The *average* orientation tuning strength is low at such singularities. This local coupling suggests that preferred orientation and orientation tuning strength might also be linked globally both being a function of the cortical surface coordinates. We demonstrate that such a link actually exists and that it can be described by a simple analytical expression. In good approximation, the map of the orientation tuning strength is obtained from the map of the preferred orientations by Fourier transformation methods. We found good correspondence between these computed maps and experimentally measured maps obtained from optical imaging of intrinsic signals (Bonhoeffer & Grinvald, Neurosci. Abs., 1990 and unpubl. data). This supports the view that a common design principle underlies the maps for preferred orientations and orientation tuning strengths and we expect this result to facilitate progress towards a more unified theory of the development of visual cortex. We thank Drs. Grinvald & Bonhoeffer for sharing with us their unpublished data in digital form.

431.6

OPTICAL IMAGING REVEALS PREFERENTIAL LABELING OF CYTOCHROME OXIDASE-RICH REGIONS IN RESPONSE TO COLOR STIMULI IN AREAS V1 AND V2 OF MACAQUE MONKEY.

C.E. Landisman, A. Grinvald, D.Y. Ts'o. Laboratory of Neurobiology, The Rockefeller University, NY, NY, 10021 and The Weizmann Institute, Rehovot, Israel.

In vivo optical imaging of the cytochrome oxidase blobs using the intrinsic (no dye) optical signals has previously been performed by localizing the centers of monocularity, during stimulation with a moving luminance grating. We now report that optical imaging maps have demonstrated cortical regions that yield a notably stronger response to low spatial frequency isoluminant chromatic gratings than to the luminance gratings. These patches of strong response to chromatic gratings coincide with the cytochrome-oxidase blobs. Results suggest, however, that the segregation of responses is not complete. There are residual responses of the interblob regions to the chromatic stimuli and of the blobs to luminance stimuli. Indeed with very low spatial frequency stimuli, striate cortex in general was more responsive to chromatic than luminance stimuli. In V2, isoluminant color stimuli preferentially labeled color-selective subcompartments of the stripes.

Optical imaging during standing versus flashing visual stimuli has also shown preferential labeling in V1 and V2. Preliminary results suggest that interblob regions and the thick stripes are less responsive to standing stimuli than flashing stimuli. Using our ability to localize the blobs *in vivo* with optical imaging, we have begun to target specific blobs with electrical recordings to explore the relationship between the optical responses to color stimuli and the electrophysiological assessment of receptive field properties. (Supported by EY08240 and the Whitaker Foundation).

431.7

ORIENTATION SELECTIVITY OF AND INTERACTIONS BETWEEN COLOR AND DISPARITY SUBCOMPARTMENTS IN AREA V2 OF MACAQUE MONKEY.

D.Y. Ts'o, C.D. Gilbert, T.N. Wiesel. Laboratory of Neurobiology, The Rockefeller University, NY, NY 10021.

By combining optical imaging maps of V2 with electrophysiology, we have studied the relationship between the stripes that contain disparity sensitive cells and stripes that contain color selective cells. Both cytochrome oxidase histology and the optical imaging maps have suggested that disparity compartments at times lie directly adjacent to or merge with color compartments, all within a single histologically-defined "stripe". Systematic electrode penetrations across these regions reveal a gradual progression of receptive field properties, from cells with sharp disparity tuning and no color selectivity, to cells having both disparity tuning and color selectivity, to color selective cells with no disparity tuning. After injections of retrograde tracers in pure color or disparity compartments, label is found in border regions containing color disparity cells, up to 6mm away.

Within the disparity zones, cells tended to have vertical or near vertical orientation preference. This result was most pronounced among cells with the sharpest disparity tuning, which were also unresponsive to monocular stimulation. In general, we found two types of regions in V2 containing oriented cells: one in which orientation was organized in a columnar fashion, where the preferred orientation was very uniform and altered very little both for the length of a vertical penetration and for neighboring penetrations. A second type of region of oriented cells exhibited no columnar organization for orientation, but instead the preferred oriented changed rapidly not only for neighboring penetrations, but also for neighboring cells within a single vertical penetration. These two types of organization for orientation were reflected in the optical imaging orientation maps. (Supported by grants EY07968, EY05253, EY08240 and the Whitaker and Rita Allen Foundations).

431.8

CHARACTERIZATION OF DYNAMIC PATTERNS OF CORTICAL ACTIVITY BY A SMALL NUMBER OF PRINCIPAL COMPONENTS.

D. Shoham, S. Ullman and A. Grinvald. Weizmann Institute, Rockefeller University and MIT.

In previous years our group (Arieli et al 88, 90) has used real-time optical imaging to study the spatio-temporal organization of the activity of large neuronal populations in cat visual cortex. The activity of a 3x3*mm* patch of cortex was imaged by an array of 120 detectors. We have observed complex coherent patterns which scanned the cortex at several frequencies. The results were presented as 'brain wave movies'. While these movies appear quite irregular and complex, a closer inspection reveals several recurring spatial patterns.

Here we attempt to measure the level of complexity inherent in those cortical waves by using Principal Component Analysis. In this method the given set of patterns is used to define a base of orthogonal patterns (principal components), which under a specific mathematical criterion is optimal for representing all the data. Each of the original patterns is decomposed into a weighted sum of the principal components. While the complete base of principal components is needed to fully represent the data, a small subset may give a good approximation in some cases where the data exhibit high regularity.

We applied this method to the brain wave movies, using all the movie frames as the data set. Surprisingly, we found that as few as 3-10 principal components (out of 120) were sufficient to approximate an entire experimental movie with an accuracy of 80-90%. An examination of the weights of the different components as a function of time revealed that the weight of the first component is proportional to the temporal signal averaged over detectors. We compared the components obtained under different stimulus conditions and found a high degree of similarity. These results suggest that further analyses might be simplified by replacing the data by the first few components. More importantly, they seem to point to a basic low-dimensionality underlying cortical activity. [Supported by The Riklis Family Foundation.]

431.9

INTRACORTICAL INTERACTIONS IN CAT VISUAL CORTEX: EVIDENCE FROM POSTSYNAPTIC FIELD POTENTIALS.

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The extensive lateral spread of the dendritic and axonal arborization of cortical neurons is not reflected in the functional organization of single-unit receptive fields which is retinotopic and columnar. We have found local postsynaptic potentials which reflect long-range lateral connectivity.

We compared extracellular single-unit responses and local field potentials in area 17 of the cat using a pair of microelectrodes (tip separation, ~300μm). Single-unit receptive fields were plotted with oriented light bars. Field potentials were elicited using grating reversal at 61 5-deg patches covering the visual field. The temporal modulation of the gratings was pseudorandom. In one experiment only one patch was stimulated at a time, while in another, all patches were stimulated simultaneously but independently.

The two modes of stimulation produced drastically different local responses.With single-patch stimulation two of postsynaptic components with different receptive-field properties were obtained: a fast retinotopic component and a slow non-retinotopic component with a very large receptive field (30 deg across). With all-patch stimulation only the fast component was present. This indicates that the slow component is subject to strong lateral inhibition. These findings suggest the presence of long-range lateral interactions of visual afferents at dendrites within visual cortex.

(USPHS Grants BRSG-05981, Core Grant EY-06883)

431.10

EXPERIENCE-DEPENDENT INTRACORTICAL INTERACTIONS IN CAT VISUAL CORTEX: EVIDENCE FROM POSTSYNAPTIC FIELD POTENTIALS.

M. Kitano, T. Kasamatsu, A. M. Norcia* and E.E. Sutter.
Smith-Kettlewell Eye Res. Inst., San Francisco, CA 94115.

Cortical neurons in area 17 receive intracortical lateral afferents from a large cortical area. Using bipolar microelectrodes, we have shown the presence of large "receptive fields" for postsynaptic potentials generated within a small cortical mass in response to contrast reversal of gratings (Kitano et al., Invest. Ophthal. Vis. Sci. Suppl., 1991). Using multi-input, non-linear systems analysis, the field potentials generated in normal visual cortex were divided into fast (retinotopic) and slow (non-retinotopic) postsynaptic potentials. The latter had a large "receptive field" and showed strong spatial interactions inside this field.

In cats which had one eye lid-sutured for 4 years from the age of one week, no single units were found in area 17 responding to stimulation of the lid-sutured eye, but field potentials were evoked. These field potentials had only one component whose waveform was similar to the non-retinotopic slow component obtained by stimulation of the normal eye. However, their receptive field was small, retinotopic and did not exhibit spatial interactions. In kittens which had received single-orientation visual experience binocularly, single units in area 17 showed normal receptive-field properties. In contrast, the characteristics of the field potentials were abnormal and essentially the same as those obtained for stimulation of lid-sutured eye. These findings suggest that normal development of extensive lateral interactions in visual cortex is essential for cortical function.

(USPHS Grants BRSG-05981, Core Grant EY-06883)

431.11

SHORT AND LONG TERM CHANGES IN RECEPTIVE FIELD SIZE AND POSITION FOLLOWING FOCAL RETINAL LESIONS.

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The classical view of cortical functional organization is that the receptive field properties of cells are fixed early in life. We have found quite to the contrary that in adult cats and monkeys rather profound shifts in receptive field size and position can be induced by sustained alterations in visual input. Focal retinal lesions at homologous retinotopic loci in the two eyes, destroying the photoreceptor layer but not the cells of the inner retina, were created with a diode laser. This effected a functional "deafferentation" of the cortex, leaving a region of cells that could not be activated by visual stimuli, which we refer to as the "cortical scotoma." The size of the retinal lesion, about 3 to 6° in diameter, was chosen to silence an area of primary visual cortex about 8mm in diameter. Within minutes after making the lesion, cells at the edge of the cortical scotoma had much larger receptive field diameters (by a factor of 2 to 4) than those recorded at the same cortical site before the lesion was made. Over a period of two months following the lesion, the cortical scotoma was entirely filled in, with cells that were originally silenced regaining visual input and shifting their visuotopic representation to retinal loci outside the lesion. For cells in the center of the cortical scotoma, this corresponded to 4mm shifts in the cortical visuotopic map. The shifted fields were orientation selective and remained enlarged relative to their prelesion state. Some cells had bipartite fields, with the subfields lying on either side of the lesion. At a time when the cortical scotoma had filled in, mapping of the LGN revealed a persistent region of unresponsive cells. This suggested that at least part of the topographical reorganization seen in the cortex was due to synaptic changes intrinsic to the cortex. Considering the extent of the alterations in the map, the long range horizontal connections may be responsible for bringing visual information into the scotoma. (Supported by NIH grants EY07968 and EY05253 and by the McKnight and Rita Allen Foundations).

431.12

CONTEXTUAL STIMULI INFLUENCE RECEPTIVE FIELD SIZE OF SINGLE NEURONS IN CAT PRIMARY VISUAL CORTEX.

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Cells in the visual cortex receive input from visual loci outside their receptive fields via intrinsic long range horizontal connections. These connections may mediate the modulation of receptive field properties by contextual stimuli. The effect of the horizontal inputs appears to be amplified by permanent alterations of sensory input, such as those induced by focal retinal lesions, which lead to lasting changes in cortical receptive field size and position. Surprisingly, large increases in receptive field sizes were observed at the edge of the cortical scotoma immediately after the placement of retinal lesions. This led us to test whether receptive field size could be influenced simply by incorporating artifical scotomata into the visual stimulus, and to determine the stimulus characteristics that are most effective for inducing the changes. We first measured the extent of the receptive fields of single cells in the superficial layers of cat primary visual cortex using the minimum response field technique. We then presented an array of moving bars covering a large area of visual field while the receptive field of each recorded cell was masked so that the stimulus would not activate the cell. This conditioning stimulus was interleaved with brief presentations of test stimuli to determine the positions of the receptive field borders. Our preliminary findings revealed that after a few minutes of conditioning, the receptive field diameters of some cells increased two- to three-fold. The effect was reversible: receptive fields could often be reduced to near their original size by presenting the stimulus pattern without the mask, and then expanded again after reintroducing the mask. These results give further evidence for dynamic changes in receptive field properties, depending on context. One may speculate that the balance between excitation and inhibition produced by the horizontal input may be altered by context, resulting in the observed changes. (Supported by NEI grant EY07968, the McKnight Foundation and the National Science Foundation).

CATECHOLAMINE RECEPTORS: DOPAMINE V

432.1

MOLECULAR CLONING AND EXPRESSION OF A NOVEL D_1 DOPAMINE RECEPTOR WITH A UNIQUE DISTRIBUTION IN THE CENTRAL NERVOUS SYSTEM.

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Cloning techniques have now established that five different genes code for dopamine receptor subtypes in the central nervous system, two D_1 receptor subtypes and three D_2 related subtypes. Here we report the cloning of a rat gene coding for a novel D_1 receptor subtype. The rat gene has an intronless open reading frame of 1425 nucleotides which encodes a protein of 475 amino acids. This protein has structural features that are similar to G protein-coupled receptors. In COS-7 cells, the expressed protein binds dopaminergic ligands with a pharmacological profile similar to the previously cloned rat and human D_1 dopamine receptor. In 293 cells, D_1-selective agonists elicite a stimulation of adenylyl cyclase which is blocked by the D_1-selective antagonist SCH23390 but not by D_2- or β-antagonists. The human homologue of this novel gene is localized to the short arm of the chromosome 4 (4p16), the same region as the Huntington's disease gene. Northern analyses revealed that the mRNA for this gene (~3.0 kb) is essentially expressed in hippocampus and hypothalamus. In situ hybridization showed that high levels of mRNA are found in distinct layers of hippocampus, the mammillary nuclei and the anterior pretectal nuclei. In contrast to the previously cloned D_1 receptor, little or no mRNA for this novel receptor was observed in striatum, nucleus accumbens, olfactory tubercle and frontal cortex. The unique distribution of this receptor in restricted regions of the brain suggests a possible role for this receptor in visual and limbic functions. Based on its characteristics we suggest that this new dopamine receptor subtype be referred as the D_{1B}.

432.2

CLONING AND SEQUENCE OF A DOPAMINE RECEPTOR FROM OPOSSUM KIDNEY (OK) CELLS THAT IS EXPRESSED IN BOTH KIDNEY AND BRAIN.

S.R. Nash*, M.G. Fornaretto*, M.D. Bates*, J.R. Raymond*, & M.G. Caron. Dept. of Cell Biology, Duke Univ. Med. Ctr., Durham, NC 27710

In the kidney, dopamine increases renal blood flow through vascular effects and promotes natriuresis and diuresis through tubular effects. Dopamine receptors have been described in the renal cortex by autoradiographic, ligand binding, and second messenger studies; however, both pharmacological and molecular biological evidence suggests that previously cloned human and rat D_1, D_2, and D_3 receptors are not expressed in the kidney. In order to clone a kidney specific dopamine receptor message, we used a probe from the 5' end of the human D_{1a} receptor message coding block to screen a cDNA library made from opossum kidney (OK) cell mRNA. This established epithelial cell line, which expresses DA1 dopamine receptors, has been shown to have biochemical characteristics of proximal tubule cells. A partial cDNA clone containing an open reading frame homologous to the amino terminal half of G-protein coupled receptors was isolated from this library. Specific oligonucleotides were used to obtain the remaining portion by RACE-PCR. The deduced amino acid sequence from the open reading frame of this 1.8 kb message is 89% and 87% identical to the previously characterized human and rat D_1 receptors (D_{1a}) respectively (Dearry *et al. Nature* **347**:72-76 (1990); Zhou *et al. Nature* **347**:76-80 (1990)). The encoded receptor protein contains all the essential hallmarks of catecholamine receptors: glycosylation sites in the extracellular domains, conserved aspartate and serine residues in the 3rd and 5th transmembrane regions, and several consensus phosphorylation sites in intracellular regions. In situ hybridization using a probe derived from the 5' end of the cDNA yielded specific hybridization signals in opossum (*Didelphis virginiana*) renal cortex and various brain regions (e.g. corpus striatum, accumbens, and olfactory tubercle). It will be of interest to determine whether this receptor represents the synonymous D_{1a} sequence in a marsupial species or a new subtype highly homologous to the CNS specific D_{1a} receptor but expressed in both kidney and brain.

432.3

THE D1 DOPAMINE RECEPTOR AND ITS mRNA ARE MODULATED BY AGONIST.

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The recent cloning of the gene for the D1 dopamine receptor has provided a molecular probe for investigating the metabolism of D1 receptor and its mRNA in SK-N-MC human neuroblastoma cells. Northern blots of SK-N-MC cell mRNA probed for dopamine D1 receptor reveal a strongly hybridizing band at 4.2 kilobases. The identity of the mRNA was confirmed by RNAse protection assays using a fragment from the 5' untranslated region of the D1 receptor. Treatment of SK-N-MC cells with 10μM dopamine for varying lengths of time caused a 40% increase in D1 mRNA by one hour followed subsequently by a rapid decline to 70% of untreated levels by 3 hours and a slower decline to 50% by 12 hours. D1 receptor levels as measured by ^{125}I-SCH23982 binding show a slight rise over one hour followed by a decline to 37% of control by 12 hours. The observed increase in D1 mRNA is probably due to increased synthesis, since nuclear runoff experiments show a two-fold increase in D1 mRNA synthesis in nuclei from cells pretreated with dopamine for one hour. D1 mRNA half-life is unchanged in treated cells, further supporting this hypothesis. The responses of D1 receptor and mRNA to agonist are similar to those found for the β2 receptor and the LH/CG receptor, raising the possibility that this is a general phenomenon associated with G-protein coupled receptors that stimulate adenylyl cyclase.

432.4

SHORTER VARIANTS OF THE D_3 DOPAMINE RECEPTOR PRODUCED THROUGH VARIOUS PATTERNS OF ALTERNATIVE SPLICING.

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Using Polymerase Chain Reaction (PCR) amplification of mRNAs from several areas of rat brain we have shown the occurrence of two shorter transcripts of the dopamine D_3 receptor gene, in addition to that corresponding to the D_3 receptor. Cloning and sequencing of these transcripts, together with the establishment of the exon-intron organization and structure of the D_3 receptor gene, shown these transcripts to result from different processes of alternative splicing. The first transcript encodes a 100 amino-acid protein produced by splicing of an exon whose absence deletes the third transmembrane domain and gives rise to a frameshift in the open reading frame. In the second transcript, an in frame 54 bp deletion is produced by splicing occurring at an internal acceptor site, suppressing half of the second extracellular loop and a part of the fifth transmembrane domain. This latter transcript was stably expressed in CHO cells which failed to reveal any dopaminergic ligand binding activity. The functionnal significance and possible role of these shorter variants of the dopamine D_3 receptor in cell signaling and aetiology of psychiatric diseases remains to be established.

432.5

STABLE TRANSFECTION OF CELL LINES WITH DOPAMINE RECEPTORS. T. M. Filtz, B. R. Monks* and P. B. Molinoff. Dept. of Pharmacology, Univ. of Penn. School. of Med., Philadelphia, PA 19104

Classically, dopamine receptors have been divided into two subtypes, D1 receptors linked to stimulation of adenylyl cyclase with high affinity for SCH-23390 and other benzazepines, and D2 receptors linked to inhibition of adenylyl cyclase with high affinity for butyrophenones and other neuroleptics. Six subtypes of dopamine receptors have thus far been cloned and sequenced. D1 and D5 receptor cDNA clones have similar coding sequences, and, when expressed in mammalian cells, have the pharmacological profile and second-messenger linkage expected of D1 receptors in striatal homogenates. $D2_S$, $D2_L$, D3, and D4 receptors are similar in coding sequence and possess the pharmacological properties associated with D2 receptors. Given the multiplicity of dopamine receptors and the complexity of the central nervous system, the pharmacological properties of these receptors are difficult to resolve *in vivo*. Selective expression of subtypes of dopamine receptors in mammalian cell lines permits unambiguous characterization of their properties. The rat D1 receptor, the human $D2_S$ and $D2_L$ receptors, and the rat D3 receptor coding sequences were excised with appropriate restriction enzymes from bacterial expression vectors and blunt-end ligated into a eukaryotic expression vector, pRC/CMV. This vector contains the cytomegalovirus promoter for high copy number in mammalian cells and a neomycin-resistance sequence for the selection of permanently transfected cell lines. COS-7, CHO, 293, and AtT-20 cells have been transiently transfected with D1, $D2_S$, $D2_L$, and D3 receptor sequences. Expression of receptors has been confirmed by radioligand binding studies using ^{125}I-SCH-23982, ^{125}I-IBZM, and ^{125}I-NCQ-298. Studies with the D2 receptor-selective antagonists ^{125}I-NCQ-298 and ^{125}I-IBZM show no differences between $D2_S$ and D3 receptors when expressed in COS-7 cells. Stable transfectants have been isolated and receptor expression confirmed by radioligand binding studies in COS-7 and CHO cells. (USPHS NS18591 and a PMA Foundation Fellowship)

432.6

Localization of D2 DA Receptor mRNA in Primary Olfactory Neurons. MICHAEL T. SHIPLEY, WILLIAM T. NICKELL, ANDREW B. NORMAN (University of Cincinnati) and CHARLES GERFEN (NIMH).

The glomeruli of the olfactory bulb are densely innervated by dopaminergic (DA) interneurons; the post-synaptic targets of these neurons are unknown. We recently showed that most DA receptors in the bulb are of the D2 subtype and are selectively localized in both the glomerular and the *olfactory nerve* layers. This suggested that DA neurons target D2 receptors on olfactory nerve terminals. Consistent with this idea, nerve lesions eliminate D2 binding in *both* the nerve and glomerular layers. If D2 receptors are present on olfactory nerve terminals, then these receptors should be synthesized in the cell bodies of primary olfactory neurons (PONs).

An ^{35}S-labelled oligonucleotide cDNA probe specific to the D2 receptor was used to localize D2 receptor sequences in rat olfactory epithelium using *in situ* hybridization.One olfactory bulb was removed from each rat 14 days prior to sacrifice. D2 mRNA labelling was present in all regions of the olfactory epithelium on the intact side; labelling was absent from the epithelium on the side of the ablated bulb. Since ablation of the olfactory bulb causes degeneration of the PONs, the present results demonstrate that the D2 mRNA is localized in the PONs and not in other cells in the epithelium.

Taken together, these findings support the hypothesis that juxtaglomerular DA neurons are presynaptic to olfactory nerve terminals. This implies that there is presynaptic DA regulation of olfactory nerve terminals. Synaptic targeting of olfactory nerve terminals by DA neurons might also explain the loss of DA and TOH in the bulb after peripheral deafferentation: Deafferentation would deprive juxtaglomerular DA neurons of their postsynaptic target. Target regulation of transmitter phenotype occurs in other neural systems. Supported by NIH-DC00347 and NS29218.

432.7

DIRECT VISUALISATION OF DIFFERENT DOPAMINE RECEPTOR CLASSES IN THE MAMMALIAN BRAIN: A CORRELATIVE STUDY USING IN SITU HYBRIDIZATION HISTOCHEMISTRY AND RECEPTOR LIGAND AUTORADIOGRAPHY.

J.M. Palacios[1,2], G.B. Landwehrmeyer*[3], G. Mengod[1,2]

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The genes coding for several dopamine receptors have been recently characterized. The D2, D3 and D4 receptor classes are all recognized by ligands previously considered to be "D2" selective. In order to establish a differential labeling of these sites with the available "D2" ligands, we first identified the brain regions enriched for mRNA coding for a specific receptor form by in situ hybridization histochemistry using ^{35}P-labelled oligonucleotides. The pharmacological characteristics of the receptors in these regions were determined by direct labeling with [^{3}H]-spiperone, [^{3}H]-raclopride, [^{3}H]-CV 205 502, [^{125}I]-iodosulpride and [^{3}H]-YM 09151-2. Furthermore, displacement experiments using unlabeled compounds like domperidone, raclopride, clozapine and quinpirole, which present some selectivity for the different "D2" receptor classes, were carried out .The results obtained show that by combining selected radioligands with unlabeled compounds experimental conditions can be defined to visualise specifically the different dopamine "D2"-like receptor classes.

432.8

REGIONAL DIFFERENCES IN DOPAMINE D_1 AND D_2 AGONIST AFFINITIES IN THE RAT BRAIN G. von Euler and P. Hedlund* Dept. of Histology and Neurobiology, Karolinska Institutet., Box 60400, S-10401 Stockholm, Sweden

Competitive-inhibition curves with dopamine on [^{3}H]SCH 23390 (a D_1 antagonist) binding and on [^{125}I]sulpride (a D_2 antagonist) binding were analyzed by quantitative receptor autoradiography on cryostat sections in order to reveal possible regional differences in agonist affinities at D_1 and D_2 receptors in the rat brain. The IC_{50} value, probably corresponding to K_{Low}, of dopamine versus [^{3}H]SCH 23390 was lower in the nucl. accumbens (32%) but higher in the tub. olfactorium (217%) than in the striatum (100%=2.74 μM). No high affinity D_1 agonist binding was observed. The K_{High} value of dopamine versus [^{125}I]sulpride was lower in the nucl. accumbens (57%) and tub. olfactorium (45%) than in the striatum (100%=1.13 nM). In contrast, the K_{Low} was higher in the nucl. accumbens (146%) and tub. olfactorium (218%) than in the striatum (100%=12.7 μM). The percentage of binding sites in the high affinity state with regard to the total number of binding sites was higher in the nucl. accumbens (69%) and the tub. olfactorium (75%) than in the striatum (64%), indicating a higher degree of G-protein association in the former areas.

Taken together, the results indicate the presence of regional differences in the agonist affinities at central D_1 and D_2 receptors, which may underlie regional differences in the postsynaptic effects of dopamine neurotransmission. The affinity changes of the D_2 receptor may reflect regional differences in the phosphorylation state of the receptor, in the distribution of D_2 receptor subtypes, or in the association to modulatory receptors such as neurotensin receptors and adenosine A_{2a} receptors.

432.9

RECOVERY OF CENTRAL D_1 AND D_2 RECEPTORS FOLLOWING ALKYLATION IN YOUNG AND ADULT RAT BRAIN. R.J. Baldessarini, N. Kula*, T. George*, A. Campbell*, V. Bakthavachalam*, and J.L. Neumeyer. Departments of Psychiatry and Neuroscience Program, Harvard Medical School; Laboratories for Psychiatric Research, Mailman Research Center, McLean Division of Massaschusetts General Hospital, Belmont, MA 02178; and Research Biochemicals, Inc. (RBI), Natick, MA 01760.

Novel D_1 and D_2 receptor-selective alkylating agents are being developed and evaluated for their ability to bind irreversibly to central dopaminergic receptor proteins. Agents tested included several D_2-selective alkylating derivatives of *p*-aminophenethylspiperone (NAPS), such as the *p*-isothiocynate (NIPS) or its bromacetamino or ethylfumaramido congeners, and other nonalkylating N-substituted spiperone derivatives, which were compared with the nonselective alkylating agent 1-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ). Rates of recovery of dopaminergic receptors were assessed by increases in binding of selective ligands to D_1 (^{3}H-SCH-23390) or D_2 sites (^{3}H-YM-09151-2) in corpus striatum tissue, as well as of behavioral arousal induced by R(−)-apomorphine in the rat from 1 to 10 days after initial treatment with a test agent. We have found an orderly progression of recovery rates in adult rat following a series of N-alkyl-aryl derivatives of spiperone (methyl, phenethyl, NAPS and NIPS), with the slowest recoveries found with the alkylating compound NIPS ($t_{1/2}$ = 1–2 days). These rates were compared with those found after treatment with EEDQ. Recovery of both D_1 and D_2 sites in brain tissue (as B_{max} values) of 25–35-day old rats was more rapid than in adults. The latter observation corresponds with relatively high concentrations of these receptor proteins in brain tissue of similarly aged rats. Agents such as those tested show great promise for the study of synthesis rates of dopaminergic receptors. [Supported by USPHS-NIMH grants MH-34006, MH-45692, and MH-47370, and an award from the Bruce J. Anderson Foundation.]

432.10

EFFECT OF SELECTIVE D1 AND D2 DOPAMINE (DA) RECEPTOR INACTIVATION IN SUBSTANTIA NIGRA (SN) ON RESPONSES OF SN DA NEURONS TO I.V. R(-)NPA. R.F. Cox and B.L. Waszczak, Pharmacol. Sect., Northeastern Univ., Boston, MA 02115.

We have shown that SN but not striatal injections of the receptor inactivator N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) reduce the ability of of i.v. R(-)N-n-propylnorapomorphine (NPA) to inhibit SN DA cell firing (Soc. Neurosci. Abstr. 16:696, 1990). The effect was revealed as a rightward shift and depressed maximum of the NPA dose-response curve. We have now used selective receptor inactivation strategies to examine contributions of SN D1 or D2 DA receptors in this response. An hour before intranigral EEDQ injections (100 nmol/ul), rats were given s.c. "protection cocktail" (prazosin, idazoxan and ketanserin) containing either SCH 23390 (SCH, 4 mg/kg) to shield D1 sites, or eticlopride (ETIC, 2 mg/kg) to protect D2 sites. Controls received protection mixtures but no EEDQ. Single unit recording studies were done a day later, followed by quantitative autoradiography to assess the degree of SN D1 and D2 receptor loss. The ED50s for NPA were elevated after pretreatment with both SCH or ETIC, compared to control rats given only the basic cocktail (ED50s: 0.3 ug/kg, control; 1.5 ug/kg after SCH; 0.9 ug/kg after ETIC). Selective SN D2 receptor inactivation further increased the NPA ED50 to 2.3 ug/kg and reduced the maximum inhibitory response to 82%. SN D1 receptor inactivation, however, caused no decline in the maximum response and gave an NPA ED50 of 1.0 ug/kg, indicating no further change in the position of the curve relative to that for protection cocktail + ETIC. Autoradiography confirmed the selective loss of each receptor, i.e. SN D2 sites declined by 74% but D1 sites dropped by only 2% after SCH protection, whereas D2 sites declined by just 9% but D1 sites fell by 84% after ETIC. Overall, these results indicate a predominant role for SN D2 receptors in mediating the inhibition of DA cell firing by i.v. NPA. However, because SCH pretreatments also elevated the ED50 for NPA, a slight contribution from D1 receptors cannot be ruled out. (Support: NS 23541)

432.11

AMPHETAMINE-STIMULATED DOPAMINE RELEASE COMPETES IN VIVO FOR ^{123}I-IBZM BINDING TO THE D2 RECEPTOR IN PRIMATES. M. Al-Tikriti, R. Malison, S. Zoghbi*, P. Hoffer*, J. Seibyl, M. Laruelle, R. Baldwin*, E. Sybirska, E. Smith, D. Charney, J. Elsworth, R. Roth and R. B. Innis Depts. Psychiatry & Radiology, VA Med. Ctr. and Yale Univ., West Haven, CT 06516.

The reversibly binding D2 dopamine receptor radioligand ^{123}I-IBZM (iodobenzamide) was used to test whether the endogenous neurotransmitter dopamine competes *in vivo* for radiotracer binding measured with single photon emission computed tomography (SPECT).

In a series of non-human primate experiments (n=27), the effects of temperature, amphetamine, haloperidol, and reserpine on brain uptake of ^{123}I-IBZM were measured. After i.v injection of ^{123}I-IBZM (1.9-17.3 mCi), specific brain uptake reached a peak by 100 min and demonstrated a gradual, apparent "steady-state" washout over the next 2 hrs. Brain uptake was temperature dependent, with rates of washout of specifically-bound radioligand greater under normothermic conditions (26%/hr: core body temperature 35-37°C) than under controlled hypothermia (11%/hr; 32-34°C). Given the greater retention of radioactivity, low temperature conditions were used in all other experiments. Administration of haloperidol (0.02 mg/kg i.v.) during the period of apparent steady state resulted in a dramatic increase in washout (60%/hr) as did d-amphetamine (1.0 mg/kg i.v.; 34%/hr). Reserpine pretreatment (1.0 mg/kg i.v.) caused greater than 90% depletion of striatal dopamine levels, blocked amphetamine-enhanced washout (10%/hr), but did not block the effects of the direct-acting D2 receptor antagonist haloperidol. Postmortem analysis of the two animals pretreated with reserpine confirmed greater than 90% depletion of tissue dopamine levels.

These results are consistent with the hypothesis that endogenous dopamine effectively competes for radioligand binding in neuroreceptor imaging studies and suggest that receptor imaging may be developed to monitor transmitter turnover.

432.12

IN VIVO HUMAN DOPAMINE RECEPTOR QUANTIFICATION IN SELF-INJURIOUS BEHAVIOR SYNDROMES. DF Wong, J Harris, S Naidu,* E Shaya,* M Yaster,* RF Dannals,* AA Wilson,* H Ravert,* HN Wagner, Jr.,* W Nyhan,* H Moser,* Div. Nuclear Medicine, Kennedy Inst. and Div. Child Psychiatry, Johns Hopkins Univ., Balto. MD. 21205

The Lesch Nyhan (LN) and Rett syndromes are neurodenerative disorders with infantile onset associated with motor handicaps and a compulsive hand to mouth behavior leading to self injury (SIB). Post mortem studies and CSF dopamine metabolite measurements show both disorders have dopamine neurotransmitter abnormalities. Six LN males (aged 4 to 23) and 4 Rett females have been studied with a quantitative model which requires 2 PET scans to determine absolute D2 receptor density B_{max} using ^{11}C NMSP. Motor handicaps are so severe that general anesthesia (nitrous oxide with rectal methohexital induction) is necessary for immobilization for the 90 minute PET procedure. When compared to normal subjects aged 18-70 years the B_{max} showed a linear fall with age. Three adult Rett Syndrome cases showed borderline low B_{max}, while LN cases showed high normal or elevated B_{max} relative to 95% confidence limits on a linear regression. Animal models of self injury respond to D1 antagonists and suggest that in vivo D1 studies in LN and Rett syndrome need further study. Investigations of the D1/D2 interaction in vivo compared to post mortem values would assist in understanding the pathogenesis. Supported by NICHD 23042, 23540, 24061.

ISCHEMIA V

433.1

TIRILAZAD MESYLATE (U-74006F) ATTENUATES THE ACCUMULATION OF NEUTROPHILIC LEUKOCYTES (NLs) IN ISCHEMIC GERBIL BRAIN. J.A. Oostveen and L.R. Williams. CNS Diseases Res., The Upjohn Co., Kalamazoo, MI.

The involvement of NLs was examined in the gerbil unilateral carotid occlusion (UCO) model of transient focal cerebral ischemia (Hall et al., 1988). Frozen coronal sections were processed with cytochrome oxidase (CO), myeloperoxidase (MPO), and cresyl violet histochemistry. In control gerbils after 3 hr UCO, NLs are clumped within the cerebral vasculature, and are commonly observed crossing the capillaries into the brain parenchyma. After UCO and 24 hr of reperfusion, there is considerable necrosis within the ischemic hemisphere, and numerous CO- and MPO-positive, degranulated NLs are dispersed throughout the necrotic tissue. Computer-assisted image analysis of the lateral cortex indicated an average (± SEM) density of 194 ± 32 (CO) and 202 ± 41 (MPO) cells/mm^2 (n=7). In gerbils treated with U-74006F (10 mg/kg i.p.), the density of CO-positive cells was significantly reduced by 66% ($p \leq 0.05$). The density of MPO-positive cells was reduced 31%. Thus, one mechanism by which U-74006F provides cytoprotection to ischemic brain tissue may be to limit the accumulation of NLs in tissue at-risk, thus limiting consequent NL-mediated oxidative tissue destruction.

433.2

MK801 INCREASES CBF IN "MODERATELY" ISCHEMIC RAT CORTEX. M. Jacewicz, U. Dirnagl and W. Pulsinelli. Dept. Neurology and Neuroscience, Cornell Univ Medical Center, New York, NY 10021

The effect of MK801 (an NMDA antagonist) on cerebral blood flow (CBF) was studied in Spontaneously Hypertensive rats under conditions in which MK801 reduces cortical infarct volume (from 169 ± 34 mm^3 to 134 ± 58 mm^3, Dirnagl, 1990, Brain Res 527:62) following middle cerebral artery and common carotid artery occlusion (MCA/CCAO). Rats received MK801 5mg/kg i.p. (N=10) or saline (N=10) 30 min before undergoing MCA/CCAO under brief halothane anesthesia. A ^{14}C-iodoantipyrine CBF study was performed in conscious rats at 2.5 h after MCA/CCAO, when ischemic injury in this model becomes largely irreversible and enlargement of the ischemic tissue volume has nearly peaked (Jacewicz et al, submitted). The volumes of ischemic cortex with CBF <35 ml/100g/min (VIC_{35}) and CBF = 35-65 ml/100g/min (VIC_{35-65}) were quantified by ^{14}C-autoradiography and computerized image analysis. Body temperatures were maintained at 37.5 ± 0.5 °C, and physiologic variables remained comparable in MK801-treated animals and controls.

MK801 caused widespread increases in CBF except in the ischemic core of the middle cerebral artery (MCA) territory. MK801 shrank VIC_{35-65} from 82 ± 25 mm^3 (controls) to 50 ± 12 mm^3 ($p < 0.05$, Student's t-test) while having no effect on VIC_{35} (79 ± 41 mm^3 and 79 ± 61 mm^3 in MK801-treated rats and controls respectively). Recent work (Jacewicz et al, submitted) has determined that rat cortex with CBF = 35-50 ml/100g/min will infarct and oligemic regions (CBF = 50-65 ml/100g/min) will suffer ischemic injury if ischemia is prolonged. In this model, the modest protection MK801 offers against infarction may involve critical CBF increases in the moderately ischemic cortex of the MCA periphery.

433.3

HYPOTHERMIA IN PERMANENT FOCAL ISCHEMIA: EVOKED POTENTIALS, REGIONAL CEREBRAL BLOOD FLOW AND QUANTITATIVE MRI. E.H.Lo and G.K.Steinberg. Department of Neurosurgery, Stanford University School of Medicine, Stanford, CA94305.

A rabbit model of permanent focal ischemia was used to study the effects of hypothermia on somatosensory evoked potentials (SEP), regional cerebral blood flow (rCBF) and quantitative magnetic resonance imaging (MRI) parameters. Three temperature (temporal muscle) groups were used: 37°C, 33°C, and 30°C (n=4 per group). SEPs were measured before and after occlusion. After 4 hrs of permanent focal ischemia, rCBF was measured using radioactive microspheres. Brains were removed immediately afterwards for MRI measurements. Improved SEP recovery was found in the 30°C and 33°C groups compared to the normothermic group ($p<0.05$). T1 and T2 MRI values of the core ischemic regions were significantly increased in the normothermic group ($p<0.05$, left vs right hemisphere). In both hypothermic groups, left vs right T1 and T2 values were not significantly different. No significant differences in rCBF were found between any temperature groups. These results demonstrate that hypothermia protects against permanent focal ischemia without altering rCBF.

433.4

CHANGES IN EXTRACELLULAR OPIOID PEPTIDES AND DOPAMINE FOLLOWING FOCAL CEREBRAL ISCHEMIA

G.K. Steinberg, N.T. Maidment, E.H. Lo, and C.J. Evans
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Increases in extracellular excitatory amino acids and dopamine have been implicated in the pathophysiology of cerebral ischemic injury. However, it is not known whether ischemia-induced release is restricted to certain neurotransmitters or is more widespread. This study used microdialysis to measure extracellular opioid peptides and dopamine in the caudate nucleus of the rabbit using ischemic episodes of varying duration. Twelve rabbits underwent stereotactic placement of a dialysis probe in the caudate. Baseline samples were collected and rabbits underwent occlusion of the left internal carotid, anterior cerebral and middle cerebral arteries. The time from occlusion to sacrifice was 6 hours but the duration of occlusion was varied between 10 minutes and 6 hours. Opioid peptides were measured with RIA and dopamine with HPLC. Ischemic neuronal damage (IND) was assesed with histopathology. Extracellular content of opioid peptides and dopamine increased markedly between 15 - 234X within the first 10 minutes after occlusion. The rate of return to preocclusion levels was dependent on the length of the occlusion period, but dopamine levels remained elevated longer than opioid peptide levels. Histopathology revealed > 75% area severe IND in the striatum when the occlusion was ≥30 minutes. These results suggest that ischemia-induced increases in extracellular neurotransmitters are a general phenomenon. If either dopamine or opioid peptide are involved in ischemic neuronal damage, a sustained elevation in their extracellular content may be required to produce such injury.

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433.5

POST-ISCHEMIC TREATMENT WITH FELBAMATE REDUCES DELAYED NEURONAL CELL DEATH. C.G. Wasterlain, L.M. Adams, and P.H. Schwartz. Epilepsy Res. Labs., VAMC, Sepulveda, CA 91343.

Rescuing neurons from delayed cell death should be one of the main goals of post-ischemic therapy. We previously reported that the anticonvulsant felbamate protects neurons from hypoxic and/or ischemic injury *in vitro* and *in vivo*. This study investigated the effectiveness of felbamate given after the hypoxic-ischemic insult in reducing delayed neuronal necrosis. We used 7-day-old rat pups subjected to bilateral carotid ligation, followed by a 1 hr exposure to 6% O_2, and sacrificed 72 hr later. 100-400 mg/kg ip injections or oral administration (300-500 mg/kg) raised serum felbamate to 100-150 μg/mL for several hours. Following bilateral carotid ligation and exposure to 6% O_2 for 1 hour (which had no effect on serum levels), felbamate (300 mg/kg ip) given immediately (0 hr), 1, 2, or 4 hr after the end of hypoxia significantly reduced the volume of neocortical infarcts by 55%, 30%, 44%, and 24%, respectively. In this model, dentate gyrus granule cells are intact at 4-24 hours after the insult but display delayed necrosis by 72 hours. Felbamate given immediately after hypoxia reduced granule cell necrosis by 92% ($p<0.001$). Single doses of 100 and 200 mg/kg were less effective given after the insult than 300 or 400 mg/kg. These data suggest that felbamate given after a hypoxic-ischemic insult is very effective in rescuing neurons from delayed post-ischemic death.

433.6

LIPOCORTIN-1 INHIBITS CEREBRAL ISCHAEMIA. J.K.Relton[*1], F.Carey[*2], D.C.U'Prichard[2] and N.J.Rothwell[*1]. [1]Dept. Physiol. Univ. of Manchester, UK. [2]ICI, Macclesfield, UK.

The objective of the experiment was to determine the effect of lipocortin-1 (annexin-1), an endogenous phospholipid and calcium binding protein, on neuronal damage following cerebral ischaemia (unilateral middle cerebral artery occlusion (MCAo)) in the rat. The extent of damage was determined using a stain for mitochondrial viability (tetrazolium blue), and results quantified using a Seescan image analysis system. Central injection (1.2μg icv) 30min prior to and 10min after MCAo, of an active fragment of lipocortin-1 (N-terminal 1-188aa) profoundly reduced (by 71.4 ± 8.5%) the extent of neuronal damage (infarct volume) assessed at 24h (81.2 ± 15.5 vs 23.2 ± 6.9mm^3, $n=8$, $p<0.01$). In a separate study, a single injection (4μl icv), 10min after MCAo, of neutralizing anti-lipocortin-1 fragment antiserum increased (by 53.2 ± 18.1%) the size of infarct assessed 2h later (22.6 ± 2.4 vs 34.6 ± 4.1mm^3, $n=10$, $p<0.05$). These findings indicate that lipocortin-1 is an endogenous inhibitor of cerebral ischaemia with considerable therapeutic potential.

433.7

Early neuronal membrane injury precedes very delayed histologic degeneration after "ischemia" in organotypic hippocampal culture. J. J. Vornov, R. C. Tasker and J. T. Coyle Depts. Neurology, Anesthesiology, Neuroscience and Psychiatry, The Johns Hopkins School of Medicine, Baltimore, MD 21205

To examine the time course of ischemic injury, we have used organotypic hippocampal culture which preserves synaptic circuitry and regional differentiation. Brief *in vitro* "ischemia" was achieved with 2-deoxyglucose (2-DG) and potassium cyanide (KCN) to inhibit glycolysis and oxidative metabolism. Propidium iodide fluorescence was used to observe early membrane injury as it occured in the living culture. Staining was most rapid following a 30 minute exposure at high concentration (5 mM 2-DG, 1 mM KCN), and only started after 2-4 hours of recovery. The staining began in CA1, then involved CA3 with sparing of the dentate gyrus. Histology after 24 hours of recovery demonstrated marked pyknosis of neuronal nuclei in the propidium stained regions. Exposures of either shorter durations (8-15 min) or lower concentrations of 2-DG and KCN produced a pattern of propidium staining which was further delayed (4-6 hours), less intense, and affected only CA1 and then CA4/CA3c, but spared CA3a,b and the dentate gyrus. Histology at 24 hours was largely normal. With longer recovery (3, 5, and 7 days), an increasing severity of pyknosis was noted in the areas showing early propidium staining. The time course and regional distribution of ischemic neuronal degeneration in organotypic culture appears to reproduce that observed in animal models. Membrane injury, as revealed by propidium staining, far precedes histologic degeneration. Furthermore, the pattern of injury is distinct from that produced by brief exposure to NMDA .

433.8

SEROTONIN MAY MEDIATE PLATELET NEUROTOXICITY. Rajiv Joseph, Chock Tsering, Saul Grunfeld and KMA Welch. Lab. of Experimental Hematology & Stroke, Henry Ford Hospital, Detroit, MI 48202.

Our earlier studies suggested that platelet secretory products have a toxic effect on acetylcholinesterase positive neurons in organotypic spinal cord cultures (Neurology 1991; 41 (Suppl.1):163). Further work was done with added refinements such as use of gel-filtered platelets, platelet membrane and red blood cells as additional controls, and using both rat and human platelets. The results indicate, as in our previous study, that platelet secretion has a morphologically demonstrable toxic effect on neurons ($p<0.0001$). In order to identify a toxic component from among the known platelet secretory products, experiments were carried out exposing the explants to 5HT, a major platelet product. Based on the concentration of 5HT in whole blood, before and after platelet activation, and the estimated content of platelets in an occlusive thrombus, we calculated that the concentration of 5HT around an acute thrombus could well reach 4000 times the concentration in whole blood with the platelets unactivated. We exposed the explant-cultures to varying concentrations of 5HT in order to obtain a dose-response curve. There was evidence of neurotoxicity at 5HT concentrations of 3.5 uM and higher. The number of surviving neurons in the treated group ([5HT] 350 uM) was 72% less than in the controls ($P<0.0001$). Considering that this effect was seen with a 5HT concentration (350 uM) that is about one-fourth the calculated concentration that could theoretically be present in the vicinity of an acute thrombus, it appears reasonable to postulate that 5HT could significantly contribute to ischemic neuronal injury.

433.9

SOMATOSTATIN AND DYNORPHIN A INDUCE INCOMPLETE FOCAL ISCHEMIC CORTICAL INJURY IN RATS. J.B. Long and F.C. Tortella, Neuropharm. Br., Dept. of Med. Neurosci., Walter Reed Army Inst. of Res., Washington, D.C. 20307.

Vasoactive peptides are evident in innervations of the cerebrovasculature and can pharmacologically alter microcirculatory function. After spinal subarachnoid injection in rats, dynorphin A (DYN) and somatostatin (SOM) cause dose-dependent reductions in blood flow that lead to neuronal loss and persistent hindlimb paralysis. We now describe parietal cortical responses to these peptides following their topical application in anesthetized rats. Videomicroscopy and laser doppler flowmetry revealed that application of 10 μl of artificial CSF containing 10-40 nmoles of these peptides caused immediate intense arteriolar vasoconstriction associated with dose-dependent 30-90% focal reductions in blood flow of 45-90 min duration. Cortical EEG monitored 1-2 mm from the site of application indicated that 70-80% reductions in blood flow induced by 40 nmoles of SOM were associated with 50-60% reduction in EEG amplitude coincident with induction of paroxysmal epileptiform activity. Evans Blue dye extravasation revealed appreciable localized breakdown of the blood-brain barrier by 2 hrs after peptide application. After 1 week, the site of peptide application was characterized by large hemorrhagic lesions, gliosis, and macrophage infiltration confined to the cortex. These results indicate that these peptides provide a novel pharmacological means to conveniently and reproducibly study the pathophysiology associated with focal incomplete ischemia/reperfusion injury to the neocortex.

433.10

REPERFUSION IN A RAT MODEL OF FOCAL ISCHEMIA. W.R. Selman*, A. Hopkins*, R.C. Crumrine, R.A. Ratcheson and W.D. Lust. Departments of Neurosurgery and Anatomy, Case Western Reserve University, Cleveland, OH 44106

Reperfusion following focal ischemia has been shown to be of little benefit in protecting tissue from injury if initiated after 2 h of occlusion. In this report, the metabolic recovery of the focal and perifocal regions was examined at 1 h of reflow either after 1 h [1,1] or 4 h [4,1] of middle cerebral artery (MCA) occlusion in spontaneously hypertensive rats. Diffusion-weighted MRIs were performed after reflow and the brains were frozen in situ at 1 h of reperfusion. Tissue sections were lyophilized and dissected based on the hyperintense areas on the MRI scans, which presumably indicate edematous changes at these early time periods.

The edematous regions corresponded with infarcted areas at 3 days post-occlusion. The area of edema in the [1,1] group was confined to the ventrolateral cortex, whereas in the [4,1] group the edema extended dorsally to include the entire ipsilateral cortex served by the MCA. Glucose levels were at or above control in all regions, suggesting that all tissues, including the ischemic core, were reperfused. ATP and P-creatine did not recover in either the ischemic core or the perifocal region in the [4,1] group. While ATP in the [1,1] group recovered to 60% of control, the P-creatine levels completely recovered in both the focal and perifocal region. Lactate remained significantly elevated in both regions of the [4,1] group and in the ischemic core of the [1,1] group. In the perifocal region of the [1,1] group, lactate levels were not significantly different from those of control.

The results indicate that metabolic recovery was severely compromised between 1 and 4 h of ischemia, supporting the histological evidence that the threshold for irreversible damage had been exceeded. The fact that the ischemic focus in the [1,1] group was destined to become infarcted despite evidence of metabolic recovery implicates a different postischemic type of cell damage.

433.11

HISTOLOGICAL ANALYSIS FOLLOWING TEMPORARY OCCLUSION OF THE MIDDLE CEREBRAL ARTERY (MCAO) IN HYPERTENSIVE RATS (SHR). R.K. Clark, E.V. Lee*, W.J. Price*, R.F. White*, C.J. Fish*, G.Z. Feuerstein and F.C. Barone. SmithKline Beecham Pharmaceuticals, King of Prussia, PA 19406

Permanent MCAO in SHR rats results in ischemic changes characterized by focal pan necrosis, inflammatory cell infiltrate, gliosis, and loss of the necrotic tissue by thirty days post occlusion. We have now characterized changes which occur after MCAO followed by reperfusion. Temporary MCAO results in infarcts which vary in size in an occlusion-time dependent manner. After one hour of reperfusion, ischemic changes were observed histologically, including microhemorrhages and the beginning of an inflammatory infiltrate in and around the vasculature. This infiltrate consisted primarily of neutrophils; however, monocytes were present. Infiltration occurred much more quickly and to a greater extent in reperfused tissues than in permanently occluded tissues, where it began approximately 12 hours post occlusion. Three days after surgery, in addition to the neutrophils, a heavy macrophage infiltrate was present in the reperfused tissues. Macrophages were a minor component of the permanently occluded lesions. Following five days of reperfusion, the necrotic tissue was largely replaced by macrophages, with numerous neutrophils distributed throughout the lesion. In contrast, in the permanently occluded tissues, macrophages were scattered through a loose extracellular matrix and remaining necrotic tissue, and neutrophils were located focally. The data indicate that duration of occlusion affected the size of the lesion, while reperfusion affected the timing, extent and composition of the response that leads to resolution of the necrotic tissue. Histologic and immunohistochemical evidence of these changes will be presented.

433.12

MIDDLE CEREBRAL ARTERY OCCLUSION (MCAO) IN THE MOUSE. FC Barone, DJ Knudsen*, AH Nelson*, GZ Feuerstein, MN Tzimas*, DB Schmidt*, EV Lee*, RK Clark and HM Sarau, SmithKline Beecham Pharmaceuticals, King of Prussia, PA 19406.

MCAO, alone or with ipsilateral common carotid artery ligation (CCAL), and sham surgeries were made in BDF and CFW mice. Histological and biochemical techniques were utilized to characterize the effects of focal ischemia 24 hr later. Hemispheric swelling (% SWELL), hemispheric infarction (% INFARCT) and infarct volume (mm^3 INFARCT) were (n=7-12, *=p<0.05 vs MCAO):

STRAIN	SURGERY	% SWELL	% INFARCT	mm^3 INFARCT
BDF	SHAM	1 ± 1	0 ± 0*	0 ± 0*
	MCAO	4 ± 2	7 ± 1	5 ± 1
	MCAO + CCAL	9 ± 2*	21 ± 5*	15 ± 3*
CFW	SHAM	2 ± 1*	0 ± 0*	0 ± 0*
	MCAO	7 ± 1	17 ± 2	15 ± 2
	MCAO + CCAL	13 ± 3*	46 ± 4*	38 ± 4*

Neutrophil adherence and infiltration was identified within meningeal and parenchymal vessels bordering infarctions at various stages of diapedesis into tissue. A Myeloperoxidase (MPO) activity assay was used to quantify increased neutrophil influx (mUnits per gram wet weight) for control (8.5±2.7) compared to ischemic (33.9±8.6, n=8, p<0.01) hemispheres of CFW mice following MCAO + CCAL. LTB_4 receptor density (fmol per mg protein) increased under the same conditions for control (3.7±0.3) compared to ischemic (5.4±0.3, p<0.01) hemispheres. Results indicate that MCAO damage in this mouse model is sensitive to strain differences and CCAL. Mouse focal ischemia is characterized by an inflammatory response with neutrophil infiltration that can be quantified by MPO activity. Increased LTB_4 receptor binding might be important in this process.

HYPOTHALAMIC-PITUITARY-ADRENAL REGULATION: MOLECULAR STUDIES

434.1

CORTICOTROPIN RELEASING-FACTOR GENE EXPRESSION IS NOT INCREASED AFTER PHARMACOLOGICAL ADRENALECTOMY. Tallie Z. Baram MD, PhD and Linda Schultz* Dept. of Neurology, University of Southern California; Div. of Neurology, CHLA; Los Angeles, CA 90054

A regimen of twice daily metyrapone injections (100 mg/kg), resulted in pharmacological adrenalectomy of pregnant rats and fetuses in utero: i.e., depression of plasma corticosterone and elevation of plasma ACTH in pregnant and in fetal rats. Toxicity was minimal on days 14-17 of pregnancy, and increased with higher maternal weight and pregnancy progression.

Maternal Corticotropin releasing-factor (CRF) messenger RNA abundance increased significantly within 48 hours of metyrapone initiation. No change in CRF gene expression in the paraventricular nucleus of fetuses (days 17-19) was seen, even after 72 hours of the regimen. This is compatible with the independence of CRF gene expression of glucocorticoid feedback in the fetal rat.

434.2

HYPOTENSIVE HEMORRHAGE ELEVATES CRH mRNA, BUT NOT AVP mRNA IN RAT HYPOTHALAMUS. D.N.Darlington, C.A. Barraclough and D.S. Gann. Depts. of Surgery and Physiology, University of Maryland, Baltimore, MD 21201 USA.

To determine if acute hypotensive hemorrhage affects mRNA for corticotropin releasing hormone (CRH) and vasopressin (AVP) in the rat hypothalamic paraventricular nucleus (PVN), 300g Sprague-Dawley male rats were cannulated (femoral artery and vein) and subjected to 15ml/kgx3min hemorrhage 4days later. Time controls received no hemorrhage. Rats were killed at 1 or 4hr (n>6/group), the hypothalamus was removed, frozen and sectioned at 12 μm. In Situ hybridization was performed using an oligodeoxynucleotide probe for CRH mRNA and AVP mRNA. Hemorrhage led to a fall in arterial blood pressure and heart rate that recovered by 1hr. Plasma ACTH and corticosterone were elevated 20, 60, 90 and 120 min after hemorrhage, but returned to control by 4 hrs. CRH mRNA was significantly elevated 1 and 4hrs after hemorrhage in parvocellular PVN. AVP mRNA did not change after hemorrhage in parvocellular or magnocellular PVN, nor in the supraoptic nucleus. These data suggest that neural signals originating from cardiovascular receptors upregulate message for CRH, but not AVP, in the PVN. Supported by DK26831 ,GM27946 and HD02138.

434.3

DISTRIBUTION OF CRF-BINDING PROTEIN mRNA AND IMMUNOREACTIVITY IN THE RAT BRAIN. E. Potter*, D.P. Behan*, P.E. Sawchenko and W.W. Vale. The Salk Institute, La Jolla, CA 92037

Initial studies on the structure and tissue distribution of the rat CRF-binding protein (CRF-BP) gene indicated that CRF-BP mRNA was expressed in the brain. We have used cRNA probes generated from non-overlapping regions of a rat cDNA clone, along with antisera raised against human recombinant CRF-BP, to analyze by hybridization and immunohistochemical means the cellular localization of CRF-BP and protein, respectively. Results from both approaches converged to indicate that CRF-BP is expressed principally in the cerebral cortex. This includes cells in layers II, III, V and VI of all neocortical fields, the hippocampal formation, the olfactory bulb, anterior olfactory nucleus, and endopiriform nucleus. Other prominent sites of mRNA and protein expression include the inferior colliculus, raphe nuclei, nuclei of the trapezoid body and lateral lemniscus and the lateral reticular nucleus. In the hypothalamus, the most prominent site of expression is in the ventral premammillary nucleus; only scattered cells have been seen in the paraventricular and dorsomedial nuclei. Dual immunostaining for CRF and CRF-BP has thus far revealed a partial overlap in some of these regions. In addition, prominent CRF-BP-stained terminal fields have been identified in CRF-expressing cell groups, including the suprachiasmatic nucleus and the bed nucleus of the stria terminalis. In the anterior pituitary, CRF-BP mRNA has been colocalized with ACTH-immunoreactivity in a majority of corticotropes. Thus, CRF-BP which reversibly neutralizes the biological activity of CRF could serve to modify the actions of CRF by intra- and intercellular mechanisms in both the CNS and pituitary. CRF-BP interferes with the binding of most CRF antisera and its distribution may explain discrepancies in CNS cell groups where CRF mRNA is readily demonstrable in the face of little or no evidence for corresponding peptide expression.

434.4

TISSUE DISTRIBUTION OF CRF OVER-EXPRESSION IN TRANSGENIC MICE. A.V. Cameron*, M.P. Stenzel-Poore*, P.E. Sawchenko, E.R. Brown and W.W. Vale, The Salk Institute, La Jolla, CA 92037.

To produce an animal model of chronic over-expression of CRF, transgenic mice have been generated bearing a CRF transgene comprising the metallothionein promoter and the CRF structural gene. We have monitored a single lineage, which like the transgenic founders, shows significantly elevated plasma glucocorticoids, obesity, and bilateral hair loss, suggestive of a Cushing's-type syndrome (Stenzel-Poore et al., J. Cell Biochem. 15A:203, 1991). Using *in situ* hybridization with a cRNA probe for CRF, the brains of transgenic individuals of this lineage compared to nontransgenic littermate controls show elevated levels of CRF mRNA in many regions which normally express the CRF gene, especially in the lateral septum, medial preoptic area, amygdala, supraoptic, arcuate, parabrachial, and Barrington's nuclei, and the inferior olivary complex. However, CRF mRNA was also observed in the medial habenula, subfornical organ, dentate gyrus of the hippocampus (granule and hilar cells), deep nuclei of the cerebellum, and anterior pituitary, which are not normally prominent sites of CRF expression. The hypothalamic paraventricular nucleus showed only a modest elevation of CRF mRNA, possibly as a result of feedback by the high levels of glucocorticoids. By Northern blot and *in situ* hybridyzation studies, CRF expression was increased in the testes of transgenic compared to control mice, but was not detected in liver and kidney, which commonly express other metallothionein fusion genes. Thus, the greatly elevated levels of CRF gene expression in these Cushingoid mice is manifest in a tissue distribution which is similar, but not identical, to normals, and which does not appear to be a simple consequence of targeting conferred by the metallothionein promoter.

434.5

DIFFERENTIAL REGULATION OF THE HUMAN CORTICOTROPIN-RELEASING HORMONE GENE IN JEG-3 AND PC-12 CELLS. H.Guardiola-Diaz, C.Boswell*, B.Mesa*, A.F.Seasholtz. Department of Biological Chemistry and Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109

Hypothalamic neurons mediate the mammalian stress response by integrating multiple synaptic inputs from higher brain centers and producing and secreting factors such as corticortopin-releasing hormone (CRH). Such integration at the hypothalamic level involves various second messenger systems and steroids that act by regulating transcription of the CRH gene. We are using gene transfer and DNaseI footprinting methods to localize regulatory elements in the human CRH gene. Chimeric genes containing human CRH gene 5'flanking sequence and the bacterial chloramphenicol acetyl transferasc (CAT) coding sequence were transiently transfected into JEG-3 (human choriocarcinoma) and PC-12 (rat pheochromocytoma) cells. A cAMP responsive element (CRE) has previously been identified for the rat CRH gene (-238 to -182) and is highly conserved in the human CRH gene. Addition of 25uM forskolin results in a 7 fold increase in CAT activity in JEG-3 cells and 15 fold increase in CAT activity in PC-12 cells. Transfected PC-12 cells respond synergistically to forskolin and dexamethasone treatment resulting in 35 fold increase in CAT activity. In contrast, forskolin treated JEG-3 cells show a 35% inhibition of cAMP-induced CAT activity after addition of dexamethasone. DNase I protection analysis has revealed a protected region across the CRE consensus sequence in the human CRH gene. Chimeric constructs containing a mutation in the CRE result in loss of both forskolin responsivcness and protein binding. We are presently localizing the positive and negative glucocorticoid responsive elements as well as glucocorticoid receptor binding sites in the CRH gene. This will aid in understanding the molecular mechanisms involved in the "crosstalk" between cAMP and steroid pathways that result in changes in gene expression. This work was supported by a NARSAD Young Investigators Award, and NIH Grant DK42730 to A.F.S.

434.6

TRANSCRIPTIONAL REGULATION OF THE POMC GENE BY CRF AND DEX IN THE FETAL AND NEONATAL RAT PITUITARY. R.E.M. Scott and J.E. Pintar, Dept Anat. and Cell Biol., Columbia P&S, NY, NY 10032.

POMC synthesis in the anterior lobe (AL) is under positive regulation by CRF and is inhibited by glucocorticoids, while in the intermediate lobe (IL) glucocorticoids have little effect which is thought to result from the absence of functional glucocorticoid receptors (GR) in the IL. Previous studies have shown that at late fetal and early neonatal ages dexamethasone (DEX) inhibits both CRF stimulated secretion and cAMP stimulated increases in POMC mRNA levels in both the IL and AL. These effects of DEX on the IL disappear by p3 and indicate transient GR expression in the IL during development. We have now extended these studies to show that the effect of DEX on CRF stimulation is at the transcriptional level and that transcriptional regulation of fetal POMC cells by glucocorticoids and CRF begins soon after their first appearance.

A POMC splice junction probe was used in an RNA protection assay to measure POMC hnRNA and provide an indirect measure of transcriptional change. We measured changes in POMC hnRNA in response to CRF and DEX at fetal and postnatal ages in both the AL and NIL after 1 hour treatment in vitro. At e15, POMC hnRNA in whole fetal pituitaries increased in response to CRF (3-fold) and was inhibited by DEX. At p1, in both AL and NIL, the CRF-mediated 2-fold increase in POMC hnRNA was inhibited by pretreatment with DEX and remained at the control level. By p10, DEX had no effect on CRF stimulation in the NIL and POMC hnRNA increased ~1.6 fold with or without preincubation with DEX. In the AL, CRF stimulated POMC hnRNA levels by ~1.5 fold, but if preincubated with DEX these levels remained unchanged. These results demonstrate that POMC transcription can be regulated by CRF and DEX by as early as e15 and also show that glucocorticoids effect POMC transcription in the IL during development and that this effect disappears as the IL undergoes a maturation process closely linked to the arrival of dopaminergic fibres at ~p3.

434.7

EFFECTS OF CHRONIC DEXAMETHASONE ON MOUSE POMC GENE TRANSCRIPTION FROM UPSTREAM START SITES. M. Woloschak*, J. R. Lundblad*, N. Levin, J. L. Roberts. Fishberg Research Center for Neurobiology, Mt.. Sinai School of Medicine, N.Y., N.Y. 10029.

Glucocorticoids inhibit transcription of the POMC gene by a proposed mechanism involving inhibition of the binding of activating transcription factors, such as fos and jun, to the POMC promoter by the glucocorticoid receptor. The POMC gene is transcribed in the pituitary to a major mRNA species of 1100 bases with minor larger and smaller species being detected in the pituitary as well as in many non-pituitary tissues. Recently many ACTH-secreting tumors have been demonstrated to contain larger mRNA species that comprise a relatively large percentage of the total POMC mRNA level, and that originate from transcription initiated upstream from the usual pituitary start site. In the present study, RNase protection assays have been used to examine the regulation of upstream POMC gene transcription in AtT20 cells treated with or without 100nM dexamethasone for 4 weeks. From a mouse 5' genomic POMC clone, a 840-base 5' POMC riboprobe was constructed extending from 40 bases of intron A upstream through the entire first exon and including 700 bases of the 5' promoter region. Treatment with dexamethasone significantly reduced POMC gene transcription from the usual pituitary start site relative to control as expected. Transcription from upstream start sites, however, was diminished with dexamethasone to a much lesser extent than that seen from the usual start site. These results demonstrate decreased sensitivity of upstream POMC promoters to glucocorticoids in the mouse and suggest differences in the interactions of the glucocorticoid receptor and activating transcription factors to upstream elements relative to those downstream. Since fos and jun have been shown to induce POMC gene transcription (Loeffler, Boufflier, Lorang, and Roberts, unpublished), further studies will be directed at the effects of glucocorticoids on fos gene expression in these cells.

434.8

CORTICOTROPIN RELEASING HORMONE INDUCES PROOPIOMELANOCORTIN mRNA EXPRESSION IN AtT-20 CELLS BY A c-FOS DEPENDENT MECHANISM. Mirela O.Fagarasan*, Francesca Aiello*, Katherin Muegge*, S.K. Durum* and J. Axelrod. National of Mental Health, Bethesda, M.D. and National Cancer Institute, NIH, Frederick, MD.

Corticotropin releasing hormone (CRH) causes a time dependent increase in proopiomelanocortin (POMC) mRNA levels and in adrenocorticotropin releasing factor (ACTH) and β-endorphin synthesis in AtT-20 cells. After treatment of AtT-20 cells 24 hours with CRH the amount of POMC mRNA was about 3 fold higher than control. We found that CRH induces c-fos mRNA expression in a time and dose dependent manner. The effect appeared in 15 minutes, was maximum after 30 minutes and returned to basal levels after 1 hr. CRH had no effect on c-jun mRNA expression. When antisense oligonucleotides to c-fos mRNA were introduced into the cells, the induction of POMC mRNA by CRH was abolished. Desensitization of protein kinase C by long term treatment with phorbol ester (TPA) partly abolished the ability of CRH to induce c-fos as well as POMC m-RNA expression.Thus the transient induction of c-fos mRNA by CRH appears to be a critical step in CRH induced POMC mRNA expression in AtT-20 cells and appears to be partly mediated by protein kinase C.

434.9

BASAL HYPOTHALAMIC-PITUTARY-ADRENAL AXIS FUNCTION IN THE AGED RAT Morano M.I., Vázquez D.M., Caamaño C.A., Kwak S.P., Watson S.J., Akil H. Mental Health Research Institute. University of Michigan. Ann Arbor, MI 48109.

The aged rat shows a delayed recovery to basal corticosterone levels following some stressors. We have previously demonstrated that there is a decrease in the binding capacity and mRNA levels of both glucocorticoid (GR) and mineralocorticoid (MR) receptors in the hippocampus of aged Fisher-344 rats, although the basal plasma corticosterone levels are similar to those in young rats. In order to further investigate the consequences of this loss of GR and MR, we have examined the basal status of the HPA axis in young (6 mo.) and old (27 mo.) male rats. CRF mRNA content in the paraventricular nucleus (PVN) was quantitated by RNase protection assay. Corticosterone, ACTH, β-endorphin (βE) and Nacetyl βE were measured in plasma. ACTH, βE or NAc βE and POMC mRNA contents were also measured in the pituitary anterior and neurointermediate lobes (AL and IL). Quantitation of POMC peptide biosynthesis was determined by [3H]Leu pulse labeling and chase of AL and IL dipersed cells, followed by immunoprecipitation and SDS-PAGE. CRF mRNA content is similar in the PVN of young and old rats. In contrast, POMC mRNA levels are lower in the AL of old animals [Y= 100 ± 8% vs O= 59 ± 9%, $p < 0.005$]. However, the POMC peptide levels are not changed in the AL and plasma of aged rats with respect to young ones. After 15 min pulse, *de novo* AL POMC synthesis of aged animals is similar to that of young rats although there are lower levels of POMC mRNA, suggesting an increase in the POMC translation rate. POMC processing is similar in the AL of young and old rats. All the parameters studied in IL are unaltered in the aged animals. We conclude that: 1) although aged rats have lower hippocampal GR and MR binding capacities, the CRF mRNA content of the PVN is normal; 2) AL POMC mRNA levels are 40 % decreased in the older animals; 3) the increase in AL POMC biosynthetic efficiency in old rats may account for the maintenance of a basal HPA function similar to that observed in young ones. Supported in part by MH42251, DA02265 and Markey Foundation.

434.10

CRH, ACTH, AND CORTICOSTERONE INDUCE THE C-FOS PROTO-ONCOGENE IN RAT BRAIN. K. Nudelman*, F.R. Sharp, and S.M. Sagar. Dept. of Neurology, Univ. of California, San Francisco, CA 94121.

We have shown that adrenalectomy (Jacobson et al., Endocrinology, 1990) induces Fos in PVNp neurons containing CRF; and stress induces c-fos mRNA in cortex, septum, hypothalamus, and other regions (Sharp et al., J. Neurosci., 1991). The present experiments examine whether stress hormones induce c-fos. A catheter was inserted into the femoral veins of anesthetized rats. 1d later subjects had 0.9% NaCl, CRH, ACTH, corticosterone (CCX), or dexamethasone (DX) injected IV. 2h after injection subjects were anesthetized, perfused, and the brains processed for immunocytochemistry using a monoclonal antibody (MicoAss) specific for Fos. Midline thalamic nuclei and scattered cortical neurons were stained in NaCl injected rats. CRH (10-120 ug/kg) and ACTH (.1-1mg/kg) induced Fos in posterior cingulate & retrosplenial cortex; lateral septum; stria terminalis; caudal ventral striatum; the central, lateral, and basolateral nuclei of amygdala; PVNp, PVNm and SON in some rats, preoptic, anterior, and posterior hypothalamus; nucleus tractus solitarius; and locus coeruleus. DX and CCX tended to induce Fos in the same regions except for SON, PVNm, and central nucleus of amygdala. Release of ACTH and CCX could account for stress induction of c-fos in some neurons.

434.11

11ß-HYDROXYSTEROID DEHYDROGENASE (11ß-OHSD) mRNA EXPRESSION IN PARAVENTRICULAR NUCLEUS: ENZYME INHIBITION REDUCES PORTAL BLOOD CRF. J.R. Seckl, R.C. Dow*, S.C. Low*, C.R.W. Edwards* and G. Fink, Dept Medicine, Edinburgh Univ. and MRC Brain Metabolism Unit, Edinburgh, UK.

Corticosteroid feedback regulation of the hypothalamic-pituitary-adrenal axis is imperfectly understood with differences in the effects of physiological and synthetic glucocorticoids. 11ß-OHSD rapidly metabolizes physiological glucocorticoids to inactive products, thus acting as a tissue-specific regulator of glucocorticoid access to and activation of peripheral mineralocorticoid and glucocorticoid receptors. 11ß-OHSD bioactivity and mRNA are also present in the brain where 11ß-OHSD might alter corticosteroid-receptor interactions. Using *in situ* hybridization with cRNA probes we found high expression of 11ß-OHSD mRNA in magnocellular and parvicellular neurons in the paraventricular nucleus, where corticotrophin-releasing peptides are synthesized. Administration of glycyrrhetinic acid (GE; a potent inhibitor of peripheral and brain 11ß-OHSD) to anaesthetized male rats, significantly decreased CRF release into hypophysial portal blood over the next 90 min (260±36 pg/45 min in controls, n=15; 123±24 pg/45 min after GE, n=10). This occurred in the presence of unchanged circulating corticosterone levels, suggesting that inhibition of 11ß-OHSD activity increases the effective intracellular feedback signal of corticosterone within paraventricular CRF neurons. Adrenalectomy reversed the fall in CRF induced by GE (241±54 pg/45 min) confirming dependence of the GE effect on adrenal products. Paraventricular 11ß-OHSD represents a novel and important level of control of early-intermediate feedback of physiological glucocorticoids on hypothalamic CRF release *in vivo*.

434.12

CALMODULIN mRNAs' IN RAT BRAIN: DISTRIBUTION AND REGULATION BY GLUCOCORTICOIDS. M. N. Gannon and B. S. McEwen. Lab. of Neuroendocrinology, Rockefeller University, New York, NY 10021.

Manipulation of the hypothalamic-pituitary-adrenal axis selectively alters Ca^{2+}/calmodulin (CaM)-dependent adenylate cyclase (AC) activity in rat brain. Since many effects of glucocorticoids are mediated through a genomic mechanism, we have attempted to determine whether changes in AC activity are secondary to, or associated with, alterations in CaM mRNA levels. Selective oligonucleotide probes for CaM mRNAs' derived from 3 separate genes (I, II, and III) were developed. By northern analysis, the probes identified a heterogeneous tissue distribution of mRNAs' for CaMI, CaMII and CaMIII, with sizes consistent with those found using cDNAs. Interestingly, *in situ* hybridization analysis, at the level of the dorsal hippocampus, revealed that all species of CaM mRNA were high in both hippocampus and cortex, and (at the light microscope level) appeared to be colocalized. Preliminary evidence (n = 5) suggests that ADX attenuates CaMIII mRNA levels in both brain regions, and that this effect is prevented by daily administration of corticosterone (50 mg/Kg ip.). ADX effects on CaMI and CaMII, if any, are currently under investigation. These results support a growing body of evidence implicating a role for glucocorticoids in modulating Ca^{2+}-dependent signal transduction mechanisms in brain.

Supported by NIMH MH-41256 (B.M) and a NARSAD Young Investigator Award (MNG).

ION CHANNELS: MODULATION AND REGULATION III

435.1

POTENTIATION OF PRESYNAPTIC CALCIUM INFLUX BY ELEVATION OF CYCLIC AMP. Ruth Heidelberger and Gary Matthews, Dept. of Neurobiology, SUNY, Stony Brook, NY 11794-5230.

Retinal bipolar cells are non-spiking interneurons that relay information from photoreceptors to amacrine and ganglion cells. Previously we have shown that depolarization induces Ca influx in synaptic terminals and somata of goldfish Mb1 bipolar neurons via L-type Ca channels (Heidelberger & Matthews, Invest. Ophth. Vis. Sci., 31, 389, 1990). Here, we present data on the modulation of this dihydropyridine-sensitive influx pathway by agents known to elevate cAMP. Goldfish bipolar cells were isolated after papain treatment, and incubated with fura-2AM for monitoring $[Ca]_i$. Cells were depolarized by elevated $[K]_o$. Depolarization-induced Ca influx following a 200 second exposure to a test compound was compared to the control response to high $[K]_o$. Peak increase in $[Ca]_i$ following incubation with 2-5 µM forskolin was 198 ± 23% of the control (mean ± s.e.m.,N=27). 1,9-Dideoxyforskolin, a forskolin analog that does not activate adenylate cyclase, had no effect on Ca-influx (93 ± 17% of control; mean ± s.e.m.,N=8). Peak Ca-influx in terminals after 1 mM CPT-cAMP, a membrane-permeant derivative of cAMP, was 227 ± 29% of control (mean ± s.e.m.,N=21). Dopamine (10 - 100 µM), which elevates cAMP levels in retina, increased depolarization induced Ca-influx in terminals to 194 ± 16% of control (mean ± s.e.m.,N=15). The potentiation of Ca influx after elevation of cAMP was seen in 81% of cells. Enhancement of influx was slowly reversible, often requiring more than 10 minutes. These results suggest that cAMP regulates the Ca channels in bipolar neurons, and therefore that transmitters such as dopamine may modulate release of neurotransmitter from bipolar-cell synaptic terminals. (Supported by NIH grant EY03821, NIMH Training Grant MH18010)

435.2

DUAL MODULATION OF A HYPERPOLARIZATION-ACTIVATED Cl^- CURRENT BY cAMP AND cGMP IN APLYSIA SENSORY NEURONS. B. Armitage, N. Buttner and S.A. Siegelbaum. Ctr. Neurobiol. and Behavior, Columbia U., New York, NY 10032

In Aplysia sensory neurons, the hyperpolarization-activated Cl^- current (Chesnoy-Marchais, Nature 1982) is increased by FMRFamide, 8-Br-cGMP, and 8-Br-cIMP in whole cell recordings (540 Cl^- inside, 600 Cl^- outside) (Buttner and Siegelbaum, Soc. Neurosci. Abstr., 1990). Here, we show the antagonistic modulation of this Cl^- current by dual second messengers.

While FMRFamide and cGMP/cIMP activate the Cl^- current, the neuropeptide SCP and 8-Br-cAMP inhibit the same Cl^- current. This inhibtion is probably mediated by cAMP-dependent protein kinase since the kinase inhibitor K252A (10 uM) decreases the response to SCP by 70% (n=4) and to 8-Br-cAMP by 60% (n=3). Activation of the Cl^- current is likely to result from activation of a cGMP-stimulated cAMP phosphodiesterase (PDE) which reduces cAMP since: 1. 8-Br-cIMP activates this PDE but not cGMP-dependent protein kinase, and 2. The PDE inhibitors IBMX and RO 20-1724 inhibit the response to FMRFamide, 8-Br-cGMP, and 8-Br-cIMP. To test whether the sensory neurons can synthesize cGMP, we applied zaprinast, a specific inhibitor of cGMP phosphodiesterase, and Na nitroprusside (1mM), which releases nitric oxide and activates guanylate cyclase in other systems. Both zaprinast and Na nitroprusside activate the Cl^- current. In addition, Na nitroprusside elevates cGMP levels in sensory neurons by 40%.

435.3

MODULATION OF ACETYLCHOLINE CHANNEL BY DBcAMP IN XENOPUS CELL CULTURE. W.M. Fu and M.M. Poo. Department of Pharmacology, College of Medicine, National Taiwan University, Taipei, Taiwan and Department of Biological Sciences, Columbia University, New York, N.Y. 10027.

Application of a membrane-permeable form of cAMP and agents that activates adenylate cyclase system to isolated embryonic Xenopus myocyte in culture markedly altered the ACh sensitivity of the muscle membrane, as assayed by focal extracellular iontophoresis of ACh: the ACh-response is elevated and accompanied by an increase rate of desensitization. Singel channel recordings showed that activation of adenylate cyclase system increases opening probability and the mean open time of ACh-channels, without affecting the single channel conductances. These effects on ACh channels disappeared in older isolated myocytes as well as at extrasynaptic regions of innervated myocytes, suggesting the confinement of susceptibility to the synaptic regions following innervation. Possible involvement of cAMP second messenger system in the physiological modulation of synaptic functions was further supported by the markedly enhancement of spontaneous activity at developing Xenopus neuromuscular synapses by agents that activates the adenylate cyclase system. Neuropeptides co-released with ACh, if acts through adenylate cyclase system, could thus help the synaptic maturation by elevating the efficacy of the synapse.

435.4

PHOSPHORYLATION MODULATES THE RATE OF VOLTAGE-DEPENDENT INACTIVATION OF THE BARIUM CURRENT IN DISSOCIATED HELIX NEURONS. J.L. Yakel, Laboratoire de Neurobiologie, Ecole Normale Superieure, 75005 Paris, France.

Whole-cell recording techniques were used to investigate how phosphorylation modulates the rate of voltage-dependent inactivation of the voltage-activated barium current in Helix neurons. The amplitude of the barium current rapidly ran down while the rate of inactivation greatly increased if either (1) neurons were not dialyzed with ATP, or (2) neurons were dialyzed with alkaline phosphatase. ATPγS dialysis partially reduced the rate of run-down in non ATP-dialyzed neurons, and dramatically reduced the rate of both activation and inactivation of the barium current. Dialysis with the protein phosphatase inhibitor okadaic acid enhanced the amplitude and decreased the rate of inactivation of the barium current, without significantly altering the rate of activation. Dialysis with either AMP-PNP, the catalytic subunit of protein kinase A, or synthetic peptide inhibitors of protein kinases A or C did not significantly alter the amplitude or kinetics of the barium current, whereas dialysis with a synthetic peptide inhibitor of the calcium/calmodulin-dependent protein kinase significantly reduced the rate of inactivation. Therefore, the rate of voltage-dependent inactivation of the barium current in Helix neurons is modulated by the state of phosphorylation of either the calcium channels themselves or an associated regulatory protein.

435.5

SYNERGISTIC ACTIVATION OF PROTEIN KINASE C BY ARACHIDONIC ACID AND OAG REDUCES *HERMISSENDA* POTASSIUM CURRENTS. R. Etcheberrigaray, C. Collin, D. S. Lester* and D. L. Alkon. *Neural Systems Section*, NINDS, National Institutes of Health, Bethesda, MD 20892.

It has been shown that protein kinase C (PKC) can be activated by either diacylglycerol (DG) or arachidonic acid (AA). The concentrations of these lipid activators commonly used to activate PKC are relatively high compared to physiologic levels. In vitro studies (Lester *et al.*, Society for Neuroscience 1991) have indicated that submaximal activating concentrations of AA and DG act synergistically to activate PKC. In the present study, we further test this hypothesis of synergistic PKC activation in a cellular system. For this purpose, we used the *Hermissenda* B photoreceptor known to have at least two PKC-regulated K^+ currents (I_A & I_C). Two-electrode voltage-clamp measured both I_A and I_C in the axotomized Hermissenda B cell. Bath application of the cell permeant diacylglycerol analog, 1-oleoyl-2-acetylglycerol (OAG 5 μgml^{-1}) did not have significant effects on the two K^+ currents after 15 min. incubation. At 30 min. OAG, a 25% increase of I_A amplitude was observed, I_C remained virtually unchanged. Staurosporine had no effect on the I_A enhancement, suggesting that this OAG effect may not be PKC mediated. Application of 5 μM AA had no effect on both I_A and I_C. Application of 5 OAG μgml^{-1} followed by 5 μM AA 15 min. later, caused a strong reduction of K^+ conductances. A 57% amplitude reduction was observed for I_A, and I_C amplitude decreased in 44%. Staurosporine blocked these effects, suggesting that these OAG-AA effects are PKC mediated. PKC regulation of these same K^+ currents has been shown to induce responses similar to those found for classical conditioning. This OAG-AA synergistic effect may, therefore be an important step in this process.

435.6

CANNABINOIDS INHIBIT A HIGH-THRESHOLD CALCIUM CURRENT. K. Mackie and B. Hille. Physiol. and Biophysics, Univ of WA, Seattle, WA 98195.

Medicinal properties of *Cannabis sativa* and its major biologically active constituent, Δ^9-tetrahydrocannabinol (THC), have been known for years. Recently a high-affinity THC receptor has been identified that has a CNS distribution consistent with the behavioral effects of THC. The cellular mechanisms of action of THC are uncertain. One action of THC is to inhibit adenylyl cyclase (AC). As neurotransmitters that inhibit AC often affect ionic channels by other pathways, we have investigated a link between cannabinoid receptors and calcium channels. In NG108-15 cells, the cannabinomimetic alkylaminoindole, WIN 55,212-2, potently, stereospecifically and reversibly inhibited a component of the high-threshold voltage-sensitive I_{Ca}. Maximal inhibition of I_{Ca} was 40% at 100 nM WIN 55,212-2. A similar decrease was found with the non-classical cannabinoid, CP 55,940 (100 nM). The inhibition is not likely to be due to a decrease in cAMP level as prior incubation with 8-(chlorophenylthio)cAMP (100 μM), dibutyryl cAMP (1 mM) and/or isobutylmethylxanthine (100 μM) did not prevent I_{Ca} inhibition by WIN 55,212-2. The components of I_{Ca} inhibited by cannabinoids are not entirely the same as the I_{Ca} inhibited by norepinephrine (NE) and adenosine (ADO) in these cells. WIN 55,212-2 inhibited a subset of the I_{Ca} inhibited by NE (1 μM) with a slower onset of inhibition. On the other hand, WIN 55,212-2 and ADO (50 μM) inhibited distinct I_{Ca}'s, however the onset of inhibition was similar for the two compounds.

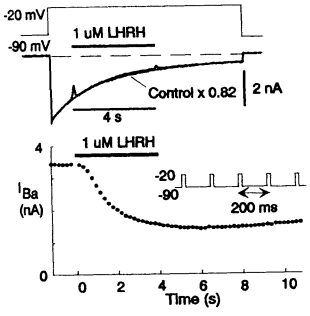
Given the central role of calcium channels in neurotransmitter release and in patterned firing of action potentials, it is possible that inhibition of these channels contributes to the profound behavioral changes produced by THC.

Supported in part by GM07604, NS08174, and by a Research Award from the McKnight Foundation.

435.7

Inhibition by LHRH of N-type Ca current in bullfrog sympathetic neurons: kinetics and state-dependence. L.M. Boland and B.P. Bean, Department of Neurobiology, Harvard Medical School, Boston, MA 02115

Inhibition by luteinizing hormone releasing hormone (LHRH) of Ca channel current was studied using whole-cell recordings from freshly dissociated bullfrog sympathetic ganglion neurons. LHRH acts through activation of G-proteins to inhibit N-type but not L-type channels. The rate of development of inhibition is faster at higher [LHRH], reaching $\tau \sim$ 2 sec at saturating [LHRH] of 0.3-3 uM. Recovery proceeds with $\tau \sim$ 19 sec and is not concentration-dependent. LHRH inhibition is strongly affected by the gating state of the channel: LHRH has little effect if applied during a long activating voltage pulse but strongly inhibits if applied when channels are mostly in the resting state. Inhibition can be partly overcome if channels are activated by short, large depolarizations or long, smaller depolarizations. The state-dependent properties are predicted by a model that assumes: 1) Inhibition results from activated G-proteins binding to the channel, 2) G-protein binding stabilizes closed gating states, and 3) activation of G-protein bound channels destabilizes G-protein binding.



435.8

WITHDRAWN

435.9

SINGLE CHANNEL PROPERTIES OF SOMATOSTATIN- OR MET - ENKEPHALIN - ACTIVATED, GTP - DEPENDENT INWARDLY RECTIFYING K-CONDUCTANCE IN CULTURED RAT BRAIN NEURONS. J.J.Grigg, T.Kozasa*, S.Nakajima and Y.Nakajima, Dept. of Anat. & Cell Biol. and Dept. of Pharmacol., Univ. of Illinois, College of Medicine, Chicago, IL 60612.

Somatostatin (SOM) and μ opioid receptors are coupled to inwardly-rectifying K-channels through a pertussis toxin-sensitive G protein in locus coeruleus neurons (North et al. PNAS, 84:5487,'87; Inoue et al., J. Physiol. 407: 177,'88). We have investigated the single channel properties of these K-channels in cultured noradrenergic neurons from the locus coeruleus, using inside-out patches. When the solution bathing the cytoplasmic face was switched from a GDP-containing to a GTP-containing solution, there was an increase in channel activity if the pipette contained either SOM or met-enkephalin. This increase in activity did not occur in the absence of agonists. At potentials more depolarized than 0 mV, GTP did not produce an increase in channel activity, suggesting an inward rectification of the single channel current. Supported by AG06093 and DA05701.

K_i = 125 mM / K_o = 100 mM; 0.5 μM SOM

435.10

MODULATION OF G PROTEINS AND HETEROLOGOUS DESENSITIZATION FOLLOWING CHRONIC MUSCARINIC AND α_2-ADRENERGIC AGONIST TREATMENT. Z.Vogel, D. Saya*, B. Attali* and S.-Y. Nah*. Dept. of Neurobiology, Weizmann Institute of Science, Rehovot 76100, Israel.

Muscarinic and α_2-adrenergic agonists inhibit adenylate cyclase and the dihydropyridine-sensitive voltage-dependent Ca^{2+} channels in rat spinal cord-dorsal root ganglion cocultured cells. For example, oxotremorine and clonidine, at 100 μM, inhibited $^{45}Ca^{2+}$ influx through the channels by 35±6 and 42±2%, respectively. This inhibition was reversed by the respective antagonists, atropine and yohimbine, and by pretreatment with pertussis toxin. Chronic treatment (≥24h) with oxotremorine or clonidine desensitized the Ca^{2+} channel response to both muscarinic and α_2-adrenergic agonists (i.e., heterologous desensitization). In addition, it led to a marked reduction (≥40%) in the capacity of $G\alpha_i$ and $G\alpha_o$ subunits to undergo pertussis toxin-catalyzed ADP-ribosylation. No difference was found in cholera toxin-induced ADP-ribosylation of $G\alpha_s$. The reduction in pertussis toxin ADP-ribosylation was attenuated when the concentration of Lubrol PX in the reaction mixture was increased above 0.01%. We propose that prolonged agonist exposure leads to alterations in the receptor-G protein transduction system, which in turn accounts for the heterologous desensitization and reduction in ADP-ribosylation. *Supported by the Israel Anti-Drug Authority, Minerva Foundation, National Institutes of Health and the German-Israel Foundation for Scientific Research and Development.*

435.11

REGULATION OF Kv1 CHANNEL GENE EXPRESSION IN CLONAL AND NATIVE PITUITARY CELLS. E.S. Levitan, J. Miklos*, C. Severns*, and B. Attardi*. Depts. of Pharmacology and Medicine, Univ. of Pittsburgh, Pittsburgh, PA 15261

We have been exploring the hypothesis that transmitters and hormones produce long-term changes in cell excitability and secretion by regulating ion channel gene expression. Previously, we reported that dexamethasone (DEX), a glucocorticoid receptor (GR) agonist that **suppresses** secretion of many pituitary hormones, **increases** voltage-gated K^+ current and mRNA encoding the Kv1 K^+ channel subunit in GH3 rat pituitary tumor cells. We have now investigated the role of GR activation, and have identified a neuropeptide that may have an opposing effect. Kv1 RNA is increased with corticosterone (COR) and hydrocortisone (HC). This induction is inhibited by the GR receptor antagonist RU38486 and is not seen with estradiol or testosterone. To explore whether this gene regulation is physiological, native rat pituitary cells were dissociated, cultured for 3 days, and then treated with steroids. 6 hour treatments with DEX or COR increased Kv1 mRNA ~3-fold. This induction was blocked by RU38486 and was not mimicked by estradiol. Therefore, GR activation increases K^+ channel gene expression in clonal and native pituitary cells. In contrast to the glucocorticoids, the neuropeptide TRH, which **increases** secretion, **decreased** Kv1 mRNA in GH3 cells in a preliminary experiment. This suppression was also seen with BAYK 8644+40 mM K^+, suggesting that cytoplasmic calcium may inhibit expression of Kv1. Thus, glucocorticoids and TRH may have opposing effects on secretion and K^+ channel gene expression.

435.12

DEVELOPMENT OF TWO TRANSIENT POTASSIUM CURRENTS IN CULTURED MOUSE HIPPOCAMPAL NEURONS. R-L. Wu* and M.E. Barish, Division of Neurosciences, Beckman Research Institute, City of Hope, Duarte, CA 91010.

We studied the potassium currents present in mouse hippocampal pyramidal neurons in dissociated cell cultures derived from embryonic day 15 embryos. Potassium currents were isolated in whole-cell gigaohm seal recordings by using a KCl/KF-based internal solution to remove Ca currents, and TTX in external solutions to block Na currents. Control experiments confirmed that the remaining voltage-gated K currents were insensitive to removal of external Ca or addition of sufficient Cd to block Ca currents. Two transient outward currents were separated based on voltage-dependence and sensitivity to 4-aminopyridine (4-AP). A-current showed fast activation and inactivation, negatively-positioned steady state inactivation, and block by millimolar concentrations of 4-AP. D-current showed slower activation and inactivation, more positively-positioned steady state inactivation, and block by 100 μM 4-AP. An additional slowly inactivating delayed rectifier (K-current) was sensitive to TEA. All outward current was blocked by a combination of conditioning depolarization to -40 mV and addition of 100 μM 4-AP and 20 mM TEA to the bath, indicating that under our recording conditions outward current could be accounted for by the combination of A-, D- and K-currents.

We observed that during the three day period following day 0 (the day of dissociation), as total outward current amplitude and whole cell capacitance increased, inactivation of total potassium current slowed and the aggregate steady state inactivation curve shifted towards more positive voltages. Separation of the various potassium currents indicated that during this period the amplitude of A-current declined while that of D-current increased. These changes in potassium currents, in combination with maturation of Na and Ca currents, may underlie developmental changes in responses of hippocampal neurons to depolarizing stimuli. (Supported by NIH, AHA and BRI/CoH).

REGULATION OF AUTONOMIC FUNCTIONS

436.1

VIBRATORY STIMULATION (VS) AND RECTAL PROBE ELECTROSTIMULATION (RPE) OF SPINAL CORD INJURED (SCI) MEN. N.L. Brackett and C.M. Lynne*. The Miami Project to Cure Paralysis, University of Miami, Miami, FL 33136

Men with spinal cord injuries are often impotent and infertile due to erectile and ejaculatory dysfunction. The present study compared the quality of erections, ejaculations and semen using the methods of VS or RPE. These measures were correlated to the subjects' neurological level and completeness of injury as well as other variables.

Thirteen SCI men were stimulated a total of 35 times. Five subjects could ejaculate with either method, five with only RPE, one with only VS and two with neither method. The ability to ejaculate using the vibrator correlated more with completeness than with level of injury. In those subjects who ejaculated, both antegrade and retrograde samples were recovered in most cases. Antegrade ejaculations produced by VS tended to be forcefully expelled compared to those produced RPE which looked more like emissions. Occurrence of erections prior to stimulation did not predict occurrence or quality of erections during stimulation. Subjects reported pleasurable feelings associated with orgasm more often when VS rather than when RPE was used. Serum levels of follicle stimulating hormone, luteinizing hormone, prolactin and testosterone ranged from low-normal to normal with the exception of three subjects in which one or more of these hormones fell below normal ranges. Higher sperm counts tended to occur with RPE compared to VS. Sperm motility was low in almost all subjects and did not change with the type of stimulation used. The normative data collected in this study are intended as a basis from which to design future studies investigating the causes and treatments of male impotence and infertility following SCI.

436.2

REDUCED URINARY BLADDER AFFERENT CONDUCTION VELOCITIES IN STREPTOZOTOCIN DIABETIC RATS. I. Nadelhaft and P. Vera. VA Med. Ctr. & Univ. Pittsburgh Med. Sch. Depts. of Neurosurgery and Pharmacology, Pittsburgh, PA, 15240, USA.

Previous experiments in our laboratory have described the method used to measure the conduction velocity distribution of a selected group of fibers (Br.Res.520:83-89[1990]). We have applied this technique to the diabetic rat. Ten female Sprague-Dawley animals were made diabetic with streptozptocin (60 mg/kg iv). Six matched animals were used as controls. Two diabetics died shortly after their injections but the remaining animals survived to the completion of the experiment (approximately two months). Glycosylated hemoglobin values measured at the time of death were 17.19 +/- 4.74 % (diabetic, n = 8) and 4.07 +/- 0.74 % (controls, n = 6). Diabetic bladders were thicker and heavier. The wet weights were 0.50 +/- 0.11 gm (diabetic, n = 7) and 0.16 +/- 0.01 gm (controls, n = 6). The conduction velocities of a total of 151 and 86 single afferent fibers were measured in the diabetic and control animals respectively. The conduction velocity distribution of the diabetics showed a shift towards slower speeds when compared to controls. The mean conduction velocities were 1.70 m/s for diabetics and 2.84 m/s for controls. The percent of units with conduction velocities greater than 2.5 m/s was 11.3 for diabetics and 27.9 for controls. This experiment demonstrates, for the first time, that diabetes causes a significant reduction of afferent conduction velocities in a functionally well-defined system.

436.3

GALANIN IMMUNOREACTIVITY IS SEXUALLY DIMORPHIC IN AUTONOMIC REGIONS OF THE RAT LUMBOSACRAL SPINAL CORD. B.W. Newton. Department of Anatomy, University of Arkansas for Medical Sciences, Little Rock, AR 72205

Galanin-like immunoreactive (GAL-LI) fibers have been demonstrated to surround preganglionic autonomic neurons; and a large group of GAL-LI neurons, which project to the thalamus, is found in lumbar laminae VII and X (Ju et al., Neuroscience, 20:439, '87). Additional characterization of the distribution of GAL-LI in the rat spinal cord reveals pronounced sexual dimorphism in the lumbosacral autonomic spinal cord. Ten male and ten female adult Sprague-Dawley rats were perfused for the PAP technique in order to reveal spinal GAL-LI (anti-GAL diluted 1:10,000; Peninsula Labs., Belmont, CA #RAS-7153-N). GAL-LI was not sexually dimorphic in the thoracic spinal cord. However, GAL-LI fibers in autonomic regions of the lumbosacral spinal cord were more abundant in males than females. The females showed a marked variation in the number of GAL-LI fibers: this variation was subjectively classified as either "average" or "heavy". The variation is probably due to fluctuations in estrogen levels during the estrous cycle. However, the number of GAL-LI fibers in any female never exceeded the number found in males. The number of previously described lumbar GAL-LI neurons was also sexually dimorphic: males have significantly ($P < 0.01$) more GAL-LI neurons (62%) than females. The number of GAL-LI neurons in females was not variable. The physiological significance of sexually dimorphic, autonomic, GAL-LI is presently unknown. Supported by BRSG RR05350 and the Arkansas Caduceus Club.

436.4

IN VITRO NEONATAL RAT: INTRACELLULAR RESPONSES OF BRAINSTEM NEURONS TO SUBDIAPHRAGMATIC VAGAL STIMULATION. C.S. Yuan* and W.D. Barber. Department of Anatomy, College of Medicine, University of Arizona, Tucson, AZ 85724.

We developed an *in vitro* neonatal rat preparation to investigate brainstem neuronal processing of subdiaphragmatic vagal input. (Barber et al., FASEB J. 5(5): A1063, 1991). Neonatal rats, 0 to 2 days of age, were anesthetized with halothane. Following a craniotomy, the brainstem was transected at the rostral medulla and the stomach with intact vagi and caudal brainstem was isolated. The preparation was superfused in a recording chamber with modified Krebs solution. The subdiaphragmatic vagal nerves were electrically stimulated by suction electrodes. Intracellular potentials, identified by orthodromic spikes resulting from supramaximal vagal stimulation, were recorded from neurons in the nucleus tractus solitarius (NTS). Electrical stimulation of the subdiaphragmatic vagal trunks produced EPSPs in some neurons but failed to produce an action potential. Some neurons discharged spontaneously, others were "silent". The background synaptic activity of some NTS neurons consisted of both EPSPs and IPSPs with amplitudes of more than 3.0 mV. The synaptic activity increased for a period of 100-300 msec following vagal stimulation. This *in vitro* neonatal preparation retains the functional circuitry to investigate brainstem neuronal responses to subdiaphragmatic vagal input without the effects of general anesthesia. (Supported by USPHS Grants NS 27972 and DK 36289).

436.5

ORGANIZATION OF MEDULLARY AUTONOMIC REFLEX ARCS. M. Anwar, D.J. Reis and D.A. Ruggiero. Div. Neurobiol., Dept. Neurol. & Neurosci., Cornell Univ. Med. Coll., NY, NY 10021.

Medullary autonomic reflex arcs are composed of 1° visceral afferents ending in nucleus tractus solitarii (NTS), relaying by direct and indirect pathways to autonomic preganglionic and respiratory lower motoneurons. Polysynaptic limbs of reflex arcs are thought to involve interneurons in the lateral tegmental field (LTF). We sought to define interneuronal networks in the medullary LTF and their relationships to the solitario-reticulospinal (transtegmental) tract bridging NTS and autonomic premotor pools in the ventrolateral medulla (VLM). In adult male anesthetized (0.5 mg/kg chloral hydrate, i.p.) rats, injections of two retrograde fluorescent tracers (rhodamine microbeads and FluoroGold) were centered on NTS and the rostral ventrolateral reticular nucleus (RVL); distributions of single and double-labeled neurons were mapped in the medulla. NTS and RVL afferent projection neurons were backfilled along elongate sheets concentrated in the intermediate reticular zone of LTF; most formed independent populations. Neurons colocalizing both tracers were detected in a dorsal reticular zone (LTFd) subjacent to NTS. PHA-L injections into LTFd confirmed divergent projections to NTS and VLM. Methods combining anterograde transport of PHA-L with retrograde tracers were developed to determine relationships between the solitario-reticular tract (SRT) and RVL afferents. RVL-fluorescent interneurons were distributed throughout LTF; and embedded within immunofluorescent fibers of SRT. An interstitial nucleus of the SRT extending from the NTS to VLM may be an anatomical substrate of multisynaptic autonomic reflex arcs.

436.6

EFFECT OF SOMATOSTATIN-28 (SS) ON VAGAL CARDIOMOTOR NEURON (VCN) FUNCTION M.M. Caverson, C.M. Willey* and E.T. Kiriakopoulos, Depts. of Anatomy & Physiology, Univ. of Western Ont., London, Canada N6A 5C1.

We have shown that SS-immunoreactive fibers are located around VCN in nucleus ambiguus (AMB). The present studies investigated the source of SS afferents to AMB and the effect of SS on VCN function. Experiments were done in adult male Sprague-Dawley rats. In the first series, rats were anesthetized with pentobarbital, the femoral artery was cannulated and heart rate (HR) was monitored. Double-barrel glass micropipettes were placed in AMB, cardiomotor regions were identified by the vagal bradycardia evoked by L-glutamate microinjections (10-30 nl;1M), and these sites received injections of the retrograde tracer Fluorogold (2%;10 nl). After 7-10 days animals were colchicinized, perfused 8-10 hrs later, and the forebrain and brain stem were processed using immunofluorescence to identify neurons containing FG and SS. Double-labeled neurons were found in the central amygdaloid nucleus, medial parvocellular divisions of the hypothalamic paraventricular nucleus and the central nucleus of the NTS. In the second study, animals (n=32) were anesthetized with alpha-chloralose, artificially ventilated and spinal cord transected at C_2-C_3. The femoral artery and vein were cannulated and arterial pressure (AP) and HR were monitored. Microinjections of L-glutamate were made to identify cardiomotor regions of AMB and these sites subsequently received injections of SS (20-100 nl;5-10mmol). SS consistently elicited a slow onset (6s-3.5min), long-duration (10-50min) increase in HR that was not accompanied by a change in AP. The HR response to SS was +22±12 bpm (5-60 bpm) from a control HR of 256±30 bpm. Control injections of 0.9% saline (n=8) did not alter HR. These data suggest that SS inhibits VCN function and that limbic, hypothalamic and/or medullary SS-containing cell groups projecting directly to cardiomotor regions of AMB may provide the anatomical pathways by which these SS effects are mediated. (Supported by the MRC).

436.7

EXCITATORY EFFECTS OF THYROTROPIN-RELEASING HORMONE (TRH) IN DORSAL MOTOR NUCLEUS OF THE VAGUS (DMV) MOTONEURONS. R.A. Travagli*, R.A.Gillis*, Y.M. Hernandez, and S.Vicini. Dept. of Pharmacology and FGIN, Georgetown Univ., Wash., DC 20007

The purpose of our studies was to characterize the excitatory effect of TRH in DMV motoneurons by using the patch-clamp technique in rat brainstem slices. In our initial studies we employed the cell-attached configuration using concentrations of TRH ranging from 3 to 30 μM. Exposure of DMV motoneurons to TRH resulted in dose-related increases in spontaneous occurring action potential firing rate (2- to 4-fold increase at the maximal TRH concentration tested). This was observed in all the DMV neurons tested (N=21) independently of their location rostral or caudal to the obex. Invariably, tachyphylaxis occurred to the excitatory effects of TRH. Subsequent experiments using current clamp confirmed the observation that TRH excites DMV neurons located rostral to the obex. However, excitation of DMV neurons located caudally to the obex was not always observed using this recording configuration. Finally, using voltage-clamp recordings from DMV neurons revealed the induction of an inward current of 7.8 ± 1.4 pA (N=5), by TRH. This current was associated with a 10% decrease in input resistance. This effect might be related to the activation of a cationic channel by TRH in DMV neurons. We also observed that TRH produced an increase in frequency of spontaneously occurring excitatory postsynaptic currents, and, in some cases, a decrease in spontaneous inhibitory postsynaptic currents in rostral and caudal DMV neurons.

436.8

EXPRESSION OF C-FOS IN NEURONS OF THE FETAL BRAINSTEM DURING HYPOXEMIA IN THE SHEEP. G.E. Hoffman, T.J. McDonald *, A. Syed, and P.W. Nathanielsz. Depts. Physiol. and Behav. Neurosci., Univ. Pittsburgh, Sch. Med., Pittsburgh, PA 15261 and Lab. for Preg. and Newborn Res., Dept. of Physiol. Cornell Univ., Ithaca, NY 14853.

The fetal pituitary adrenal axis is responsive to hypoxemia; fetal ACTH levels rise and c-fos expression in corticotrophin releasing hormone (CRH) neurons as well as other neurons in the paraventricular nucleus (PVN) of the hypothalamus is induced. Within the brainstem, the ventrolateral medulla and nucleus of the solitary tract (NTS), are sensitive to hypoxia and can influence the hypothalamus. Using c-fos as a marker for neuronal stimulation we examined the relationship between activation of these brainstem centers (including catecholamine neurons within them) and the PVN. Fetuses (123-125 days gestational age) were made hypoxemic by reducing the maternal oxygen to 60% of normal for 20-60 min and the brains were perfused and immunocytochemically stained for c-fos and CRH (hypothalamus)or DβH (brainstem). Following hypoxemia, the induction of c-fos in CRH and other PVN neurons neurons was accompanied by a marked activation of c-fos in the ventrolateral medulla and NTS; activation included catecholamine neurons in the A1/C1 and A2/C2 cell groups. The degree of c-fos activation of brainstem neurons was correlated with the magnitude of PVN activation.

These data support the hypothesis that fetal CRH neurons are stimulated during fetal stress, and suggest that catecholamine systems might mediate that stimulation.

Supported by NIH HD 21350 and NS 28477, and NS 23858.

436.9

PARAVENTRICULAR NUCLEUS (PVN) OXYTOCIN NEURONS ARE RESISTANT TO THE EXCITATORY NEUROTOXIN, IBOTENIC ACID. M.F. Callahan, M.J.A. Rocha*, G. Cai*, D.K. Sundberg*, and M. Morris. Dept. of Physiology and Pharmacology, Wake Forest University Medical Center, Winston Salem, NC, 27103.

Ibotenic acid lesions of parvocellular PVN neurons attenuate the tachycardia and plasma oxytocin (OXY) responses to stress. We examined the effects of this lesion on brain OXY mRNA and peptide levels in plasma, posterior pituitary (PP), brainstem (BS), olfactory tubercule (OLT) and spinal cord. Male rats received bilateral PVN injections of ibotenic acid (5.0 μg/0.5 μl) and were sacrificed at 7 days. The brains were fixed and processed for *in situ* hybridization using an oligonucleotide probe. Ibotenate produced moderate to extensive loss of cells and infiltration of glia into the parvocellular region of the PVN. There was heavy labeling for OXY mRNA in the magnocellular region and isolated labeling in parvocellular region for both groups with no apparent differences in the pattern or intensity of OXY mRNA. The lesion had no effect on OXY levels in BS (1.21±0.5 ng -v- 0.8±0.2 ng); PP (1.2±0.3 ug -v- 1.2±0.2 ug), or OLT (381±68 pg -v- 345±62 pg; control -v- lesion). Lesion had no effect on plasma OXY. OXY in the cervical-upper thoracic cord showed a 50% decrease. Although the neurotoxin destroys predominantly parvocellular PVN neurons, the current findings indicate that BS projecting OXY cells are not affected by the toxin. This indicates that a subpopulation of OXY cells, perhaps small magnocellular neurons, are resistant to the effects of ibotenic acid. (Supported by NIH HL-43178).

436.10

BARORECEPTOR NERVES INFLUENCE HYPOTHALAMIC PEPTIDE mRNA EXPRESSION. M.J.A. Rocha*, M.F.Callahan, D.K. Sundberg*, M. Morris. Department of Physiology and Pharmacology, Bowman Gray School of Medicine of Wake Forest University. Winston-Salem, NC 27103

Neuroanatomical and physiological evidence indicates that baroreceptors influence hypothalamic vasopressin (VP) and oxytocin (OT) neurons. Experiments were conducted to evaluate the effect of sinoaortic denervation (SAD) on the expression of VP and OT mRNA under basal and stimulated conditions. Male control or SAD rats (10 days post oper) were given water or 2% salt to drink for 72 hrs. A second control group (salt intake limited) was included because the SAD rats consumed less salt solution. Two experiments were performed 1) *in situ* hybridization using oligonucleotide probes and 2) measurement of tissue peptide levels in the paraventricular (PVN) and supraoptic (SON) nuclear regions. Salt loading produced an increase in plasma osmolality, VP and OT with no differences observed between the groups. There was, however, a greater depletion of neurohypophyseal peptides in the denervated group. There was a 72% and a 71% depletion of VP and OT in the SAD rats as compared to 33% and 48% in the controls. Salt loading produced an increase in PVN and SON VP content in control animals (1.0+0.2 vs 2.8+0.5 ng/PVN and 1.6+0.2 vs 3.2+0.4 ng/SON; water vs salt loading). No changes were observed in SAD animals. There were no changes in central OT content with respect to the groups or treatment. Evaluation of message expression showed that there was increased expression under basal and stimulated conditions in the denervated animals. This suggests that denervation activates the hypothalamic neurosecretory neurons at the level of message expression and that activation is not reflected in the plasma peptide response. The fact that there is a greater salt-induced peptide depletion in the SAD rats indicates an inability to maintain peptide synthesis. (Supported by HL 43178.)

436.11

MAMMALIAN AUTONOMIC NEURONS EXPRESS APLYSIA BIO-ACTIVE NEUROPEPTIDES. D.A. Ruggiero[1], M. Anwar[1], A. Alevizos[2], D. Karagogeos[3] and K.R. Weiss[4]. Dept. Neurol. & Neurosci., Cornell Univ. Med. Coll., NY 10021; [2]Kaftatzoglou, Athens, Greece; [3]Dept. Med. Sci., Univ. Crete, Crete Greece; [4]Dept. Physiol., Mt. Sinai Med. Sch., NY 10029.

The R15 polyprotein gene in a marine mollusk Aplysia encodes a primary mRNA transcript, alternatively spliced in different neurons to generate overlapping sets of neuropeptides (Buck et al., *Cell* 51:127, 1987), including R15α_1 (38 a.a.) and R15α_2 (24 a.a.), which exert coordinated actions on invertebrate renovascular, digestive and reproductive systems. We sought to determine whether R15α_1 and R15α_2 are expressed in rodent brain and adrenal gland. Adult male anesthetized rats were perfused; tissues incubated in rabbit polyclonal R15α_1 (16 mer difference) or R15α_2 antisera and immunoprocessed. R15α_1-lir was restricted to locus ceruleus, ependymal and adrenal chromaffin cells; whereas R15α_2 was expressed by neurons in nucleus tractus solitarii, paraventricular and supraoptic (SO) hypothalamic neurosecretory nuclei, and processes in neurohypophysis. To determine whether R15α_2 is coexpressed in vasopressinergic cells, tissues were sequentially incubated in R15α_2 antiserum (24h; PAP) and arginine vasopressin (AVP) antiserum (24h; immunofluorescence). Neurons expressing R15α_2 and AVP were topographically segregated. Distributions of R15α_2-lir neurons in parvicellular divisions of PVN were skewed anteroventromedially to the AVP population. In the posterior magnocellular division, R15α_2-lir neurons formed a shell encircling an AVP-lir cell column. Striking parallels exist in localization of bioactive invertebrate neuropeptides to autonomic and neuroendocrine control sites in the mammal.

436.12

EFFECTS OF OVINE AND HUMAN CORTICOTROPIN-RELEASING HORMONE ON RESPIRATORY AND CARDIOVASCULAR FUNCTIONS IN HUMANS. M.Lehnert, M.Wink*, U.Krause*, E.Salomon*, J.Aldenhoff and J.Beyer*. Dept. of Internal Medicine - Endocrinology, Mainz University, 6500 Mainz and Central Institute of Psychiatry, 6800 Mannheim, FRG

It has convincingly been shown that corticotropin-releasing hormone (CRH) affects cardiovascular and respiratory parameters through extrahypothalamic pathways. Thus, in experimental animals, centrally applied CRH induces a specific stimulation of ventilation and an increase of arterial pressure and heart rate. In humans, we have previously demonstrated stimulatory effects on ventilation by exogenous CRH. In a group of 11 healthy subjects the cardiovascular and respiratory effects of 100 to 300 µg ovine (o) and human (h) CRH were now evaluated. During steady state ventilation was monitored pneumotachographically and by continuous registration of ventilatory gases. These parameters were determined following i.v. application (bolus over 30 sec vs. infusion over 30 min) and compared to placebo. Both hCRH and oCRH increased minute ventilation in all individuals by 60 to 90%. These effects lasted for 5 to 10 minutes following hCRH and for 10 to 15 minutes following oCRH after a single bolus injection. Another 100 µg of CRH infused over 30 minutes prolonged the effects on ventilation, in particular following oCRH. Heart rate were increased by 10 to 15% following hCRH, no major changes were detected in blood pressure. The mode of action of systemically applied CRH is still under investigation, but it appears possible that exogenous CRH may reach brain sites via circumventricular organs and thus alter autonomic outflow.

LEARNING AND MEMORY—PHYSIOLOGY V

437.1

THE NOVELTY-INDUCED INHIBITION IN BRAIN DNA SYNTHESIS IS MEDIATED BY THE DORSAL NORADRENERGIC BUNDLE. T.Menna*, A.Cerbone[2], A.G.Sadile[2], C.Lamberti-D'Mello[2]*, C.Buono*, F.Rafti* and A.Giuditta[3]*. (SPON: European Brain and Behaviour Society). IIGB, CNR, Naples; [2]Dip. Fisiol.Umana e Funz.Biol.Integr. "F.Bottazzi"; and [3]Dip. Fisiol. Generale ed Ambientale, Univ. Naples "Federico II", Naples, I.

The aim of this study was to investigate the role of the brain noradrenergic systems in modulating brain DNA synthesis (BDS) during nonassociative learning (Soc.Neurosci.Abstr., 16, 916, 1990). Adult male Sprague-Dawley rats were randomly assigned to one of six groups. Group A and B were bilaterally lesioned in the dorsal noradrenergic bundle (DNB) by injection of 6-OH-DA, group C and D were sham-lesioned, and group E and F were unoperated uninjected controls. All rats were given 50µCi ^{3}H-thymidine intraventricularly and sacrificed 0.5h later. The exposed rats were injected 15 min before test trial, Groups B, D and F were exposed for 10min to a a Làt-maze, and corner-crossings and rearings were monitored per 1min-blocks. BDS was measured in several brain regions by the incorporation of ^{3}H-thymidine into DNA. DNB-lesioned rats exposed to the Làt-maze did not show the marked inhibition in BDS observed under comparable conditions in control rats. The results support a role of noradrenergic systems in modulating brain DNA synthesis during learning. **(Supported by CNR and MURST 40% grants).**

437.2

Physical Activity Enhances Spatial Learning and Associated Hippocampal, not Parietal or Frontal Cortical, Cholinergic Function in F344 Rats. D.E. Fordyce[1,3] and R.P. Farrar[1,2,3]. [1]Institute for Neuroscience, [2]Department of Pharmacology and [3]Department of Kinesiology, The University of Texas at Austin, Austin, TX.

The effects of physical activity on spatial learning performance and associated cholinergic function were examined in F344 rats. Cholinergic analysis included resting and depolarization-induced activation of high affinity choline uptake (HACU) and muscarinic receptor binding in the hippocampus, parietal cortex and frontal cortex. Chronic-run rats demonstrated enhanced performance on the spatial learning task, indicated by second trial latencies and first and second trial proximity ratio scores ($p<.002$), and a reduction in hippocampal HACU, upregulation of muscarinic receptor density, and an increase in HACU 24 hours after spatial memory testing ($p<.05$). In addition, spatial memory tested rats demonstrated enhanced depolarization-induced activation of HACU compared to all other groups (62 vs 37 pmol/mg/4min, $p<.001$). Rats that were yoked for swim time to spatial memory tested rats did not show any spatial memory-induced alterations in HACU. Cholinergic alterations induced by chronic running were observed only in the hippocampus, not the parietal or frontal cortex. The data from this study, therefore, indicate that the chronic running-induced hippocampal alterations in HACU and upregulation of muscarinic receptor density, in combination with enhancement of HACU related to spatial memory testing, may contribute to the enhanced spatial learning performance in chronic-run rats.

437.3

[^{3}H]-CHOLINE UPTAKE IN THE FRONTAL CORTEX OF MATURE AND AGED RATS IS PROPORTIONAL TO THE ABSOLUTE ERROR IN THE CONTENT OF TEMPORAL MEMORY. W.H. Meck, Department of Psychology, Columbia University, New York, NY 10027.

The relationship between the magnitude of the error in the content of temporal memory and sodium-dependent high affinity choline uptake (SDHACU) in the hippocampus and frontal cortex was examined in mature (10-16 mo) and aged (24-30 mo) rats. The peak time of the response rate distribution that relates the probability of a response to signal duration in a 20-sec peak-interval (PI) timing procedure was used to index the remembered time of reinforcement. Discrepancies in temporal memory produce stable horizontal displacements of timing functions such that they can be centered at times that are either less than or greater than the actual time of reinforcement. Typically, the average remembered time of reinforcement for a group of mature rats would be very close to actual time of reinforcement,with a symmetrical distribution of individual peak times centered around that time. In contrast, as rats age they demonstrate a proportional rightward shift in their timing functions, indicating that remembered durations overestimate the actual time of reinforcement by a constant percentage. Regression analyses indicated that SDHACU in the frontal cortex, but not in the hippocampus, of both mature and aged rats is proportional to the absolute error in the content of temporal memory. Correlation coefficients averaged over both age groups were $r = .75$, $p < .001$ and $r = .06$, $p > .05$ for the frontal cortex and hippocampus, respectively.

437.4

EFFECTS OF ACIDIC FIBROBLAST GROWTH FACTOR ON MEMORY IN RODENTS. K. Sasaki[1], Y. Oomura[2,3], A. Li[2], K. Hanai[4], I. Tooyama[4], H. Kimura[4], and H. Yaqi[1] Fac. Engi., Toyama Univ[1]., Toyama Med. & Pharm. Univ., Toyama 930-01[2], Inst. Bio-Active Sci., Nippon Zoki Pharm. Co., Hyogo, 673-14[3], Inst. Molec. Neurobiol., Shiga Med. Univ., Shiga 520-01[4], JAPAN

Acidic fibroblast growth factor (aFGF) is produced in the ependymal cells of the cerebroventricular system and released into cerebrospinal fluid (CSF) by glucose. The aFGF in rat CSF increased 1000 times in a 2 hr period after intraperitoneal (IP) or intracerebroventricular (ICV) glucose infusion, and diffused into the brain parenchyma and was taken up into neurons in the hypothalamus, hippocampus and other parts of the brain. We investigated the effects of endogenous and exogenous aFGF on memory. IP glucose injection 2 hr before an acquisition trial of foot shock in a passive avoidance task significantly increased retention in mice when tested 24 hr later. In a Morris water maze task, IP glucose injection 2 hr before the first trial block reduced latency to escape onto a platform hidden just below the surface of the water. These glucose effects were abolished by ICV anti-aFGF antibody injection 30 min before the glucose injection. Continuous ICV infusion of aFGF into rats also significantly increased retention latency in passive avoidance task. Results suggest that aFGF facilitates memory in the central nervous system. Memory facilitation by aFGF was significantly attenuated by CA$_1$ neuron death in the hippocampus caused by 5 min ischemia of the brain.

437.5

BEHAVIORAL EVIDENCE FOR THE NAPLES HIGH AND LOW-EXCITABLE RATS AS A GENETIC MODEL TO STUDY HIPPOCAMPAL FUNCTIONS. A.G.Sadile, A.Cerbone, T.Sagvolden[2]*, H.Welzl[3], Dip.Fisiol.Umana "F.Bottazzi", Univ. Naples, I; [2]Inst.Neurophysiol., Univ. Oslo, N.; [3]Inst.Behav.Biol., ETH, Zurich, CH.

The behavioral evidence supporting the hypothesis that the Naples High (NHE) and Low-Excitable (NLE) rats may be a genetic model to study hippocampal functions (Adv.Biosci., 59:299, 1986) is presented. It comprises: (1) Differential activity in a spatial novelty situation (selection trait), proportional to the stimulus complexity rats are exposed to. NHE are hyper- and NLE- rats hypoactive; (2) Impaired acquisition rate of water motivated operant conditioning in both lines vs. controls; and (3) Impaired working memory in a non reinforced tunnel maze with 6 asymmetrical arms in both lines compared to random-bred controls (Adv.Biosci., 70:177, 1988). Thus, a defective hippocampus-dependent spatial processing is inferred. The behavioral evidence (qualitatively similar in both lines), along with histochemical (Behav.Brain Res., 24:111, 1987), electrophysiological and neurochemical one (Adv.Biosci., 59:299, 1986; Behav.Brain Res., 33:287, 1989), suggests that both NHE/NLE are disintegrated at the hippocampal interface. Moreover, far from fitting in current theories on hippocampal functions, they lend support to its modulatory role in the parallel processing of cognitive and non-cognitive (attentional, emotional) informational components. **(Supported by CNR and MURST 40% grants).**

437.6

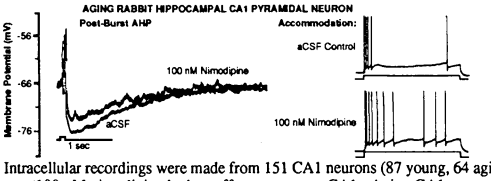
COGNITIVE AND NEUROCHEMICAL CHANGES INDUCED BY EXPERIMENTAL AUDITORY DEFICIT IN AGED RATS. F. Drago, L. Nardo* and F. Spadaro* Department of Pharmacology, University of Catania Medical School, Catania, Italy.

In aging, auditory deficits occur frequently and have been involved in cognitive impairment. We studied the effects of an experimental acoustic deafferentation on learning and memory processes and on some neurochemical parameters of aged rats. Sprague-Dawley male rats, 18 months old, were used. A solution of fibrinogen, thrombine and Factor XIII was injected once in a week for three months in the middle ear of aged male rats. Learning and memory capacity, assessed with passive and active avoidance tasks, was reduced in rats with experimental auditory deficit as compared to control animals. In the despair test, a behavioral model for depression, animals with the auditory impairment showed a more serious depressive attitude than controls. After the behavioral tests were performed, an auditory recovery of a group of animals was allowed suspending the injections. A month later, the behavioral testing was repeated. The animals that recovered from the auditory deficit showed a normalization of the behavioral pattern. A neurochemical study showed a reduction in DA and DOPAC content and in CAT and AChE activity in various brain ares of animals with auditory deficit. These results show that prolonged acoustic deafferentation can induce reversible cognitive changes. This may be of clinical relevance in elderly people with an acoustic deficit without hearing aids.

437.7

NIMODIPINE INCREASES EXCITABILITY OF CA1 NEURONS IN AGING RABBITS. J.R. Moyer, Jr., L.T. Thompson, J. Black', and J.F. Disterhoft. Department of CMS Biology, Northwestern Univ. Med. Sch., Chicago, IL 60611 and 'Dept. of Psychobiology, UC Irvine, Irvine, CA 92717.

Nimodipine, a dihydropyridine calcium channel antagonist, facilitates learning in aging rabbits (Deyo *et al.*, 1989) and increases spontaneous firing rates of CA1 pyramidal neurons *in vivo* (Thompson *et al.*, 1990). CA1 neurons from trace conditioned rabbits also exhibit learning-specific afterhyperpolarization (AHP) reductions (deJonge *et al.*, 1990). To investigate the mechanism by which nimodipine exerts its behavioral effects, we studied the AHP before and after application of nimodipine in both aging and young adult CA1 neurons *in vitro*.



Intracellular recordings were made from 151 CA1 neurons (87 young, 64 aging). Doses ≤100 nM nimodipine had no effects on young CA1. Aging CA1 neurons showed significantly reduced AHP amplitudes and areas, and decreased accommodation after 100 nM nimodipine (extracellular levels in behavioral studies). Aging CA1 neurons also showed significantly larger AHPs than young CA1 neurons. These data support the hypothesis that aging neurons have altered calcium regulation (perhaps via decreased calcium buffering capacities) resulting in larger AHPs, and that in aging CA1 neurons, nimodipine can decrease the AHP, thus increasing cellular excitability in a manner facilitating learning. (Supported by 1 RO1 AG08796 & The Miles Institute).

437.8

ARE THERE PLACE CELLS IN THE PRIMATE HIPPOCAMPUS ? E.T.Rolls and S.M.O'Mara*. University of Oxford, Department of Experimental Psychology, Oxford OX1 3UD, England.

Previous work on the role of the primate hippocampus in spatial processing has shown that there are cells in the macaque hippocampus which respond when the monkey looks at particular positions in space, and that some of these cells respond in allocentric (world-based) rather than egocentric (body-centered) coordinates (J.D.Feigenbaum & E.T.Rolls, 1991, Psychobiology 19: 21-40). Such cells were named 'space' cells, to distinguish them from 'place' cells in the rat which respond when the rat is in a particular place in an environment. In the experiments described here, we investigated activity changes of cells when macaques (Macaca fascicularis) were moved in a small chair on wheels to different places in a cue-controlled testing environment (a 2m x 2m chamber). Many cells with spatial responses were recorded in the primate hippocampus and parahippocampal gyrus. For a number of cells, it was possible to show that firing rate depended on where in space the monkey looked, and not on the place in the environment, or on head direction. The responses of some cells were affected by the removal of cues in the environment. Other cells responded in relation to whole body movement of the monkey in the testing environment, with some specific for head-centred rotation, and others for linear translation. Cells which respond according to the place where the monkey is located and independently of local view and of head direction have not yet been found, but testing is continuing.

437.9

SINGLE NEURONS IN PRIMATE TEMPORAL CORTEX CODE BOTH OF PAIRED ASSOCIATES STORED IN VISUAL LONG-TERM MEMORY. K. Sakai* and Y. Miyashita. Dept. of Physiology, Sch. of Medicine, Univ. of Tokyo, Tokyo 113, JAPAN.

Human memory is assessed by the paired associate learning test, in which the subject is shown a series of paired figures and then asked to retrieve the associate of a cue figure. We prepared 24 computer-generated patterns and sorted geometrically distinct figures into pairs. Two monkeys (*Macaca fuscata*) were trained to memorize these pairs. In each trial, a cue stimulus was presented on a video monitor, and choice stimuli were shown after a delay period. Each monkey obtained a fruit-juice reward for correctly touching the paired associate. We found two types of neurons in the anteroventral temporal cortex. One type selectively responded to both of the paired patterns during the cue period. The other type, which responded to one pattern optimally, exhibited tonic activity during the delay period when the associate of that pattern was used as a cue. These results provide direct evidence that the single neurons acquired the response selectivity through associative learning, representing long-term memory of paired associates.

437.10

RAPID AND CONSOLIDATED ASSOCIATION OF CORTICAL MEMORY CIRCUITS BY THE HIPPOCAMPUS: "TRACING-CIRCUIT ASSOCIATION" MODEL FOR HUMAN MEMORY.

Yoichiro Kuroda Dept. of Neurochem., Tokyo Metropolitan Institute for Neurosciences, Fuchu-shi, Tokyo 183 Japan.

The hippocampus has long been considered important in the consolidation of memory. In 1989 , a "tracing circuit" model of human memory was proposed where in consolidation occurs by "impulse tracing" to stabilize connections by strengthening synapses in closed circuits in the cortex (*Neurochem.Intern.* 14,309). The existence of reciprocal cortico-hippocampal projections suggests that closed circuits between cortex and hippocampus can be formed and can further consolidate the tracing circuits in the cortex. Here, using recent compelling data on a longitudinal(septotemporal) axis of the hippocampus, I propose a "tracing circuits association" model, in which association of episodic memories of different modalities is formed in the hippocampus by intra-hippocampal connections of cortico-hippocampal closed circuits. When tracing impulses carrying simultaneously presented information of different modalities can form a new closed circuit through the hippocampus by reciprocal projections with the association cortex, the tracing circuits are further stabilized, with prolongation of firing in closed circuit. Since all CA3 neurons appear to project to CA1 neurons distributed along the long axis of the hippocampus (Ishizuka et.al., *J.Comp.Neurol.* 295, 580,1990), simultaneous excitation of closed circuits of different modalities can easily be connected by cross projection of CA3 neurons in one cortico-hippocampal closed circuit to CA1 neurons in a different circuit. Such inter-closed circuit CA3-CA1 synapses are repetitively stimulated during the prolonged firing, induced LTP and other facilitatory processes which stabilize these synaptic connections over long periods, enabling reactivation of the associative memory circuit.

437.11

A SPIKING NEURON NETWORK MODEL OF DYNAMIC SHORT-TERM MEMORY. B. Kehoe* and D. Zipser, Department of Physics, CSUF, Fresno, CA 93740 and Department of Cognitive Science, UCSD, La Jolla, CA 92093.

The firing patterns of cortical neurons in monkeys engaged in short-term memory tasks (see, e.g., Fuster, J.M., *J. of Neurophysiology*, **36**, 61-78, 1973) have been shown to be similar to the activity patterns of units in a recurrently connected, discretely updated, artificial neural network (ANN) trained as a gated sample and hold register (D. Zipser, *Neural Computation*, **3**, 178-192, 1991). This similarity suggests a dynamic storage mechanism for short-term memory utilizing a recurrent network. The discrete time model has been extended to an approximation of a continuous time ANN and also to a model of a spiking network based upon the continuous model, trained using the backpropagation through time algorithm subject to biologically based constraints. The characteristic features of the discrete network corresponding to the neurobiological data are preserved. Large networks have been trained with significant levels of noise to reflect the variability of average spiking rate of real neurons. The resulting trained network spontaneously learns to utilize all of its resources by creating clusters of similarly connected units that represent what were single units in smaller networks. These clusters presumably help to overcome the effects of noise by averaging. Networks made up of spiking units, with biologically based parameters and connections determined by the continuous network, are being studied. Their interspike interval distributions model those found in the data. The attractor dynamics of spiking networks, and the implications of those dynamics for purturbed neurons in such a network, are explored as a guide to further experimental testing of the recurrent model of dynamic short-term memory.

437.12

NMDA-BASED PATTERN DISCRIMINATION IN A MODELED CORTICAL NEURON. B. W. Mel, Computation and Neural Systems Program, Caltech, 216-76, Pasadena, CA, 91125.

Compartmental simulations of an anatomically characterized cortical pyramidal cell were carried out to study the integrative behavior of a complex dendritic tree (anatomical data from Rodney Douglas and Kevan Martin). Theoretical considerations had suggested a possible role for cooperative interactions among groups of neighboring synapses in the generation of cell responses (Mel, B.W., Soc. Neurosci. Abst., 208.5, 1989). In earlier compartmental modeling studies of a layer 2-3 pyramidal cell (Mel, B.W., Soc. Neurosci. Abst., 205.4, 1990) it was found that when dendritic spines contained a high proportion of voltage-dependent NMDA channels, the pyramidal cell responded selectively to spatially-clustered, rather than random, distributions of activated synapses. The cluster-sensitivity effect has now been replicated in a layer 5 pyramidal cell of very different morphology, where it is shown to persist under wide ranging biophysical parameter variations. In a demonstration of the possible biological utility of cluster sensitivity, the NMDA-rich neuron is shown to be capable of solving a complex image discrimination task within its dendritic tree. We conclude that a relatively simple statistical characterization of the input-output behavior of the cortical pyramidal cell may be both possible and appropriate.

437.13

CORTICAL ASSOCIATIVE MEMORY FUNCTION AND ACETYLCHOLINE: A COMPUTATIONAL MODEL.

M.E. Hasselmo, Dept. of Psychology, Harvard University, Cambridge, MA 02138.

Psychopharmacological evidence suggests a role for acetylcholine in memory function. Computational modeling of cortical associative memory function may help link this role in memory to the specific effects of acetylcholine on cortical neurons. Acetylcholine (100μM, presented with 1μM neostigmine) selectively suppresses intrinsic fiber synaptic transmission in slice preparations of piriform cortex, while leaving afferent fiber synaptic transmission unaffected (Hasselmo and Bower, J. Neurophysiol., in press). A model of piriform cortex shows that this selective suppression of excitatory feedback, applied during learning of new input patterns, can greatly enhance associative memory performance. Without this suppression, excitatory feedback generates previously stored patterns during the learning of new patterns which activate any of the same neurons. This leads to the strengthening of connections between all neurons activated by overlapping input patterns, resulting in considerable interference between stored patterns. Cholinergic suppression of excitatory feedback can prevent this problem by decreasing the activity of neurons not receiving direct afferent input during learning, thereby satisfying the following condition:

$$\mu, \Omega > a_i = \sum_{j=1}^{n} (1 - c)B_{i,j}\, g(a_j) - H_{i,j}\, g(a_j)$$

where μ = synaptic modification threshold, Ω = output function threshold, a = neuron activation, c = cholinergic suppression, B = excitatory feedback strength, H = inhibitory feedback strength, g(a) = neuron output function. This has an effect similar to clamping neuron activity to the input pattern during learning, a technique used in the modeling literature, but not previously justified by neurophysiological data. Memory deficits associated with presentation of cholinergic antagonists show some characteristics of the interference predicted by this model. This work suggests how the memory deficits associated with Alzheimer's disease could arise from a loss of cortical cholinergic innervation.

(Support: NIH grant NS07251, and The French Foundation for Alzheimer Research.)

CHEMICAL SENSES: PERIPHERAL MECHANISMS III

438.1

ROLE OF cAMP PHOSPHODIESTERASE IN OLFACTORY SIGNAL TRANSDUCTION. F.F. Borisy-Rudin[1], G.V. Ronnett[1], A. Cunningham*[1], J. Beavo*[2], S.H. Snyder[1]. [1]Depts. of Neuroscience, Neurology, and Molecular Biology & Genetics and the Howard Hughes Institute, Johns Hopkins University School of Medicine, Baltimore, MD 21205. [2]Dept. of Pharmacology, SJ-30, University of Washington, Seattle, WA 98195.

To investigate the mechanisms by which the olfactory signal is terminated we have characterized the phosphodiesterase (PDE) activities present in cilia and homogenates of olfactory turbinates. At least two forms of PDE, Ca^{2+}/calmodulin PDE (CAM-PDE) and rolipram-sensitive cAMP selective PDE (RS-PDE), are present in olfactory turbinates as determined by biochemical and immunohistochemical methods. High affinity CAM-PDE appears to be the predominant form in washed preparations of olfactory cilia and is fivefold greater than in brain when assayed at low micromolar cAMP. By comparison, in turbinates about half the PDE activity is sensitive to rolipram. Following bulbectomy, CAM-PDE activity is significantly depleted in turbinates. Immunocytochemical staining of the rat olfactory mucosa reveals selective expression of CAM-PDE in mature olfactory receptor neurons with immunoreactivity most pronounced within the dendritic knob and cilia, as well as the axon bundles. Neither sustentacular cells nor basal cells display any immunoreactivity. Bulbectomy greatly depletes the population of CAM-PDE positive cells. We propose that Ca^{2+} activation of CAM-PDE may be involved in termination of olfactory signals.

438.2

OLFACTORY RECEPTOR NEURONS OF THE MALE MOTH MANDUCA SEXTA EXPRESS SECOND MESSENGER MODULATED ION CHANNELS IN VITRO. M. Stengl[1], R. B. Levine[2], J. G. Hildebrand[2]. [1]Fak. für Bio., Univ. Konstanz, Postfach 5560, 7750 Konstanz, BRD. [2]ARL, Neurobio., Univ. of Arizona, Tucson, AZ 85721

Primary cultures of identifiable olfactory receptor neurons (ORNs) from male Manduca sexta pupae facilitated studies of olfactory transduction mechanisms. About 30% of the 21 d cultured ORNs responded to puffer-pipette-applied DMSO extracts of female pheromone glands. In most cases pheromone-dependent cation channels with subconductance states opened, with reversal potential around 0 mV, non-linear I/V relationship, and no discrimination between K^+, Na^+ or Cs^+. Patch-excision opened channels which closely resembled the pheromone-dependent cation channels. These channels were blocked by TEA and cGMP+ATP. In the whole-cell configuration with other channels blocked via TTX and Cs^+, transient cation currents were elicited via perfusion of the cells with GTPγS+ATP, IP_3, or 10^{-6} M Ca^{++}. These currents resembled the pheromone-dependent cation currents in reversal potential and ion selectivity. At least a subpopulation of these currents were blocked by TEA or bromo-cGMP+ATP. These Ca^{++}-permeable channels opened only transiently with mM concentrations of Ca^{++} outside. Application of protein kinase C (PKC) activators to cultured ORNs opened Ni^+-blockable Ca^{++} channels and opened cation channels which resembled the pheromone-dependent cation channels. The Ni^+-blockable Ca^{++} channels also opened when internal Ca^{++} was buffered to less than 10^{-8} M. Future studies will examine our working hypothesis that pheromone leads to transient opening of second messenger-dependent Ca^{++} channels which then lead to opening of Ca^{++}-dependent cation channels, both of which change their sensitivity to Ca^{++} via PKC-dependent phosphorylation.

[Supported in part by NIH grant AI-23253.]

438.3

EXPRESSION OF 5'-ECTONUCLEOTIDASE AND PHOSPHATASE ACTIVITY FROM THE OLFACTORY ORGAN OF THE SPINY LOBSTER IN XENOPUS OOCYTES. H.G. Trapido-Rosenthal, M.A. Holman, R.M. Greenberg, R.A. Gleeson* and W.E.S. Carr.* Whitney Laboratory, University of Florida, St. Augustine, Florida 32086.

The olfactory organ of the spiny lobster, Panulirus argus, consists of a dense array of aesthetasc sensilla on the lateral filament of the antennule. Electrophysiological studies have shown that sensilla include populations of receptor cells that respond to the odorant adenosine 5'-monophosphate (AMP). Biochemical studies have shown that sensilla also contain a potent extracellular enzymatic activity that rapidly dephosphorylates this odorant. In this study, we report that messenger RNA (mRNA) isolated from lobster olfactory organ can induce the expression of ectonucleotidase/phosphatase activity in oocytes of the frog Xenopus laevis. Oocytes microinjected with mRNA from lobsters' lateral antennular filaments exhibited an increase in their ability to dephosphorylate exogenous AMP, relative to non-injected and water-injected control oocytes. The amount of ectoenzymatic activity expressed depended upon the amount of lobster mRNA injected. Injection with non-messenger RNA did not enhance the ability of oocytes to dephosphorylate AMP. Heterologous expression in Xenopus oocytes may be of general value for studying odorant-degrading enzymes, since the biochemical assays used to monitor expression are both simple and highly sensitive. This work was supported by grants from the National Science Foundation (BNS-8908340 and BNS-8805885) and the University of Florida (D-50-8990 and RDA-1-9).

438.4

STEREOSPECIFIC DETECTION OF AMINO ACIDS BY LOBSTER OLFACTORY RECEPTOR NEURONS. W.C. Michel[1], H.G. Trapido-Rosenthal[1], E.T. Chao[1,2*] and B.W. Ache[1,3]. [1]Whitney Lab. and [3]Depts. of Zool. and Neurosci., Univ. of Florida, St. Augustine, FL. 32086, [2]Dept. of Psychol., Berea College, Berea, KY, 40404.

Olfactory receptor cells of crustaceans detect many small, water-soluble organic compounds, including amino acids. Previous investigations of receptor cell specificity in crustaceans have focused on L-amino acids. Here we report that lobster olfactory receptor cells also detect D-isomers of amino acids and do so in a stereospecific manner. Specific binding for L- and D-alanine to an enriched olfactory ciliary membrane fraction was *ca.* 2241 and 792 fmoles/mg protein, respectively. The association of both isomers to the membrane fraction was rapid and saturable; disassociation was also rapid. Distinct receptor sites for the enantiomers were indicated by the preferential displacement of radiolabelled L- or D-alanine by the like isomer. Scatchard analysis identified two binding sites for each isomer. A higher affinity site (K_d *ca.* 5 μM) was interpreted as receptor binding while a lower affinity site (K_d *ca.* 150 μM) may reflect binding to a transport system. Alanine binding was not uniformly distributed across alanine-sensitive receptor cells. Electrophysiological recordings identified D-alanine (n=2) and L-alanine (n=1) specific neurons, as well as neurons with nearly equal sensitivity for the two isomers (n=4). Stereospecific neurons were also identified for 17 of 19 other amino acids tested. Of 89 amino acid-sensitive cells, 35 and 19 were stereospecific for L- and D-isomers, respectively. Collectively, our data suggest that lobster olfactory receptor cells express stereospecific receptor proteins. The non-uniform distribution of receptors across the olfactory receptor cell population presumably allows across fiber discrimination of amino acid isomers. (Funded by NSF Grants BNS 88-10261 & BNS 89-08340 and ONR Grant N00014-90-J-1566.)

438.5

MODEL FOR THE DISCRIMINATION OF ODORS BY 7-TM DOMAIN RECEPTORS AND BY A GROUP OF OLFACTORY NEURONS. P. Nef*, I. Hermans-Borgmeyer*, G. P. Gasic, S. Heinemann, V. Dionne*. Molecular Neurobiology Laboratory, The Salk Institute & UCSD, San Diego, CA 92037

Using PCR amplification, we recently cloned a novel multigene family of putative receptors characterized by 7 transmembrane (7-TM) domains and expressed only in olfactory epithelium. Experiments are in progress to determine wether the proteins encoded by these clones represent functional olfactory receptors which play a key role in the detection of odorants. These putative receptors probably constitute an integral part of the molecular mechanisms involved in discriminating among hundred or even thousand of different smells.

In situ hybridization analysis with some of these putative olfactory receptors revealed patterns of expression that correspond to groups of olfactory neurons topographically distributed within the olfactory epithelium. We have named these patterns "primary olfactory maps". We predict that the total number of primary olfactory maps is not infinite mainly because of the overlaping distribution of individual olfactory 7-TM receptors in the epithelium.

Currently, we propose a model for the discrimination of an odorant in which a given odor activates a group of olfactory receptors as well as a group of olfactory neurons. The combination of these two interactions produces "odorant finger-prints" consisting of a pattern of specific depolarizations that are sent to the CNS and further processed by the olfactory bulb.

438.6

THE CLUSTERING OF AN AXONAL SUBSET WITHIN THE PLANE-TO-POINT HOLOGRAPHIC OLFACTORY PROJECTIONS IN TROUT. B. Oakley and D. R. Riddle[1]. Dept. of Biology, Univ. of Michigan and [1]Dept. of Neurobiology, Duke Univ. School of Medicine.

In rainbow trout (*Oncorhyncus mykiss*) a given cluster of olfactory receptor neurons (ORNs), labeled by HRP, makes highly divergent projections to the glomerular layer. In corresponding retrograde tracing experiments, fluorescent latex beads were picked up and transported from small glomerular sites to ORNs scattered widely in all lamellae of the epithelial plane. Thus, instead of a point-to-point or regional topographic map, there is a plane-to-point or holographic projection, since every point in the glomerular layer receives information from the entire olfactory epithelial plane. Such iterated mapping of the rosette could arise if each of many intermingled sets of ORNs converged its axons into a glomerular subregion. To approach this issue we used the lectin, pokeweed agglutinin (PWA). PWA reacted with an ORN subset whose somata were highly dispersed in the olfactory epithelium and whose axons were widely scattered in the olfactory nerve. Yet, at the nerve-bulb interface the PWA positive axons converged into fascicles that terminated in two-thirds of the glomerular layer. The two glomerular fields lacking PWA positive axons received many other olfactory axons, perhaps from dispersed receptor cells having other molecular commonalities. Fourteen weeks after olfactory nerve transection, new axonal projections replicated the nine glomerular fields, including the two that were PWA positive. Evidently, the olfactory epithelium of rainbow trout makes a stable holographic projection, within which one or more subsets of scattered ORN axons may converge to glomerular subregions.

438.7

MULTIPLE NEURAL SOURCES OF VIP-LIKE IMMUNOREACTIVITY IN THE SALAMANDER OLFACTORY MUCOSA. M.L. Getchell[1,2] & T.V. Getchell[1,2,3]. [1], Div. Otolaryngol., Dept. Surgery; [2], Sanders-Brown Center on Aging; [3], Dept. Physiol. & Biophys., Univ. of Kentucky Coll. of Med., Lexington, KY 40536.

The olfactory mucosa may receive extrinsic innervation from 3 sources: terminal nerve, trigeminal nerve, and autonomic fibers accompanying the trigeminal nerve. Based on cell structure, staining pattern, and localization of immunoreactive elements, we have correlated VIP-immunoreactive innervation with its neural source. Immunofluorescence was used to identify and localize VIP innervation. Terminal nerve innervation was characterized by granular staining in occasional bipolar cells with thick, non-varicose processes localized within immature neuroepithelium, at the base of mature neuroepithelium, and in the olfactory nerve. Trigeminal innervation was associated with agranular staining in thin, varicose fibers within nerves, located at the base of the lamina propria, whose branches terminated within glands and blood vessel walls. Immunoreactivity for substance P, also present in fibers of these nerves, was not colocalized with VIP. Thus, VIP-immunoreactive innervation from different neural sources is associated with different structures and targets in the olfactory mucosa. Supported by NSF BNS-88-21074 (MLG) and NIH DC-00159 (TVG).

438.8

VOLTAGE-SENSITIVE DYES LOCALIZE TRANSDUCTION EVENTS WITHIN OLFACTORY RECEPTOR NEURONS. R.C. Gesteland, J. Brouwer* & P. Farmer*, Anatomy & Cell Biology, Univ. of Cincinnati Medical Center, OH 45267.

Voltage-sensitive dyes confirm whole-cell patch recording evidence that transduction processes differ in frog and in salamander olfactory receptor neurons. Patch-clamp studies show that in frogs the dendritic and somal membranes are inactivated at rest, with unusually low conductances. In salamanders sodium channels are available for excitation and respond to stimulation with increases in sodium conductance. Voltage dye measurements in slices of olfactory epithelium with the laser scanning confocal microscope discriminate voltage gradients within single neurons. They show that an effective stimulus depolarizes only receptor cilia and the knob on the apical end of the dendrite in the frog. The membrane potential of the dendrite and soma is unchanged, within the sensitivity limits of the technique. In salamander olfactory receptor neurons, stimulus-induced depolarization invades the dendrite and soma. It appears that receptor currents in the frog are funneled from the receptor cilia to the axon for action potential initiation through the cytoplasm without significant electrotonic energy loss. In the salamander, conventional electrotonic and active processes conduct the stimulus message from the cilia to the axon spike generator. In epithelium preparations viewed *en face* odor response properties of different neurons are easily compared to allow studies of stimulus selectivity among neuronal subpopulations. These are difficult with microelectrode methods. Some voltage-sensitive dyes preferentially light up supporting cells in the olfactory epithelium. These cells show stimulus-evoked hyperpolarization. This may be a measure of potassium uptake by these cells which are thought to regulate the epithelial extracellular ionic environment. Resolution of spatially separate events within cells of the olfactory epithelium is a unique property of high-resolution fluorescent voltage-sensitive dye microscopy.

Supported by NIH grants DC00342 and DC00347, University of Cincinnati Research Challenge and the Mark P. Herschede Fund.

438.9

CLONING OF GUSTATORY SPECIFIC G PROTEINS FROM RAT. Susan McLaughlin*, Zhou Hao*, Peter McKinnon*, Robert F. Margolskee. Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110.

The guanine nucleotide binding proteins (G proteins) mediate signal transduction in olfactory, visual, and hormonal systems. The G proteins comprise a family of proteins which transduce an extracellular signal into an intracellular second messenger (e.g. cAMP, cGMP, IP_3). In the vertebrate taste cell, electrophysiological, and biochemical evidence suggest that G proteins are involved in the transduction of both bitter and sweet tastants. The bitter compound denatonium raises the intracellular calcium concentration in rat taste cells, apparently via G protein induced IP_3. Sucrose causes a G protein-dependent generation of cAMP in rat taste cells which in turn depolarizes the cell. To identify and characterize those proteins involved in the taste transduction process, we have cloned G proteins from rat taste cells. Isolated rat circumvallate and foliate taste papillae were used to make polyA^+ RNA and a taste cell enriched cDNA library. Control RNA and cDNA was also made from regions of the lingual epithelium devoid of taste cells. Using degenerate primers corresponding to conserved regions of G proteins, the polymerase chain reaction (PCR) was used to amplify and clone taste cell G protein cDNAs. Hybridization with G protein cDNA probes was also used to screen this library for G protein clones. To date, seven distinct G protein cDNAs have been isolated, cloned and sequenced from the taste cell library. Four different G protein clones correspond to G_q and G_i subtypes. The G_q and G_i clones are more highly expressed in the RNA from taste cells vs. non-taste cells. Three other G protein clones are expressed in taste cell RNA but not in non-taste RNA, one of these clones ($G_{gustatory}$) is novel and only distantly related to other known G proteins. Presumably, G_{gust} is specifically involved in taste signal transduction.

438.10

POSTSYNAPTIC RESPONSES IN BASAL CELLS ARE ELICITED BY STIMULATING RECEPTOR CELLS IN *NECTURUS* TASTE BUDS. Douglas A. Ewald & Stephen D. Roper Dept of Anat & Neurobiol, Colo St U, Ft Collins CO 80523 & the Rocky Mtn Taste & Smell Cntr, Denver CO 80262

Receptor cells comprise the majority of cells (90%) in *Necturus* taste buds. Receptor cells extend apical processes into the taste pore. Basal cells, which comprise only 10% of the total population, lack apical processes yet are involved in the majority of the morphologically-identified synapses in the taste bud (Delay & Roper, J. Comp. Neurol. **277**: 268, 1988). In thin (150-300 μm) slices of lingual epithelium, chemical stimulation of receptor cells with focally applied KCl (140 mM) elicited receptor potentials in receptor cells and long-latency depolarizing responses in cells that were subsequently identified by dye injection as basal cells (Ewald & Roper, Neurosci. Abstr. **16**: 25, 1990). We have made simultaneous intracellular recordings from receptor cell-basal cell pairs to study the long-latency responses and test whether they represent synaptic coupling from receptor to basal cell. Varying the magnitude of the KCl-evoked receptor potentials revealed a threshold for basal cell responses of a membrane potential between -30 to -10 mV. In about 20% of the simultaneous impalements, electrical stimulation of the receptor cell elicited small depolarizing responses in the basal cells. The magnitude of basal cell responses evoked by electrical stimulation of a single receptor cell (mean = 0.53 ± 0.06 mV, N=10) was 14% of the responses elicited by KCl stimulation of the entire population of receptor cells. The threshold for electrically-evoked responses was in the same range as for those elicited by chemical stimulation. The lack of a long latency in responses elicited by electrical stimulation suggests that the long latency in response to KCl stimulation resulted from the time for the chemically-evoked receptor potential to reach threshold. The incidence (20%) of synaptic coupling between receptor cells and basal cells and the ratio of amplitudes of direct, electrically-evoked responses to KCl-evoked responses (14%) suggests extensive convergence of receptor cells to basal cells. The role of this type of synaptic connection in integrating or modulating the response to gustatory stimulation is under investigation. Supported by NIH grants DC00244, DC00374 and AG06557.

ALZHEIMER'S DISEASE: AMYLOID II

439.1

AGGREGATION AND AMYLOIDOGENICITY OF FRAGMENTS OF THE ß/A4 AMYLOID PROTEIN PRECURSOR IN EUCARYOTIC CELLS. A. Weidemann*, R. Prior*, T. Dyrks*, T. Hartmann*, C. L. Masters, H. Monyer and K. Beyreuther*. Center for Molecular Biology Heidelberg, 6900 Heidelberg, F.R.Germany.

In Alzheimer's disease the typical amyloid deposits are mainly composed of ß/A4 amyloid protein being synthesized as part of a larger precursor protein (APP) from which it is released by so far unknown proteolytic breakdown events. We have begun to examine the biochemical mechanisms by which ß/A4 bearing APP fragments are generated and deposited *in vitro*. Expressionplasmids coding for various C-terminal fragments of APP which include the ß/A4 region were introduced into mammalian cell lines. The corresponding gene products were analyzed with antibodies directed against the ß/A4 region and the cytoplasmic domain of APP. The C-terminal fragments of APP are proteolytically processed giving rise to a complex pattern of breakdown products. Fragments that react with á ß/A4 antibodies accumulate within the cells and form aggregates that are not soluble in SDS buffers. Immunostaining of transfected cells reveals the presence of dense aggregates in the cytoplasm. These structures are ß/A4 positive and can be stained with Thioflavin S, suggesting that the aggregates adopt the characteristic ß-pleated sheet conformation of brain ß/A4 amyloid. The accumulation of stable ß/A4 bearing APP fragments within cells offers the possibility to study the proteolytic processing events and aggregational properties of APP and its fragments in eucaryotic cells.

439.2

Effects of Alzheimer Amyloid in Rat Brain. G. M. Cole, S.A. Frautschy and A. Baird. Dept. of Neurosciences, UCSD, La Jolla, Ca. 92093 and Dept.of Cellular and Molecular Biology, Whittier Institute, 9894 Genesee, La Jolla, Ca.92037

Because they have not been directly induced in an animal model, the significance of beta-amyloid deposits in Alzheimer's disease has remained unclear. Although recent data suggest that injection of solublized beta protein and related peptides may have acute toxic or pharmacological effects, there is no data on the existence or levels of soluble beta protein or related peptides in either normal or Alzheimer brain making it difficult to assess their possible physiological or pathological significance. We have injected Alzheimer's amyloid cores into rat cortex and hippocampus and similarly isolated control lipofuscin fractions on the contralateral side. Rat brain sections were silver and immunohistochemically stained for Alz 50, ubiquitin, and beta protein at 2, 7, 30 and 60 days after injection. At 2 days prominent Alz 50 immunoreactivity was observed along the needle track on both the amyloid and lipofuscin sides and this effect was less by 7d. In contrast, at 30d, marked ubiquitin and Alz 50 immunoreactivity was present only on the amyloid injected side. Only the amyloid injected side displayed abberrant silver reactive profiles and marked neuronal loss and this was in close association with beta protein immunoreactive material. These results indicate that complex and fully developed human amyloid deposit fractions can be neurotoxic when injected into adult rat brain.
Supported by NIH grants AG09009(G.M.C), DK18811(A.B.), and NS-28121(A.B.) and Calif. Dept. of Health Service (G.M.C.)

439.3

THE FAMILIAL ALZHEIMER'S DISEASE MUTATION DOES NOT PRODUCE GROSS ALTERATIONS IN APP SYNTHESIS OR PROCESSING. D.H. Gabuzda, J. Busciglio and B.A. Yankner. Dept. of Neurology, Children's Hospital and Harvard Medical School, Boston, MA 02115. A point mutation resulting in a single amino acid substitution of isoleucine for valine at residue 717 in the amyloid precursor protein (APP) has recently been identified in some pedigrees with familial Alzheimer's disease (Nature 349: 704, 1991). To determine whether this mutation alters APP synthesis or processing, eukaryotic expressor plasmids containing cDNA's encoding the 695 and 751 isoforms of APP were constructed and site-directed mutagenesis was used to introduce the isoleucine for valine substitution. The wild-type and mutant APP expressor plasmids were transfected into COS cells. Cell lysates and supernatants were analyzed by radioimmunoprecipitation using antibodies which recognize the APP N-terminus, APP C-terminus and the amyloid β protein (AβP). In cell lysates, the N- and C-terminal antibodies both identified 3 APP bands at 100-125 kD (APP_{695}) and 110-135 kD (APP_{751}). In the supernatants, the N-terminal antibody identified 3 bands at 90-115 kD (APP_{695}) or 100-125 kD (APP_{751}). The AβP antibody identified the 3 APP bands in cell lysates and supernatants and, in addition, recognized a unique smaller species in supernatants. The same bands appeared at similar intensities in cells transfected with the wild-type or mutant APP cDNA's. These results suggest that this mutation in APP does not produce a gross alteration in APP synthesis or processing, but do not exclude the possibility that the mutant APP may be processed differently in other cell types or in vivo as a function of age.

439.4

FATE OF AMYLOID PRECURSOR PROTEIN IN THE NERVOUS SYSTEM. S.S. Sisodia*, E.H. Koo, P.N. Hoffman, V.E Koliatsos, G. Perry and D.L. Price. Neuropathol. Lab., The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205; Dept. of Pathology, Case Western Reserve School of Medicine, Cleveland, Ohio 44106.

In Alzheimer's disease, amyloid plaques contain β/A4, a 4-kD peptide derived from an amyloid precursor protein (APP). *In vitro*, APP matures through a constitutive pathway and, following a membrane-associated cleavage, C-terminally truncated APP is secreted into the media. *In vivo*, truncated precursor is present in the spinal fluid, suggesting that similar maturational processes occur in the CNS. We have demonstrated that holo-APP is rapidly transported in the PNS, but little is known about the processing of APP by neurons in the CNS. The present study examined the biology of neuronal APP, detected by several specific polyclonal antisera. When retinal ganglion cells were labeled by injection of [^{35}S] methionine into the vitreous, full-length APP was transported to terminals in the geniculate. In separate experiments, synaptosomal preparations were shown to contain APP. This system should be useful for studying processing of neuronal APP in young animals, and the possible abnormalities of processing in aged animals.

439.5

A SERINE PROTEASE FROM MONKEY AND ALZHEIMER'S BRAIN AND A CYSTEINE PROTEASE FROM ALZHEIMER'S BRAIN DEGRADE THE AMYLOID PRECURSOR PROTEIN. C. R. Abraham, B. Razzaboni and G. Papastoitsis*. Arthritis Ctr, Boston Univ. Sch. of Med., Boston, MA 02118.

Alzheimer's disease (AD) brains exhibit abnormal deposits of amyloid ß-protein, a 4Kd fragment of the larger ß-protein precursor (ßPP). The finding of the abnormally processed ß-protein and a protease inhibitor (α1-antichymotrypsin) in the amyloid deposits prompted us to search for proteases which may generate the ß-protein. We here report the presence and partial purification of two such proteolytic activities from AD brain. AD brain homogenates were fractionated and proteases were detected by incubating the fractions with an iodinated peptide flanking the N-terminus of the ß-protein. The cleaved radio-labeled products were separated by thin layer chromatography and exposed to X-ray film. Two distinct active fractions were further purified and characterized by using a battery of protease inhibitors. One activity appears to be a calcium-activated serine protease which cleaves the peptide between the methionine and aspartic acid. A similar proteolytic activity was purified from saline-perfused, fresh-frozen monkey brain. The calcium-activated protease is also able to degrade the full length ß-PP (a gift from S. Sisodia, E. Koo and R. Siman). The second proteolytic activity seems to be a metal-dependent cysteine protease which prefers the met-asp bond as the cleavage site, thus also being able to cleave at the N-terminus of the ß-protein. Finding the protease(s) which generate the ß-protein can lead to the design of protease inhibitors aimed to arrest amyloid deposition which is believed to cause neuritic abnormalities, neuronal cell death and dementia. Supported by NIA, Alzheimer's Association and Cephalon, Inc.

439.6

CHARACTERIZATION OF NEURITE-STIMULATING ACTIVITY FROM CONDITIONED MEDIA OF TRANSFECTED PC12 CELLS THAT OVEREXPRESS ß/A4-AMYLOID. R.E. Majocha, B. Tate, M. Ventosa-Michelman and C.A. Marotta. Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114.

Conditioned media (CM) from transfected PC12 cell lines permanently expressing the A4 to C terminal portion of the amyloid precursor protein cause significant increases in the area of the cell and length of neurites of non-transfected PC12 cells. The CM factor(s) responsible for the effects have apparent MW above 5000. Both alkaline and acidic treatment reduce CM activity. The CM factor(s) are resistant to denaturation at 90° C for 60 minutes. The CM effects are significantly reduced by protease treatment. However, heparinase could not destroy the stimulatory activity. Absorption of CM with Sepharose conjugated heparin sulfate or dextran sulfate do not completely remove stimulatory activity. Antibodies to ß/A4-amyloid are only partially effective in removing activity. These results suggest that multiple stimulatory factors are released from PC12 cells overexpressing ß/A4 peptide. Supported by NIH AG02126 and the Metropolitan Life Foundation.

439.7

ß-AMYLOID PRECURSOR PROTEIN EXPRESSION IN RAT BRAIN. INCREASED LEVELS OF KUNITZ DOMAIN CONTAINING FORMS AFTER NEURONAL LESIONS. G. Mengod [1,2], C. Solà[1], F.J. Garcia-Ladona [3], M. Sarasa [4], P. Frey [3], A. Probst [3] and J.M. Palacios [1,2].
1 CID-CSIC Barcelona Spain, 2 Sandoz Pharma Ltd, 3 Dept. of Pathology Univ. Basel Switzerland, 4 Fac. of Veterinary Zaragoza Spain.

We have examined the effects of neuronal lesions in amyloid expression in the rat brain. Two types of neuronal lesions were induced in rats 1) icv injection of kainic acid and 2) axotomy of motor neurons of cranial nerves. The effect of these lesions on the expression of the different forms of the ß-amyloid precursor protein (ßAPP 695, 714, 751, 770) were examined using in situ hybridization histochemistry (ISHH) with oligonucleotide probes specific for each form and by immunohistochemistry with antibodies raised against different synthetic peptides of the ßAPP.
Kainic acid injections resulted in neuronal cell death and glial reaction in the hippocampus. As previously described (Siman et al, 1989) some of the glial cells display positive staining for ßAPP. The ISHH experiments revealed a loss of ßAPP concurrent with neuronal loss. In contrast, the Kunitz domain containing forms 751 and 770 were dramatically increased in correspondance with the glial reaction. Axotomy of the facial and hypoglossal motor nerves resulted in increased ßAPP immunoreactivity in these motor neurons shortly after axotomy. The mRNA coding for the different ßAPP forms were also increased. After regeneration, the level of ßAPP mRNA and protein returned to normal. These results suggest different modes of response of the ßAPP protein to neuronal injury that affect differentially the forms containing the protease-inhibitor domain.

439.8

MICROGLIAL CLUSTERS GENERALLY LACK AMYLOID PRECURSOR PROTEIN (APP) mRNA IN ALZHEIMER'S DISEASE (AD). S.A. Scott, S.A. Johnson, C.E. Finch, and L.S. Perlmutter. Dept Neurology and Andrus Gerontology Center, University of Southern California, Los Angeles CA 90033

The distribution of beta amyloid in the brains of AD patients is well-described, yet its origin remains unclear. Human studies using in situ hybridization and Nissl staining indicate that APP mRNA is synthesized primarily by neurons, but also several other cell types including microglia. This study combines in situ hybridization with immunocytochemistry to confirm the microglial localization of APP mRNA. Using 6 AD and 5 non-AD subjects, aldehyde-fixed hippocampal sections were processed immunocytochemically using the microglial antibody LN3, and then hybridized with sense or antisense APP cRNA probes.

For both AD and non-AD cases, our preliminary conclusion is that microglia generally lack APP mRNA signal, especially when compared to the strong neuronal pattern observed using this probe. Most microglial clusters in regions of high plaque density (with a few exceptions) did not contain APP mRNA. Microglia with specific signal were sporadically found in white matter tracts (e.g. alveus) as well as in the dentate molecular laminae. On the other hand, many signal aggregates resembling the shape of pyramidal neurons appeared to be surrounded by clusters of LN3+ microglia.

To summarize, these data suggest that plaque-associated microglia do not produce amyloid *de novo*, although microglial processing of exogenous APP cannot be ruled out.
Supported by AG07909 (CEF), AG07127, Calif. AD Program #90-11101 (LSP)

439.9

ß/A4 AMYLOID PRECURSOR PROTEIN IN ALZHEIMER'S PAIRED HELICAL FILAMENTS. F.P. Zemlan, G.E. Dean and G.D. Vogelsang. Alzheimer's Research Center, University of Cincinnati College of Medicine, Cincinnati, OH 45267-0559.

Several laboratories have demonstrated that antibodies (Abs) against restricted regions of the ß/A4 amyloid precursor protein (APP) label neurofibrillary tangles in Alzheimer's brain. The present study examines whether APP is loosely associated with paired helical filaments (PHF) in tangles or is directly incorporated into PHF core protein. Alzheimer's PHF was purified and solubilized as previously reported by our laboratory, then examined on immunoblots with Abs raised against recombinantly expressed APP. Anti-$APP_{590-695}$ selectively labeled the monomeric 66 kD PHF core protein (AD66 protein) on immunoblots while anti-APP_{20-304}, anti-$APP_{444-592}$ and anti-KPI did not. As $APP_{590-695}$ includes the ß/A4 amyloid region, Abs directed against synthetic 42-mer ß/A4 amyloid and purified ß/A4 protein were examined. Both selectively labeled AD66 PHF protein on immunoblots. To confirm these results, immunocytochemical studies were performed on fresh, unfixed AD cortical tissue. Consistent with the above studies, Abs raised against synthetic ß/A4 protein labeled both intracellular and extracellular tangles in unfixed AD cortex. The present data are consistent with the hypothesis that a restricted region of the APP is directly incorporated into the 66 kD PHF core protein in AD brain. This incorporated region appears limited to the ß/A4 protein which suggests that about 4 kD of the 66 kD PHF core protein is comprised of ß/A4 amyloid with the bulk of the AD66 PHF core protein consisting of MAP tau which has been post-translationally modified.

439.10

BIOLOGICALLY ACTIVE SECRETED AMYLOID PRECURSOR PROTEINS EXPRESSED BY RECOMBINANT BACULOVIRUS INFECTED INSECT CELLS. **R.Bhasin, W.vanNostrand, T.Saitoh, M.A.Donets*, E.A.Barnes*, W.W.Quitschke and D.Goldgaber. Dept. of Psychiatry, SUNY, Stony Brook, NY 11794, Dept. Micro. and Mol. Gen., Univ. California, Irvine, CA 92717, Dept. Neurosc., Univ. California, San Diego, CA 92093.**

Three alternatively spliced forms of the amyloid precursor protein (APP), APP695, APP751 and APP770, were expressed in the baculovirus expression vector system. The recombinant proteins were secreted into the culture medium by infected insect cells and APP molecules were detected in insect cells and media two days after infection with the recombinant APP baculoviruses. A partial sequence of the amino terminus of the secreted protein revealed identity with the native secreted protein and showed that the signal peptide was recognized and properly cleaved in insect cells. Purified secreted recombinant APP751 co-migrated with protease nexin II (PN-2) purified from platelets and fibroblasts. A 15 Kda C-terminal fragment of APP was also detected in cells infected with the recombinant baculoviruses suggesting that the recombinant APP proteins were cleaved at the C-terminal end like the native APP protein. Recombinant APP751 and APP770 formed complexes with epidermal growth factor binding protein (EGF BP) whereas APP695 did not. In addition, recombinant APP751 and APP770 inhibited trypsin and chymotrypsin activity whereas APP695 did not. A human fibroblast cell line, A-1, that required APP for complete growth, had its growth restored upon addition of secreted recombinant APP695 or APP751. Thus the appropriately sized, secreted recombinant proteins produced in this expression system are biologically active.

439.11

MUTATIONAL ANALYSES OF *AMYLOID PRECURSOR PROTEIN-LIKE* GENE IN *DROSOPHILA*. L. Luo and K. White, Department of Biology, Brandeis University, Waltham, MA 02254.

The *Drosophila Amyloid precursor protein-like* gene encodes a protein with strong sequence homology, similar biochemical properties and tissue distribution compared with APP695, a neural-specific form of the amyloid precursor protein associated with Alzheimer disease (Rosen et al, PNAS **86**, 2478-2482; Luo et al. J. Neurosci. **12(10)**, 3849-3861). We are using a genetic approach in *Drosophila* to investigate the *in vivo* function of this class of molecules.

By deficiency duplication combination, we have generated null *Appl*$^-$ flies by the criteria of PCR, Southern and Western analyses. These *Appl*$^-$ flies are viable and morphologically normal, however, they exhibit behavioral deficits. A phototaxis behavior assayed by a counter current apparatus was chosen for further analysis because the quantitative difference could be partially rescued by transduced heat-shock promotor-*Appl* cDNA construct. This behavioral abnormality provides a potential *in vivo* assay for APPL protein function.

We have also generated by *in vitro* mutagenesis constructs expressing secretion-defective or constitutively secreted APPL proteins. These constructs are being introduced into flies through germ-line transformation. Such transduced genes, when introduced into *Appl*$^-$ background, may provide insight about which form is biological active by their ability to rescue the behavioral deficit. In addition, they could be used to study the cell biological properties of various forms of APPL protein in developing nervous system.

439.12

BINDING BY A HIGH MOLECULAR WEIGHT HEPARAN SULFATE PROTEOGLYCAN TO THE EXTRACELLULAR DOMAIN OF THE BETA-AMYLOID PROTEIN OF ALZHEIMER'S DISEASE. A.D. Snow*, M.G. Kinsella*, R.T. Sekiguchi*, D. Nochlin and T.N. Wight*. Dept. of Pathology, Univ. of Washington, Seattle, WA 98195.

Previous studies have shown the accumulation and co-localization of a basement membrane derived heparan sulfate proteoglycan (HSPG) to the amyloid deposits containing beta-amyloid protein (BAP) in neuritic plaques and amyloidotic blood vessels in Alzheimer's disease (AD) brain. In the present study the potential binding interaction between different PGs and the extracellular domain of the BAP was explored. In situ and affinity column chromatography experiments utilizing isolated ^{35}S-sulfate labelled PGs from medium or cell layer of bovine aortic endothelial cells or smooth muscle cells, demonstrated that a high MW HSPG (MW ~ 600 kd) binds relatively tightly, 2 smaller dermatan sulfate/chondroitin sulfate (DS/CS) PGs (MW ~100-250 kd) bind weakly, and a large chondroitin sulfate PG (of smooth muscle cells) did not bind to the BAP. Competitive inhibition studies using ^{125}I-labelled HSPGs in the presence of a variety of different unlabelled ligands suggest that both the core protein and GAG chains of the HSPG are involved in BAP binding. The specific binding of the high MW HSPG to the BAP may explain its constant co-localization to BAP amyloid deposits in AD brain, and further implies a possible role for this particular PG in BAP accumulation in AD.

COCHLEAR NERVE, MICROMECHANICS AND RECEPTORS

440.1

RESPONSES TO CLICKS OF THE CHINCHILLA BASILAR MEMBRANE. M.A. Ruggero, N.C. Rich* and A. Recio*. Dept. of Otolaryngology, Univ. of Minnesota, Minneapolis, MN 55414.

Basilar membrane responses to clicks and tones were studied in chinchilla cochleae using a new application of laser velocimetry (Ruggero and Rich, Hearing Research, 51: 215-230, 1991). Basilar membrane velocity at a site located 3.5 mm from the round window [characteristic frequency (CF): 8-10 kHz] was determined from the Doppler frequency shift of laser light reflected from glass microbeads (10-30 μm) placed on the basilar membrane. Responses to clicks in healthy cochleae consisted of relatively undamped transient oscillations with periodicity close to 1/CF. Intense rarefaction clicks caused initial basilar membrane motion toward scala vestibuli after a latency of about 90 microseconds (measured from the onset of inward stapes displacement). The initial response oscillations grew linearly with stimulus intensity but later response cycles grew nonlinearly, at rates less than 1 dB/dB. Thus, with increases in stimulus level, the response envelopes became progressively more asymmetrical, with maxima shifting to earlier times. The frequency spectra of click responses were computed by Fourier transformation. As click level increased, sharpness of frequency tuning diminished and the maximal spectral response component shifted to lower frequencies. Spectral components immediately below CF phase lagged responses evoked by less-intense clicks, while those just above CF led lower-level responses. In spite of the CF-specific nonlinearities, the magnitude and phase frequency spectra of click responses resembled those for responses to tones. Upon cochlear damage or death of the chinchilla, all nonlinearities disappeared; the gain spectra became broadly tuned and insensitive and thus very similar to those of responses evoked by intense stimuli.

[Supported by NIH (NIDCD) grants DC-00110 and DC-00419.]

440.2

RESPONSES OF SPIRAL-GANGLION UNITS TO ELECTRICAL STIMULATION OF THE AUDITORY NERVE ROOT. M.C. Brown. Depts. of Physiol. and Otol. and Laryngol., Harvard Medical School, and Eaton-Peabody Lab., Mass. Eye & Ear Infirmary, Boston, MA 02114.

The mammalian auditory nerve is composed of thick, myelinated axons from type-I neurons and fine, unmyelinated axons from type-II neurons. Type-II axons number only 5-10% of all axons in the auditory nerve and their sound-evoked responses have not been documented. Since their fine axons should have long impulse conduction times, type-II units recorded peripherally in the spiral ganglion should have long antidromic latencies to shocks from a stimulating electrode placed centrally in the auditory nerve root. Single units were recorded with micropipettes from the basal-turn spiral ganglion of the anesthetised guinea pig. Most units (n=129) had short-latencies (0.3-1.0 msec) to shocks and sound-evoked activity typical of type-I units (Liberman, 1982, Science, 216:1239-1241). A second class (n=24 units) had sound-evoked activity typical of olivocochlear efferent fibers (Brown, 1989, Hearing Res. 40:93-110); and sometimes responded to high-level shocks with latencies of 1.5-2 msec. Presumably at high shock levels, the current spreads to the nearby vestibular nerve where olivocochlear fibers run. A rare class of units (n=5) had much longer latencies (5-8 msec), appropriate for the caliber of type-II axons (Brown, 1987, J. Comp. Neurol. 260:591-604). These units had moderate to high electrical thresholds. One long-latency unit (from a preparation with good type-I thresholds) responded to high-level noise bursts. It may be suggested that these long-latency units correspond to type-II neurons, although an unusual type of olivocochlear neuron cannot be entirely ruled out.

(Supported by NIH grants DC00351 and DC00119.)

440.3

GLUTAMATE RECEPTOR mRNA EXPRESSION IN THE RAT COCHLEA. A.F. Ryan, D. Brumm* and M. Kraft*. Division of Otolaryngology and Department of Neurosciences, UCSD Medical School and VA Medical Center, La Jolla, CA 92093.

Pharmacological evidence suggests that glutamate may be the transmitter between cochlear hair cells and afferent neurons. Recently, a gene family encoding subunits of non-NMDA glutamate receptors has been recognized, and several genes cloned (Hollmann et al., Nature 342:643, 1989; Boulter et al., Science 249:1033, 1990). To determine whether these receptors participate in cochlear neurotransmission, adult rat cochleas were screened for expression of the genes GluR1, GluR2, GluR3 and GluR4 by in situ mRNA hybridization. GluR1 riboprobes showed no hybridization with any cochlear cells. GluR2 and GluR3 riboprobes both showed intense hybridization with spiral ganglion neurons (SGNs), consistent with a major role for these receptors in SGN neurotransmission. GluR4 riboprobes showed positive but lower levels of hybridization to SGNs. The most likely site for GluR2, GluR3 and GluR4 receptor subunits is on SGN afferent dendrites beneath the cochlear hair cells, since presynaptic endings are relatively sparse on SGN axons in cochlear nucleus and there is no evidence that the olivocochlear efferents use glutamate as a neurotransmitter. These results support the hypothesis that non-NMDA glutamate receptors mediate neurotransmission between cochlear hair cells and SGNs.

Supported by DC00139 and the VA Research Service. Glutamate receptor cDNAs were provided by J. Boulter of the Salk Institute.

440.4

ANATOMICAL DISTRIBUTION OF CHOLERA TOXIN BINDING SITES IN THE COCHLEA. P.A. Santi and P. Mancini*. Dept.of Otolaryngology, Univ. of Minnesota Medical School, Minneapolis, MN 55455.

The B subunit of cholera toxin specifically binds to the carbohydrate residues of the GM1 ganglioside. A FITC-labelled B subunit of cholera toxin was applied to fixed, cryosections of the mammalian cochlea in an attempt to localize the GM1 ganglioside within cochlear tissues. The cholera toxin probe strongly reacted with cells of the organ of Corti, interdental cells, Boettcher cells, inner and external sulcus cells, spiral prominence cells, interosseous globuli, and cochlear nucleus cells that extend into the internal auditory canal. Less reactivity was observed in cells of the stria vascularis, spiral ganglion, modiolus, and Reissner's membrane. No reactivity was observed in the connective tissue cells of the spiral ligament, spiral limbus, basilar membrane or perilymphatic scalas. Controls consisted of probe binding to normal brain tissue, absorbing out the probe with bovine GM1, binding inhibition of the probe with unlabeled cholera toxin, and ethanol extraction of GM1 from cell membranes prior to probe exposure. The functional significance of cholera toxin binding sites and their relationship to the GM1 ganglioside in the cochlea will be discussed. (Research supported, in part, by NIDCD and DRF).

440.5

SENSITIVE PERIOD FOR α-DIFLUOROMETHYLORNITHINE (DFMO) OTOTOXICITY IN THE DEVELOPING PIGMENTED RAT. C. M. Henley*, G. K. Martin and B.L. Lonsbury-Martin, Dept. of Otorhinolaryngology, Baylor College of Med., Houston, TX 77030.

To determine the potential role of the ODC/polyamine system in the functional development of the rat cochlea we quantified ODC and polyamines in developing cochlear tissues and then determined the effects of postnatal DFMO treatment on both cochlear function and on cochlear polyamine metabolism. DFMO, an inhibitor of ODC, is a useful biochemical tool in studying polyamine-dependent processes. Rats (n=3 to 5) were terminated at postnatal ages ranging from 3-60 days and cochlear tissues were analyzed for ODC/ polyamines. Cochlear ODC increased rapidly during the first 10 days of life, peaked on days 9-10, then rapidly declined. ODC in the lateral wall-organ of Corti was greater than in the cochlear nerve in developing, but not in adult rats. Polyamines were elevated during the period of differentiation of various cell types comprising the lateral wall-organ of Corti. The spermidine/ spermine ratio was high in the cochlear nerve, even in adults, indicating the continued elevated level of protein and nucleic acid synthesis necessary for nerve function. This may explain the reversibility of DFMO ototoxicity in humans, since the drug is cytostatic and not cytotoxic. Rats treated with DFMO (500 mg/kg/day) during the period of increased polyamine biosynthetic activity demonstrated age and frequency-dependent deficits in outer hair cell function, as assessed by acoustic distortion-products. DFMO significantly inhibited ODC during this specific period. The developing rat appears to be hypersensitive to the effects of DFMO during the period when polyamine biosynthetic activity is increased. We are currently evaluating the effects of DFMO on cochlear polyamine levels to correlate biochemical effects with observed functional losses. These studies suggest that the polyamines are critical for the normal development of cochlear function.

440.6

SENSITIVITY OF AUDITORY NERVE FIBERS TO THE RELATIVE PHASE OF FREQUENCY COMPONENTS IN COMPLEX SOUNDS. D.A. Bodnar and R.R. Capranica, Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853.

A complex sound is uniquely specified by the magnitude and phase spectra of its individual frequency components. Excitatory tuning curves of individual auditory nerve fibers only reflect the selectivity to single tone stimuli. Thus, tuning curves do not provide any information regarding the encoding of the relative phase spectrum in more complex signals.

To address the question of peripheral sensitivity to phase spectrum, we recorded from single units in the VIIIth nerve of the bullfrog (*R. catesbeiana*) using multi-tone stimuli. These stimuli consisted of the first 10 components of a harmonic series with a fundamental frequency of 200 Hz, and spanned most of the frequency range of the bullfrog's vocal repertoire. For all units the same standard reference stimulus was initially presented and then the relative phases of single harmonic components were varied systematically. Most of the cells exhibited pronounced changes in their spike rates when the relative phase of only a single component was varied, and were most sensitive to changes in phase of the component closest to their best excitatory frequency. Plots of spike rate vs. phase angle revealed a wide variety of patterns in spike rate changes. Most cells also showed significant changes in their coefficients of synchronization to the fundamental period of the stimulus. The shapes of cycle histograms, plotted on the fundamental period, changed with shifts in the relative phase of a single harmonic.

The phase relationships of individual frequency components affect the fine temporal structure of the acoustic signal waveform. The observed sensitivity of auditory nerve fibers to these relative phases suggests that complex waveform features of auditory stimuli are encoded by the peripheral auditory system.

This research is supported by NIH grant NS 09244.

440.7

DUALITY OF ENCODING WITHIN THE TUNING CURVES OF AUDITORY NERVE FIBERS IN THE TOKAY GECKO.

E. Dodd and R.R. Capranica, Section of Neurobiology & Behavior, Cornell University, Ithaca, NY 14853.

Excitatory tuning curves of auditory nerve fibers in the Tokay Gecko (*Gekko gecko*) have typical "V" shapes with a distinct best excitatory frequency (BEF) and a pronounced low-frequency tail that can encompass several octaves at moderate stimulus intensities. Since these remarkable lizards are highly vocal and produce several different types of loud calls whose waveform periodicities normally fall within the low-frequency tail regions, we explored to what extent different regions within a tuning curve preferentially dominate the fiber's spike pattern. This relative effectiveness was determined by first measuring spike rate and coefficient of synchronization intensity functions to a sequence of tonal triplets whose frequency components were the BEF and the two 30 dB cutoff frequencies on the opposing skirts of the tuning curve, while the relative amplitude of the BEF component was attenuated in 5 dB steps from 0 to -30 dB relative to the other two equal-amplitude components. Next, a set of harmonic series with fundamental frequencies of 100, 400 and 600 Hz were presented at 70 dB SPL in which the phases of the individual components were shifted systematically while cycle histograms were generated for each of the harmonic components in each series. The results clearly verify that each auditory nerve fiber selectively phaselocks to the lowest frequency component in a complex signal that falls within its excitatory tuning curve. The integrated energy contributed by the higher frequency components, especially weighted in the vicinity of the BEF, governs the corresponding spike-rate intensity function. These results therefore lead to a duality of spike train dependency: The low frequency tail below the BEF dominates the temporal phase-locked spike pattern whereas the absolute overall spike rate is governed by a complex summation of energy contributed by the higher frequency components in the stimulus. The spike response patterns to the Tokay's natural calls corroborates this interpretation. [Supported by NIH grant NS-09244, Danish Research Academy (F8900930) and the Carlsberg Foundation (88-0125)].

440.8

ENCODING OF AMPLITUDE-MODULATION (AM) RATE AND TEMPORAL-GAP IN FROG AUDITORY NERVE FIBERS. Albert S. Feng. Department of Physiology & Biophysics, University of Illinois, Urbana, IL 61801

Recordings were made from single auditory nerve fibers in the 8th nerve of northern leopard frogs to assess quantitatively their abilities to encode the AM rate and detect signal gaps, and how these abilities change with the duration of the AM signals. The goal was to test a hypothesis that signal gap is the factor that determines the upper limit of time-locked responses of these fibers to rapidly-fluctuating pulsed AM stimuli shown in an earlier study (J. Neurophysiol. 65: 424-445, 1991).

Acoustic stimuli comprised AM tone pulses of different durations presented as: (1) a pair in which the silent interval or gap was systematically varied from 0.1 to 100 ms, or (2) a train (which mimics frog's natural vocalizations) in which the AM rate was orderly varied whereby the gap between tone pulses covaried from 0 to > 100 ms. Responses of auditory nerve fibers to these two sets of stimuli were measured in terms of synchronization coefficient, mean spike count, and firing rate; these measures were plotted against the stimulus variable, e.g., gap or AM rate, to construct gap- or modulation-response-functions. These responses were compared to test the working hypothesis. Results to date indicate that the signal gap exerts a strong influence that determines the range of AM rate to which each fiber can faithfully respond. Moreover, the gap detection ability is dependent on signal duration, being superior when the signal duration is shorter. (Supported by NSF grant 88-09490)

440.9

KAPPA BUT NOT MU NOR SIGMA AGONISTS REDUCE AUDITORY THRESHOLDS AND INCREASE AUDITORY SENSITIVITY. TL Sahley, FE Musiek and DW Hoffman. (Spon. F Musiek) Dept. of Psychiatry, Pharmacology, Medicine and Surgery, Dartmouth Medical School, Hanover, NH 03756.

Little is known about how abused opioid drugs affect sensory systems. Opioid peptides and receptors are present in many nervous system structures mediating sensory function. This laboratory is studying the effects of intravenously administered opioid agonists and antagonists on auditory-evoked potentials. N1 and N2 amplitudes or latencies were not altered by the opioid receptor antagonist naloxone or the μ-receptor agonist fentanyl . The κ-receptor agonist, μ-receptor antagonist (-)-pentazocine or its racemate caused marked increases (up to 263% over baseline) in N1 and N2 amplitudes at and near threshold stimulus intensities, effectively increasing auditory sensitivity. Similar effects were seen with the κ-agonist U50488H. These actions were reduced by nor-binaltorphimine but not naloxone. (+)-Pentazocine had no effects. No changes were seen in the cochlear microphonic, supporting a site of action of these effects at the lateral olivocochlear efferent terminals on auditory nerve dendrites under inner hair cells, which contain enkephalins, dynorphins and acetylcholine. Similar results were obtained for far field auditory evoked responses. This action of κ-agonists on auditory-evoked potential amplitudes may represent a physiological role of the lateral olivocochlear efferent innervation. The different olivocochlear efferent neurotransmitters may have antagonistic or synergistic actions pre- and/or post-synaptically. [Supported by the Deafness Research Foundation.]

440.10

MINERALOCORTICOID (TYPE I) RECEPTORS IN THE INNER EAR. D.Z. Pitovski[1], M.J. Drescher[1,2*], and D.G. Drescher[1,2]. [1]Dept. of Otolaryngology, [2]Laboratory of Bio-otology, Wayne State University School of Medicine, Detroit, MI 48201.

The presence of mineralocorticoid (Type I) receptors in the mammalian inner ear was previously suggested by aldosterone modulation *in vivo* of ^{3}H-ouabain binding measured *in vitro* (Pitovski et al., Assoc. Res. Otolaryngol. Abstr. 13: 303, 1990). ^{3}H-Aldosterone binding has now been measured in the lateral wall of the basal turn of the cochlea and in the ampulla of the semicircular canal in male Hartley guinea pigs. Microdissected inner-ear tissues were incubated with 40 nM ^{3}H-aldosterone and binding determined. Specific binding was defined as total binding in the absence (experimental) minus binding in the presence (control opposite ear) of 2000-fold excess of unlabeled hormone. Binding values were also corrected for ^{3}H-aldosterone present intercellularly, monitored with ^{14}C-sucrose and normalized to tissue dry weight. Specific binding, thus measured, was 9.1 ± 1.5 (4) and 9.8 ± 1.5 (4) fmol per mg dry tissue [mean ± SEM (n)] in the lateral wall of the basal turn of the cochlea and in the ampulla of the semicircular canal, respectively.

These results demonstrate significant aldosterone binding and the presence of mineralocorticoid (Type I) receptor sites correlating with aldosterone-sensitive Na, K^{+}-ATPase activity and suggest that these inner ear tissues are a target site of mineralocorticoid action.

Supported by NIH Clinical Investigator Development Award DC 00046-01 to D.Z.P. and NIH Grant DC 00156.

441.1

MAGNITUDE MEASUREMENT OF HINDLIMB WITHDRAWALS TO GRADED NOXIOUS HEAT IN CONSCIOUS RATS. R.L. Kitchell, D. Ansley* and E. Carstens. Depts. of Animal Physiology and Veterinary Anatomy, Univ. of California, Davis, CA 95616.

Most animal pain models measure the latency (ie., threshold) of nocifensive responses. However, measurement of response magnitude would be desirable in showing psychophysical stimulus-response relationships throughout the noxious range. We therefore measured the magnitude of the conscious rat's hindlimb flexion withdrawal reflex.

Sprague-Dawley rats were habituated to a restrainer through which the left hindlimb protruded. The ventral hindpaw was taped against a Peltier thermode. Hindlimb flexor EMGs during attempted withdrawals elicited by graded noxious heat pulses (40-52°C, 5 s) were monitored via teflon-coated microwires in biceps femoris. Responses were quantified (area beneath integrated EMG for 7 s after heat onset) and normalized to % maximum. Humans tolerated all stimuli and rats showed no signs of distress.

Integrated EMGs increased in a graded fashion from 42-52 °C (N=12); no rats withdrew at 40°. The slope of the stimulus-response function was reduced, with no threshold change, 20-40 min after systemic administration of morphine (3.5 mg/kg i.p.) in a naloxone-reversible manner.

This relatively simple method measures reflex magnitude from threshold to tolerance levels of noxious heat, is sensitive to the opiate analgesic morphine, and might therefore serve as a useful new quantitative pain model.

441.2

QUANTIFICATION OF THE RAT TAIL-FLICK REFLEX (TFR). E. Carstens and C.G. Wilson*. Dept. of Animal Physiology, Univ. of California, Davis, CA 95616.

The TFR is a widely used pain test measuring the latency for a rat's tail to be flicked away from an unregulated noxious heat source. However, it is unknown if TFR magnitude increases in a graded or ballistic way with stimulus intensity, clouding the interpretation of all-or-none increases (to cutoff) in TFR latency (threshold?) often seen following analgesics. We therefore developed a method to measure TFR force in conscious rats.

Radially arranged transducers measured the force of horizontal, dorsal and rostrally directed components of TFRs elicited by feedback-controlled noxious radiant heat pulses (40-58°C, 5 s) delivered unilaterally to the tail. Areas beneath force traces were integrated to calculate TFR force vectors. Stimuli evoked no signs of distress.

The rostral force components of TFRs were much larger than horizontal/dorsal components. Most rats showed a preferred horizontal direction of TFRs (left or right in approximately equal numbers) regardless of the side of tail stimulation. TFR force vectors increased linearly from 40-48°C and then levelled off (N=10). Responses to 44°C stimuli decreased over repeated trials (2 min interval) and recovered following a 15 min break. Responses to repeated 50°C stimuli did not decrement.

Thus, the TFR is graded over a narrow range of noxious heat intensities and may habituate at low temperatures.

441.3

ULTRASTRUCTURAL AND NEUROPHYSIOLOGICAL ANALYSIS OF THE INTERNAL ARCHITECTURE OF INTACT HUMAN NERVES **R.G. Hallin,* T.Carlstedt, R. Ekedahl* and Y.-X. Yu,* Dept Clin Neurophysiol, Huddinge Univ Hospital, 141 86 Huddinge, Sweden.**

Single units within the entire nerve fibre spectrum can be studied in man. Furthermore, thin concentric needle electrodes are useful as a tool for integrative analysis of both functional and structural aspects of human peripheral nerves.

In this study, we used concentric needle electrodes (O.D. 200-250 μm, inner core diameter 10-70 μm) for percutaneous median nerve exploration. The firing characteristics of A and C fibres and their relative localization with respect to each other were studied in adjacent recording sites in the nerve. Electrodes with thicker cores were used to screen intraneural fascicular organization. Individual cutaneous fascicles destined to the hand were mutually organized in a somatotopic manner at both wrist and elbow level. Electrodes with a thin core were used to study the detailed intrafascicular fibre organization. Many observations suggested the presence of an orderly organization also within the individual fascicle, which extended to even the single unit level.

The traditional view that the fibres are spread out in disarray proximally in limb nerves was not substantiated by these studies. Instead we feel tempted to suggest that our data reflect the existence of a pervading somatotopic organization which starts in the periphery of the neuraxis and then extends to central levels of the nervous system. Some clinical implications of the obtained data will be discussed.

441.4

THE FAILURE OF CONDUCTION OF IMPULSES IN LONG RANGE AFFERENTS RUNNING IN RAT SPINAL CORD. PD Wall* and SB McMahon. Dept Anatomy, University College London, UK and Dept Physiology, St Thomas' Hospital Medical School, London, UK.

When myelinated sensory afferents enter the spinal cord, they form a T branch running rostrally and caudally. The rostral branch may run as far as the dorsal column nuclei. However, the caudal branch may also run for many segments and extend with dorsal horn terminals far beyond the region in which such afferents have been shown to produce postsynaptic effects. 50% of lower thoracic afferents extend 3 segments caudally and 5% extend 8 segments (20mm) caudally (PD Wall & P Shortland (1991) Phil Trans Roy Soc, *in press*). For 70 myelinated fibres, we have tested the possibility that these long range caudal axons do not normally conduct orthodromic impulses by measuring their partial refractory period either following local microelectrode stimulation in the dorsal columns or following the injection of an orthodromic impulse from the dorsal root. This measure showed that orthodromic impulses may fail to penetrate the caudal branch at distant caudal sites in all fibres tested. In contrast, the rostral branch always appeared to conduct orthodromic impulses. Furthermore, this apparent orthodromic blockade is relieved 2-10 days after the neighbouring dorsal roots have been sectioned.

441.5

ACTIVITY OF NEURONS IN THE ROSTROVENTRAL MEDULLA WHICH RESPOND TO NOXIOUS HEAT ARE CORRELATED TO CHANGES IN ARTERIAL BLOOD PRESSURE. C.L. Thurston & A. Randich, Dept. of Psychology, Univ of Alabama at Birmingham, Birmingham, AL 35294

Extracellular recordings were made of neurons in the rostroventral medulla (RVM) in methohexital-anesthetized rats. The neurons were classified as ON, OFF, or NEUTRAL cells depending on their response to noxious heat applied to the tail. ON cells were excited just prior to the tail flick (TF) and OFF cells were inhibited just prior to the TF. Both ON and OFF cells showed spontaneous fluctuations in activity that were correlated with fluctuations in arterial blood pressure (ABP). When ON cells are active and OFF cells are inactive, ABP is lower than when OFF cells are active and ON cells are inactive. Further, changes in neuronal activity precede changes in ABP. In addition, electrical stimulation of the cervical vagus produces a depressor response at intensities which increase ON cell activity and decrease OFF cell activity. These data suggest that ON and OFF cells in the RVM are important in autonomic regulation.
Supported by NIH grants NS24958 and NS22966.

441.6

COACTIVATION OF THE NUCLEUS RAPHE MAGNUS (NRM) BY LATERAL HYPOTHALAMUS (LH) AND THE PERIAQUEDUCTAL GRAY (PAG). M.M. Behbehani, M. Jiang* and S.D. Chandler. Depts. of Physiology and Biophysics and Anesthesia. U. of Cincinnati. Cincinnati, OH 45267-0576.

There is evidence that NRM receives input from both the LH and PAG. However, it has not been established whether a given cell in NRM responds to stimulation of both sites. We tested this possibility by extracellular and intracellular recording from NRM neurons and measurement of their response to electrical stimulation of LH and PAG. In addition, we examined the role of neurotensin (NT) in coactivation of NRM. Extracellular recording was made from 100 NRM neurons of adult male rats; in 10 cells intracellular recordings were made. Cells were identified according to their response to peripheral stimulation. Stimulation of both sites excited 15 cells and inhibited 23 cells; 29 cells responded in opposite direction to LH/PAG stimulation. The excitatory response to PAG and LH had an onset latency of 12.45±4.4 and 14.9±9.8 and duration of 22.6±15.5 and 33.9±31.5 respectively. The inhibitory response had an onset latency of 14.75±8.34 and 11.14±10.12 and duration of 91.5±31.6 and 62±29.54 respectively. In 2 cells recorded intracellularly, IPSPs produced by stimulation of LH had a larger magnitude and duration than IPSPs produced by stimulation of PAG. In 56 cells the effect of NT on NRM was tested, 31 cells were excited, 14 were inhibited and 11 were unresponsive to NT. Comparison of the response of NRM cells to LH, PAG and NT showed no significant interaction. It is concluded that 1) a significant number of NRM neurons are coactivated by PAG and LH, 2) the inhibitory response is more dominant and 3) NT is unlikely to be the mediator of the coactivation response.
Supported by PHS grant NS20643.

441.7

NOCICEPTIVE RESPONSES OF RACCOON THALAMIC NEURONS. B. H. Pubols, D. A. Simone, N. A. Bernau, and M. E. Hanson. Good Samaritan Hospital & Med. Ctr., Portland, OR 97209.

Raccoon thalamic neurons with receptive fields (RFs) totally (N = 10) or partially (N = 10) on glabrous skin of the forepaw were examined for their responsiveness to noxious mechanical and thermal stimuli. Recording loci were in the core of the ventrobasal complex (VB; N = 8), the ventral periphery of VB (N = 4), or the medial division of the posterior nuclear group (POm; N = 8).

Eleven VB neurons were classed as multireceptive (MR) and one as nociceptive specific (NS); five POm neurons were classed as MR and three as NS. All MR neurons had larger high threshold RFs than low threshold RFs, and this difference was greater for POm neurons than for VB neurons. 85% of the total were rapidly adapting and 15% slowly adapting.

For 16 out of 19 neurons tested, the thermal threshold was greater than 53°C. Thermal thresholds were between 51°C and 53°C in three MR units. Sensitization following a mild burn (53°C for 30-90 sec) to the RF, persisting for up to one hour, occurred in four of eight units tested.

These data indicate that raccoon thalamic cells responsive to nociceptive input are not segregated within VB, but are located throughout this nucleus as well as in POm. Many of these cells may become sensitized following an injury, and thus contribute to hyperalgesia. [Support: NS-19486, USPHS.]

441.8

SOMATOSENSORY EVOKED MAGNETIC FIELDS STUDIED WITH A 19-CHANNEL FIRST-ORDER GRADIOMETER SYSTEM.

I. M. Tarkka, R. Laudahn* and B. Bromm*. Institute of Physiology, University Hospital Eppendorf, D-2000 Hamburg 20, F.R.G.

Novel 19-channel MEG equipment was used to record somatosensory evoked fields (SEF) in normal subjects. The channels were arranged in a hexagonal configuration with the distance between nearest neighbors being 2.5 cm. The SQUIDs were connected to axial first-order gradiometers with 2.0 cm diameter and 5.0 cm baseline. All channels were placed in parallel. The exact relationship between the subject's head and the recording sensors was monitored using three 3-D coils placed on the scalp (international 10-20 locations Fz, Cz and Pz). The coils served as a defined magnetic source giving the coordinates of each coil relative to the gradiometers. Somatosensory stimuli were electrical pulses of 0.2 ms duration given to the right median nerve at wrist. The stimulus intensity was adjusted above the individual motor threshold. 100 sweeps were averaged and a recording window of 250 ms was used. The bandpass was 0.5 - 250 Hz. The following components were identified: M20, M35, M80, M110 and M140. Phase reversal was observed for M20, M35, M80 and M110 components within one recording location posterior to C3. Addional locations were recorded for finding the maxima of the fields of interest. The current dipole estimations confirmed multiple generators in the somatosensory cortex. The estimated dipoles were superimposed on individual MR images of the subjects.

441.9

THREE DIMENSIONAL ANALYSIS OF CENTRAL TERMINATIONS OF TRIGEMINAL PRIMARY AFFERENTS IN CATS. K.Otani*, H.Ikeda*, O.Takahashi and T.Shimosato*, Dept.Oral and Max-Fac. Surg. II, Hiroshima Univ. Sch.of Dent., Hiroshima,Japan.

We have previously reported morphological feature of trigeminal primary afferent fibers which showed electrophysiological fast adapting (FA) and slowly adapting (SA) type of afferents. In the present paper, we would like to submit more detailed quantitative analysis of them with special reference to morphological features of FA and SA types of primary afferents. Under sodium pentobarbital anesthesia, trigeminal primary afferent fibers of FA and SA type were characterized electrophysiologically and they were stained with horseradish peroxidase (HRP) with intracellular technique. The terminal and en passant boutons of them were observed under a microscope, and then analysed three-dimensionally using computer. The densities of boutons in each trigeminal subnucleus were calculated by defining a volume of terminal arbor. The density of FA-type boutons showed no significant difference between that in the trigeminal principal nucleus (Vp) and that in the oral subnucleus of the spinal trigeminal nucleus (Vo). On the other hand, the density of SA type boutons in Vp were higher than that in Vo. These results suggested that FA- and SA-type primary afferents had characteristic features in terms of the density of boutons.

441.10

COMPUTER SIMULATIONS OF THALAMOCORTICAL RESPONSE TRANSFORMATIONS IN THE WHISKER/BARREL SYSTEM.

H.T.Kyriazi and D.J.Simons. Dept. of Physiology, Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA 15261.

A model of a somatosensory cortical neuronal network (Neurosci.Abstr.15:313,1989) has been tested for its ability to simulate barrel neurons' responses to different whisker stimuli. The model incorporates known anatomical and physiological features of a barrel in cortical layer IV and is activated by actual pre-recorded spike trains from thalamic barreloid neurons. Initially, responses of spiny and smooth neurons to deflection of the principal whisker (PW) alone were studied. Model parameters were adjusted to produce accurately the reductions, relative to thalamic input, in the sizes of spiny cell ON and OFF responses and the increases in these responses for smooth cells. Subsequently, the same model parameters correctly produced the disproportionately smaller ON responses evoked in spiny cells by adjacent whisker stimuli. Also in both cell types, movements of adjacent whiskers generated post-excitatory inhibition and realistic response suppression to subsequent deflections of the PW. Finally, when the duration of the PW alone stimulus was increased, the simulated OFF response increased appropriately. Thus, circuitry within a barrel is sufficient to produce significant changes in spatial and temporal characteristics of the thalamic input signal. Supported by NS19950 and the Pittsburgh Supercomputing Center.

441.11

SELECTIVE POST-DISCRIMINATION SIGNALS IN THE MOTOR AND PREMOTOR CORTICES. V. B. Mountcastle, P.P. Atluri, & M.A. Steinmetz, Bard Labs Dept Neurosci. Johns Hopkins Un Sch of Med,Baltimore, MD 21205

Earlier studies were made of cortical neural activity in waking M.mulatta discriminating between the frequencies of mechanical sinusoids delivered to the glabrous skin of one hand. They projected the other to one of two targets to indicate whether the comparison stimulus of a pair was higher/lower in frequency than that of the base stimulus; e.g., 30 vs 32 Hz. Such stimuli evoke periodic activity in postcentral neurons (areas 3b and 1) related to Meissner afferents innervating the hand. The harmonics of respon se trains reflect precisely stimulus frequencies; the neural discriminandum is the difference in the cycle lengths in the neural periodicities; it is a sequential order code.

Identical experiments have now been made in the motor and premotor cortical areas of the hemisphere opposite the projecting arm (5 hemispheres, 1191 neurons). A selective signal of the correct discrimination occurs during one or the other sets of comparison stimuli, often beginning about 200 msec after onset of the comparison stimuli and frequent ly continuing into the reaction time. The discharge is aperiodic. No similar responses were observed when simi lar stimuli were delivered to the hand of an alert but idling monkey. We interpret this to be a post-discrimination signal projected trans-callosally from the contralateral sensory hemisphere to the motor & premotor areas.

441.12

A COMPETITIVE DISTRIBUTION THEORY OF NEOCORTICAL DYNAMICS *J.Reggia, C.D'Autrechy*, G.Sutton*, & M.Weinrich**. Neurology & Comp.Sci. Depts., Univ. of Maryland, Baltimore MD 21201.

Peristimulus inhibition in sensory pathways is generally attributed to lateral inhibitory connections. While this is convincing in prethalamic pathways, the situation is less clear in the thalamus and neocortex. Thalamocortical circuitry is incompletely defined at present, and in some cases there is an apparent mismatch between observed inhibitory effects and intracortical inhibitory synaptic connections. This paper puts forth the hypothesis that a different mechanism, competitive distribution of activation, underlies some inhibitory effects observed in neocortex. Analysis of a mathematical model based on this hypothesis predicts that peristimulus inhibitory effects generally attributed to lateral inhibitory connections can alternatively be explained by competitive distribution of activation in the absence of such inhibitory connections. Computer simulations verify these predictions by demonstrating Mexican Hat patterns of lateral interactions, transformation of diffuse activity patterns into tightly focused "islands" of activity, and edge enhancement. Analysis and simulations also show that the amount of inhibition can be adjusted by varying the intensity of the underlying competitive process. The concept of competitive distribution of activation provides an important alternative perspective for interpreting neocortical and thalamocortical circuitry and can serve as a guide for further morphological and physiological studies. For example, it provides an explanation for the existance of cortex-to-thalamus connections, and predicts that analogous circuitry must exist in neocortex.

442.1

MOTION OF CERVICAL VERTEBRAE DURING DIFFERENT HEAD MOVEMENT TASKS IN HUMAN. D.H. Wang, W. Graf, C. de Waele, P.P. Vidal and B. Jaenisch. The Rockefeller University, N.Y., NY 10021; Lab. Physiologie Neurosensorielle, CNRS, 75270 Paris, France; Schildautal-Klinik, 3370 Seesen/Harz, FRG.

Our previous cineradiographic studies on human head movements in the sagittal plane indicated that head rotation results from simultaneous individual translations (slidings) of all cervical vertebrae. The present study investigated the distribution of vertebral motion at different cervical levels during different head movement tasks. Subjects were asked to perform swinging and nodding movements of the head in the sagittal plane. The orientation angle of the head and the translation angle of each cervical vertebra were measured from fluoroscopy images. During swinging movements, translational movements of the cervical vertebrae were uniformly distributed along all segments. Thus, a global center of head rotation could be calculated around C6. During the nodding task, however, the upper segments participated to a larger extent. In this case, the global center of head rotation could be approximated around C3. Furthermore, significant degrees of pure rotation at the upper joints (C0 to C1, and C1 to C2) contributed to head rotation when the head reached extreme flexion and extension. This result suggests that the biomechanical constraints within the cervical spine (i.e. passive or effected by intervertebral muscles) may be the main factor for different kinematics of the two types of head movements studied. (Supported by NIH grant EY-04613)

442.2

FORCE CONTROL IN PRECISION GRIP: EXISTENCE OF SYNERGIES ?
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Isometric force generation in the precision grip requires the delicate interaction of many muscles in timing and activation levels. We have quantitatively examined the participation of each muscle in force control and the putative existence of muscular synergies.

Subjects produced force between index finger and thumb with visual feedback in a ramp-and-hold paradigm with 3 consecutive force steps up to 3.0 N. Full-wave rectified and averaged EMGs of 15 muscles were used for computing correlations between muscle activity and force, as well as correlations and cross-correlations (CC) between the activity of muscles recorded simultaneously. The results are, that the majority of the thumb and index finger muscles increased their activity monotonically as a function of force, though with considerable scatter. For some muscles, such as AdP and 1DI, an organized, non-random scatter and high correlations were found, suggesting a role as prime force generators. Correlations between the activity of two muscles showed muscular coupling in 50% of the pairs tested with some variability. Most prominent was coactivation (80%), and trade-off was rare (12%). Synergies between muscles of a single finger were almost twice as common as those between a thumb-and-index finger muscle pair. Temporal coactivation within muscle pairs was present in 20% of the cases, which all had clear CC peaks deviating no more than 8 ms from zero.

These data provide evidence, first for flexible muscle synergies that are not necessarily linked by biomechanical constraints, such as between flexor and extensor or between index and thumb muscles, and second for close synchronicity of a few muscle pairs, which in the majority are innervated by the same peripheral nerve.

442.3

SENSORY TRIGGERING OF A SEQUENCE OF POSTURAL RESPONSES
P.J. Cordo, Cent. Medico Riabil., Veruno,; Univ. Studi, Milano

Sudden destabilization of the body center of gravity with respect to the feet is known to trigger short latency postural responses in humans. In the present study, we show that this short latency response is only the first of a sequence of responses and that the sensory information used by the nervous system to trigger each response in the sequence is different.

Seven normal humans stood on a posture platform and were translated forward or backward from 3 initial postures, leaning forward or backward, or standing erect, to enhance or reduce the destabilizing effect of the platform movement. Subjects were instructed to take a step only if absolutely necessary.

When subjects reduced the effect of platform movement, they produced the well-known response in which the stretched ankle and knee muscles returned the center of gravity back over the feet ("torquing"). When subjects stood erect, they first produced the torquing response and then rose up on the heels or toes to shift the fulcrum point ("rising"). When subjects enhanced the effect of platform movement, they followed the first 2 responses with a step ("stepping").

The first 2 responses are most likely triggered by somatosensory input--for the torquing response, probably nonspecifically related to the movement, and for the rising response, specifically related to the change in ankle angle. The stepping response is related to the velocity of the center of gravity, and is more likely triggered by visual or vestibular input.

442.4

CHANGES IN TORQUE/EFFORT RATIO REVEAL CHANGES IN INVOLUNTARY MOTOR FUNCTION. P.R. Burgess and T.A. Cooper*, Dept. Physiol., Univ. Utah Sch. Med., Salt Lake City, UT 84108.

Standing subjects generated isometric tension by pulling upward on a bucket handle attached to one end of a cable with the elbow joint at 90° and the forearm horizontal and supinated. Other joints were braced to insure that the elbow flexors were the prime movers for the task. The moment arm of the isometric load was changed by anchoring the other end of the cable at different points so that the angle (θ) between the cable and the forearm varied between 90° and 150°. The subjects made the same effort at each anchor point in a given trial; low, medium and high efforts were made in different trials. The torque/effort ratio for the elbow flexors [torque = cable tension (sin θ)] was found to vary in a characteristic fashion, first increasing as the angle increased and then falling below the 90° value between 138° and 145°. Increases in torque/effort ratio were more pronounced when the effort was high and presumably reflect involuntary motor behavior designed to enhance cable tension until θ is sufficiently large (>140°) that the line of action is toward the body.

Supported by NIH grants NS21972, NS07172 and NS07938.

442.5

DIRECTIONAL INFORMATION DURING SLOW GOAL-DIRECTED ARM MOVEMENTS. J.B. de Graaf*(1), J.J. Denier van der Gon*(2), A.C. Sittig*(1) (SPON: European Neuroscience Association) (1) Product ergonomics, Delft University of Technology, Jaffalaan 9, NL-2628 BX Delft, The Netherlands, (2) Medical and Physiological Physics, University of Utrecht, Princetonplein 5, NL-3584 CC Utrecht, The Netherlands.

Recently we have shown that when subjects are asked to make slow arm movements to visual targets they start the movements to most targets in a direction that deviates consistently from the direction of the straight line between initial position and target position(de Graaf et al. 1991 Exp Brain Res 84: 434-438). To explore this phenomenon we did the following experiment with two congenitally blind subjects and two sighted blindfolded subjects. The subjects were seated at a table. They were asked to make slow arm movements to proprioceptive targets (the index-finger of the other hand) at a distance of 30 cm. We analysed the initial movement direction as well as the curvature of the movement trajectories. The results show that 1. the sighted subjects as well as the blind subjects started their movements to most targets in directions comparable to the directions we found in earlier experiments, and 2. the blind and sighted subjects showed more or less the same curvature in their movement trajectories. These results suggest that the movement trajectories we found in our earlier experiments do not originate from visual experience of the sighted subjects.

442.6

SENSITIVITY OF THE MANDIBULAR STRETCH REFLEX DURING CYCLIC JAW MOVEMENTS IN MAN.
A. van der Bilt*, F.A.M. Ottenhoff*, H.W. van der Glas and F. Bosman, Dept. of Oral Pathophysiology, University of Utrecht, Padualaan 14, 3584 CH, Utrecht, The Netherlands.

The reflex sensitivity was determined during various phases of rhythmic mandibular open-close movements. Subjects made such movements at their natural chewing rate controlled by a metronome. An external force, supplied by a solenoid in a magnetic field, acted on the mandible in a downward direction during the closing phase, simulating food resistance like in chewing. In addition to this force a downward directed force-pulse (10 ms; 5 N) was randomly given at various phases of the open-close cycle. The jaw movement and force as well as the activity of masseter, temporal and digastric muscles were recorded. Only weak or no reflexes were observed during the opening phase. A mono-synaptic reflex was observed in the elevator muscles if the force-pulse was given during the closing phase of the chewing cycle. The ratio of the reflex activity and the background muscle activity was largest at the beginning of the closing phase. It may be concluded that the fusi-motor system plays an important role in controlling muscle activity at the first engagement of the food between the teeth and that it is also involved in further load compensation during the closing phase.

442.7

SOLEUS H REFLEXES ARE INHIBITED DURING PEDALLING, BUT THIS IS NOT DEPENDENT ON SPEED OF MOVEMENT. J.D.Brooke, P.J.Whelan*, D.F.Collins and W.E. McIlroy, Lab. of Neurophysiol. School of Human Biology, University of Guelph, Canada, N1G 2W1.

Magnitudes of H reflexes are depressed during walking and running, compared to controls at a similar stimulation intensity and contraction level in the target muscle. We asked (a) is there such depression during pedalling and (b) if so, is the depression dependent on movement velocity? Specifically, as movement velocity increases from zero (sitting or standing), is the depression partly associated with the potential for the reflex latency to cause excitation out of phase in the cycle? For a range of pedal rates, the bursts of EMG in soleus and tibialis anterior did not systematically change their phase positions. During pedalling at 60 rpm, soleus H reflexes were significantly depressed when soleus activity was low [mean (n = 4) 0.09 mV, versus sitting mean 3.44 mV]. However, when the pedal rate was lowered to 30 rpm (perceived as very slow for rhythmic leg movement), reflexes were not significantly different from those evoked at 70 rpm. We conclude that soleus H reflexes are inhibited during pedalling compared to sitting, and that the inhibition is not due to the leg velocity being too high. Supported by NSERC (Canada) Grant #A0025.

442.8

BIPEDAL AND UNIPEDAL CYCLING BOTH DEPRESS H REFLEXES, COMPARED TO SITTING. D.F.Collins, J.D.Brooke, W.E.McIlroy, and P.J.Whelan* Lab. of Neurophysiol., School of Human Biology, University of Guelph, Ontario, Canada, N1G 2W1.

H reflex magnitude in the human soleus muscle is depressed during pedalling, compared to control reflexes when sitting (Brooke et al. Neurosci. Abs. 1991). We currently investigated whether this inhibition is dependent on the bipedality of the rhythmic task. H reflexes were recorded at four phases of the pedal cycle (90°, 180°, 270°, 360°) from five subjects while sitting, pedalling with both legs and pedalling with one leg. Soleus contraction magnitude and ankle angle were matched between conditions. Across all subjects, during one-legged pedalling reflex magnitude was significantly depressed ($p<0.05$) in the active leg at 180° and 270° compared to the stationary leg (to 21.9% and 8.8% of stationary, respectively) and compared to sitting (to 13.1% and 4.2% of sitting, respectively). Reflexes evoked in the single active leg were not significantly different from those when pedalling with both legs. Obviously, the observed reflex depression did not require bipedality. However, reflexes in the stationary leg were depressed when the contralateral leg was active. Thus, there was some bilateral interaction in reflex depression. Supported by NSERC (Canada) Grant #A0025.

442.9

CENTRAL MOTOR DRIVE AND ANKLE ROTATION DID NOT ACCOUNT FOR OBSERVED H REFLEX INHIBITION DURING PEDALLING. W.E.McIlroy, D.F.Collins, P.J.Whelan*, and J.D.Brooke. Human Biology, University of Guelph, Canada, N1G 2W1.

Human soleus H reflexes are depressed during walking, running and pedalling. What remains unclear are the sources of this inhibition. This study looked at two potential sources: 1) ankle rotation and 2) the central motor drive to move the limbs. These two factors were selected because in other paradigms they have been shown to strongly influence spinal reflex transmission, and they can be distinguished as peripheral and central influences, respectively.

Ankle Rotation: Subjects pedalled a cycle ergometer with and without a brace which prevented ankle rotation. H reflexes sampled in the recovery phase during pedalling with the brace were not significantly different from those during normal pedalling. (Mean H reflexes: Normal 5.3% SD 4.8, With brace 9.4% SD 6.0 - expressed as percent of control H reflexes). H reflexes from both pedalling trials were significantly lower than control H reflexes during sitting.

Central Drive: Subjects remained relaxed, seated on a tandem cycle ergometer, while the limbs were moved throughout the pedal cycle by a second cyclist. H reflexes sampled in the recovery of this passive movement were not different than those recorded during active pedalling. However they were both significantly lower than the control H reflexes (Mean H reflexes: Normal 6.9% SD 6.0, Passive 9.5% SD 11.0 - expressed as percent of control H reflexes).

The presence of substantial inhibition in the absence of that central motor drive may implicate peripheral inputs as an important source of inhibition. In spite of this we did not see a significant change in H reflex magnitude when ankle rotation was suppressed. It is possible that summed convergence from several peripheral sources is largely responsible for the inhibition of H reflexes during pedalling. (Supported by NSERC.)

442.10

SUPERPOSITION OF RHYTHMICAL AND DISCRETE MOVEMENT. A.G. Feldman, M.F. Levin and S.V. Adamovich*. Institute of Biomedical Engineering, University of Montreal, Rehabilitation Institute of Montreal, Canada H3S 2J4 and Institute of Information Transmission Problems, Moscow, USSR.

Control strategies underlying the simultaneous performance of rhythmical and discrete elbow movements were investigated. Two hypotheses regarding their combination were considered. Either movements are combined according to the principle of superposition (Feldman 1980) or they are integrated and therefore, interdependent. Subjects produced rhythmical flexion and extension movements about a target position which was shifted after a fixed period of time. After presentation of the second target, subjects made a fast movement to the new target while continuing the oscillation. EMG was recorded with surface electrodes from two elbow flexors and two elbow extensors. Position of the shoulder, elbow and wrist were recorded with light emitting diodes. Position and velocity traces were averaged to eliminate the rhythmical component of the movement and then compared to similar data from discrete movements to the same target positions. The results showed no difference in the discrete movement component produced in the two experimental conditions, a finding consistent with the hypothesis of superposition of control signals and movement patterns.

442.11

RECIPROCAL (R) AND COACTIVATION (C) COMMANDS FOR WRIST MOVEMENTS. M.F. Levin, A.G. Feldman, T.E. Milner and Y. Lamarre, Rehabilitation Institute of Montreal, Canada H3S 2J4; CRSN & Institute of Biomedical Engineering, University of Montreal, Canada. H3C 3J7

According to the equilibrium-point hypothesis, movements are produced by means of displacement of the invariant torque/angle characteristic (IC) of the joint and change in its slope. Displacement is produced via the central R command while the C command specifies the slope of the IC. These commands were investigated in single joint wrist movements by perturbation methods. Subjects made free flexion movements to a target at 30° or movements against randomly presented opposing or assisting loads. They were instructed not to correct the deflections arising in response to perturbations. Both peak velocity and EMG patterns were strongly affected by load conditions. Subjects undershot or overshot the target when flexion was perturbed by opposing or assisting loads respectively. However, after unloading (700 ms later), the target position was regained indicating that the IC was stable despite the perturbation. In two other experiments, subjects initially trained to reach the target with opposing or assisting loads, while the load was not presented in some trials. Depending on load conditions, the subject established different final IC positions. The slope of the IC was the same regardless of the magnitude of its shift. It is concluded that R and C commands can be specified independently. In addition, although kinematics and EMG are strongly dependent on peripheral conditions, the same does not appear to be so for basic central commands (R and C).

442.12

DETERMINANTS OF JAW MOVEMENT IN MASTICATION AND SPEECH. D.J. Ostry, A.G. Feldman, J.R. Flanagan, & K.G. Munhall. McGill University, Montreal, Canada, Institute of Biomedical Engineering, University of Montreal, Montreal, Canada, Queen's University, Kingston, Canada.

We report recent X-ray microbeam studies on human jaw movement kinematics in mastication and speech. Empirical findings are compared to simulations of jaw movement based on the equilibrium point hypothesis (λ model). In speech trials, subjects produced consonant-vowel-consonant sequences at either normal or loud speech volumes. In the mastication trials, subects chewed unilaterally on rubber tubing which varied in diameter and compliance. During mastication, we recorded the compression of the bolus. This was transformed into estimates of bite force using separately obtained functions relating applied force to tubing compression. Our previous work showed that jaw rotation and translation in speech can be separately controlled. For example, when rotation was plotted against translation, straight line paths were observed whose slopes depended on the consonant composition of the utterance. The present study extends this work by showing that rotation and translation are separately controlled in chewing. A version of the λ model for jaw motion which incorporates multiple jaw muscles, accounts well for the empirical bite-force pattern in mastication and for the kinematics of mastication and speech. The model permits separate central commands to be defined for jaw rotation, jaw translation and co-activation of muscles without motion. Superposition of commands can predict observed combinations of jaw rotation, translation and bite force.

443.1

OPTIMAL STIMULATION OF PARASAGITTAL MOTOR CORTEX USING THE MAGNETIC COIL. Joan B. Cracco*, Nasser F. Hassan*, Roger Q. Cracco, Paul J. Maccabee, Mahendra Somasundaram* and Vahe E. Amassian. SUNY Health Science Center at Brooklyn, Brooklyn, NY 11203

We investigated the most effective method of exciting parasagittal motor cortex with a magnetic coil (MC) in 4 subjects. A round (9.4 cm diameter) MC which delivered an essentially monophasic pulse (1st phase 3X larger than 2nd) was moved over right parasagittal cortex from various directions; CMAPs were recorded from contralateral left leg and foot muscles. The threshold was determined before and after the direction of current in the MC was reversed by a switch.

The threshold for left foot and leg responses was always lowest when the MC approached parasagittal cortex from the front and the current was counter-clockwise in direction in the MC, i.e. the induced current flowed clockwise in the cerebral cortex from right to left parasagittal cortex and then from left to right more anteriorly. Therefore, this location, orientation and current direction are optimal for excitation of projection neurons in right parasagittal cortex.

Defining the optimal position and orientation of the MC in exciting parasagittal motor cortex is of particular importance in the study of patients with spinal cord disease and in intraoperative monitoring.

443.2

RAPID REVERSIBLE REORGANIZATION IN MAPS OF OUTPUTS OF HUMAN MOTOR CORTEX FOLLOWING TRANSIENT RESTRICTIVE DEAFFERENTATION . L. G. Cohen, J. Brasil-Neto*, A. Pascual-Leone, R.T. Wall*, F.K Jabir* and M. Hallett. Human Cortical Physiology Unit, Human Motor Control Section, NINDS, NIH, Bethesda, MD 20892.

To evaluate timing of reorganization of motor outputs in human motor cortex, we studied motor evoked potentials (MEP) to transcranial magnetic stimulation (TCMS) in two normal volunteers immediately before, during and after unilateral anesthetic block of the distal arm below a pressure cuff inflated at the elbow. Anesthetic block was maintained for 70 and 45 minutes in the two cases. Electromyographic recordings were made from muscles immediately proximal (biceps, deltoid) and distal (abductor pollicis brevis) to the pressure cuff and, in one subject, contralateral muscles. With anesthesia, the MEP in abductor pollicis brevis became gradually smaller and eventually disappeared. Over the same time course the MEP became progressively larger in biceps ($p<0.0001$), but was unaffected in deltoid. In the subject studied, MEP amplitudes from muscles contralateral to the deafferented limb were unchanged. Reversal of the changes were observed within minutes of discontinuation of anesthesia. The rapid time course in reorganization of human motor outputs, by which a certain region of motor cortex can reversibly influence a new set of muscles, is compatible with unmasking of preexistent connections, perhaps due to disinhibition mechanisms.

443.3

THE ROLE OF THE SUPPLEMENTARY MOTOR AREA IN THE CONTROL OF SELF-PACED LIMB MOVEMENTS: A PET STUDY.

T.A. Zeffiro, C. Kertzman, C. Pelizzari and M. Hallett Medical Neurology Branch, 10-5N226, National Institutes of Health, Bethesda, MD, 20892.

In order to examine the hypothesis that different cortical motor areas are involved in the generation of either self-paced or externally-triggered limb movements, we used H_2O^{15} positron emission tomography to record regional cerebral blood flow (rCBF) in 10 subjects who performed unilateral finger flexion tasks while fixating a visual target. We studied three different tasks. In the self-paced (SP) condition, the subjects moved a joystick handle by finger flexion once every 2-3 seconds. Each movement caused the fixation target to briefly flash. In the visually-triggered (VT) condition, subjects were instructed to move in response to the target flash. In the control condition the target was flashed at the same rate; the subjects were instructed to maintain fixation but not respond. Movement onset times were recorded in the SP condition and then used to pace the subsequent two conditions. Difference images representing net rCBF changes were obtained by task pair subtraction. Response localization was determined by 3D registration of each subject's blood flow and MRI. A repeated measures ANOVA model was used for statistical analysis of rCBF changes.

We found equal rCBF increases in the SP and VT tasks in the precentral and lateral superior frontal gyri ($p<0.01$). Responses were more strongly lateralized in the precentral gyrus. In contrast, the contralateral medial superior frontal gyrus (corresponding to the supplementary motor area) was preferentially activated during the SP movements ($p<0.01$). These results provide further evidence for regional cortical specialization for control of kinematically similar limb movements that differ markedly in their behavioral context.

443.4

PRIMATE FRONTAL CORTEX: VISUOSPATIAL VS. VISUOMOTOR ACTIVITY S.P. Wise and G. di Pellegrino. Laboratory of Neurophysiology, NIMH, Poolesville, MD 20837

We examined neuronal activity in the premotor (PM) and prefrontal (PF) cortex while a rhesus monkey performed a visuomotor, spatial matching task. The monkey fixated and held a manipulandum beneath a central light emitting diode (LED), which had 8 LEDs arranged in a circle around it. On each trial, after 1 of the 8 LEDs illuminated for 0.5 s (the prime stimulus, PS), 0 to 4 of the other LEDs illuminated sequentially for 0.1 s at delays of 0.55 or 0.75 s each. At the end of this delay period, the PS illuminated again for 0.1 s whereupon the monkey had to execute a forelimb movement within 650 ms (and could break fixation). There were 2 response conditions: the monkey moved the manipulandum (1) to a predetermined target regardless of PS location (the *incompatible* condition) or (2) to the PS location (the *compatible* condition).

Because the visuospatial stimuli were physically and retinotopically identical in the two conditions, and because the monkey had to attend to (or remember) the PS location identically, the behavioral design allowed us to test the hypothesis that task-related activity simply reflects a sensory response. The alternative is that the motor instructional significance of the stimuli affects the cell activity. Second, because in the *incompatible* condition a single motor response followed a variety of visuospatial cues, we could examine whether the activity reflects simply motor aspects of the animal's behavior. The alternative is that some aspect of the visuospatial cue affects cell activity. Our results from 94 PM cells show that for many cells both hypotheses can be rejected. Accordingly, PM activity, regardless of any temporal correlation with either the sensory signals or the onset of movement, reflects integrative aspects of the visuomotor reaching behavior, i.e., aspects of behavior that cannot be simply characterized as either sensory or motor in nature. A sample of 55 PF cells suggests a higher proportion of cells there simply reflect the sensory, possibly visuospatial, aspects of the stimuli.

443.5

SPECIFIC EFFECT OF MOTOR LEARNING ON SET-RELATED CELLS OF THE PREMOTOR CORTEX IN PRIMATE. L. Germain, Y. Lamarre, M.T. Parent, Centre de recherche en sciences neurologiques. Université de Montréal, Québec, Canada H3C 3J7.

In order to assess the effect of learning on set-related cells in the precentral cortex and cerebellar nuclei we trained one primate to perform elbow flexion (F) or extension (X) according to a two choice instructed delay paradigm. During training (A) the presentation of the auditory instruction (400 Hz tone for X or 1000 Hz tone for F) was not randomized and the performance was at chance level (50%). After training (B) the presentation of the auditory instruction was randomized and the performance was (95%). Each trial started with a 500 msec control period followed by a 400 msec auditory instruction then by a randomized immobile waiting period of 500 to 1500 msec terminated by a small kinesthesic go-signal. A total of 386 movement - related cells were recorded and 267 set-related cells were found in this sample. A specific and significant increase of the proportion of set-related cells was observed in the premotor cortex from A) 22/45 (49%) to B) 65/68 (95%) ($\chi^2 = 33.5$, $p < 0.005$) furthermore the proportion of directional set-related cells increased in this area from A) 11/22 (50%) to B) 54/65 (83%) ($\chi^2 = 9.4$, $p < 0.005$). The mean coefficient of directionality (t value between F and X) of set-related cells increased specifically and significantly from A) 3.7 ± 1 to B) 7.6 ± 4 ($t = 3.19$, $n = 63$, $p < 0.005$) in the premotor cortex. The proportion of cells with auditory responses increased from A) 1/45 (0.02%) to B) 15/68 (22%) in the premotor cortex.and also from A) 7/30 (23%) to B) 13/30 (43%) in the dentate nucleus. These data suggest a predominant effect of learning an arbitrary association between an auditory instruction and the direction of an intended movement on set-related cells in the premotor cortex. (Supported by MRC of Canada group grant in Neurological Sciences).

443.6

INTRINSIC CONNECTIONS OF CAT MOTOR CORTEX: AXONAL PROJECTIONS OF SUPERFICIAL PYRAMIDS A. Keller and H. Asanuma. The Rockefeller University, New York, NY 10021

The functional organization of layer II-III pyramidal neurons in area 4γ of cat motor cortex were examined after intracellular injections of biocytin. All of the cells examined produced long-term potentiation (LTP) in response to tetanic stimulation of the somatosensory cortex. Their primary axon, originating from the soma or a basal dendrite, emitted intracortical axon collaterals before entering the subcortical white matter. The axon collaterals had both vertical and horizontal patterns of termination. Collateral branches formed a local terminal field in the immediate vicinity of their parent somata and dendrites. In addition, most neurons had axon collateral branches that projected horizontally for long distances. These long range, horizontal branches projected to and terminated within the same layer their parent soma was located, and ranged in length from 1 to 4 mm. Relatively shorter horizontal branches were emitted from the primary axon in deep layer V and in layer VI. Within the superficial layers, the long collaterals typically formed clusters of terminal fields, having a diameter in the order of 1 mm. The spatial arrangements of these intracortical connections indicate that these neurons are involved in 'clustered' networks of excitatory connections within the motor cortex. Specifically, these findings predict that the induction of LTP in neurons in the superficial layers of the motor cortex would affect the firing patterns of cells located in spatially segregated cortical columns. Supported by NSF grant #9021162 (A.K.) and NIH grant # NS-10705 (H.A).

443.7

COULD THE CORPUS CALLOSUM PREVENT INTERHEMISPHERIC TRANSFER OF LEARNING TO ENABLE HEMISPHERIC SPECIALIZATION? INTERMANUAL TRANSFER OF SENSORY-MOTOR LEARNING IN NORMAL AND SPLIT-BRAIN RHESUS MONKEYS. B.Preilowski*, H.J.Engele*, M.Reger*, W.Pöll*, Weissenau-Field-Station, Tübingen University, Rasthalde 3, D-7980 Ravensburg, FRG.

In a series of experiments the development of distal and proximal sensory-motor skills and their intermanual transfer was studied in normal and callosotomized rhesus monkeys. When the monkeys had to learn to produce a specific force between the fingers of one hand, no transfer of learning to the opposite hand was observed in intact animals; in a comparable task involving force production by arm movements delayed transfer was observed. Delayed transfer was also found in a callosotomized animal postoperatively trained and tested for transfer in the finger force discrimination task. Furthermore, when animals were alternating between hands during training on the finger task, performance with both hands tended to become equalized after callosotomy.

The results, so far, suggest that the corpus callosum has variable functions in sensory-motor control. Among others, it may modulate bilateral sensory-motor processing taking place in both hemispheres, and, during prolonged unilateral training, it may inhibit interhemispheric transfer allowing for independent skill development and performance with either extremity. If confirmed, this would carry important implications for a possible theory of handedness and laterality as well as bilateral motor functions. (Supported by the German Research Foundation.)

443.8

EFFECTS OF MUSCIMOL INJECTION INTO THE DORSAL AND VENTRAL ASPECTS OF THE PREMOTOR CORTEX IN MONKEYS PERFORMING A CONDITIONAL MOTOR TASK. K. Kurata, D. S. Hoffman, and I. Kawamoto*. Department of Physiology, Tohoku University School of Medicine, Sendai, 980, Japan, and Research Service (151), VA Medical Center, Syracuse, NY 13210.

Muscimol (Sigma, 5μg/μl solution) was injected (1.0μl) into the dorsal and ventral aspects of the premotor cortex (PM) of two Japanese monkeys (*Macaca fuscata*), while they were performing a motor task that required wrist flexion or extension to a target. The correct movement was instructed by either: (1) conditional color cues (green or red LED) equidistant from the targets or (2) directional cues toward extension or flexion (right or left LED). When the green or right LED was illuminated, extension was to be done. When the red or left LED was illuminated, flexion was required. The movement was triggered by a visual stimulus either after a variable delay or simultaneously with the instruction stimulus. Before muscimol injection, single unit recordings were made to select injection sites (1) in the dorsal aspect of the PM (PMd) around the superior precentral sulcus where typical set-related activity was frequently recorded and (2) in the ventral aspect of the PM (PMv) immediately caudal to the genu of the arcuate sulcus where movement-related neurons were densely located. When muscimol was injected into the PMd, there was an increase in the number of direction errors primarily when the conditional cues were presented. The initiated movements were similar in amplitude and velocity to the preinjection behavior. In contrast, when muscimol was injected into the PMv, many of the initiated movements showed smaller amplitudes and slower velocities, but few direction errors were made. These results suggest that the PMd and PMv play differential roles in motor control.

443.9

DEFICITS OF VISUAL REACHING BEHAVIOR BY LOCAL INJECTIONS OF $GABA_B$ AGONIST AND ANTAGONIST (BACLOFEN AND PHACLOFEN) INTO THE PREMOTOR & MOTOR CORTEX OF THE RHESUS MONKEYS. K. KAWAHIRA* and K. KUBOTA, Dept. of Neurophysiology, Primate Res. Inst., Kyoto Univ., Inuyama, Aichi 484, Japan

The role of intracortical $GABA_B$ergic inhibition in the performance of Visual Reaching was studied in two monkeys (Macaca mulatta). Baclofen (2.5 μg/μl) or Phaclofen (0.2-1 μg/μl) was injected locally into the hand motor area (MC) or the post-arcuate hand premotor area (PM), while monkeys were performing the task by hand. When Baclofen was injected into the MC (10 sites), neuronal bursts and convulsions of forearm muscles appeared gradually. Numbers of the task performance decreased gradually with decreased correct-performance. Monkeys released his hand slowly from hold key to reach target key and pressed the target key for longer periods. Number decrease was due to prolonged ITIs produced by that monkeys tended continuously to press the target key. Both reaction and movement time were prolonged. Similar effects, though weak, were produced when injected into the PM (6 sites). When Phaclofen was injected into the MC (6 sites) and the PM (5 sites), essentially similar changes as in Baclofen were induced in respective areas. Dual actions of $GABA_B$ agonist (autoreceptor presynaptic and postsynaptic) appear to be antagonized by dual actions of $GABA_B$ antagonist.

443.10

PERSISTENT DEFICITS OF EYE MOVEMENTS, GOAL-DIRECTED ARM MOVEMENTS AND VISUO-SPATIAL ORIENTATION FOLLOWING SMALL UNILATERAL POSTERIOR PARIETAL LESIONS. W. Heide*, B. Wild*, D. Petersen*. Depts. of Neurology and Neuroradiology, University of Tübingen, W-7400 Tübingen, FRG (SPON: European Brain and Behaviour Society).

Various visuo-spatial, visuo-vestibular and visuo-motor functions have been ascribed to the posterior parietal cortex. In a lesion study, we tried to correlate deficits of these functions with each other and with the cortical areas involved in 8 patients (5 left- and 3 right-hemispheric) with small chronic unilateral lesions around the intraparietal sulcus, according to MR imaging, compared to 10 normal controls. Besides DC-electrooculography of visually and vestibularly induced eye movements and psychophysical assessment of the subjective straight ahead (SSA), the subjective visual vertical (SVV) and the latencies of vection under optokinetic full-field stimulation, fast goal-directed arm movements were recorded by driving a pen to visual targets on a digitizing tableau.

Results showed that the patients' acute symptoms (visual hemineglect, spatial disorientation, optic ataxia, constructive apraxia, deviation of SSA) had almost disappeared. 3 of them, however, had a marked contralateral tilt of their SVV (>2°), associated with impaired circular vection, asymmetries of optokinetic and vestibular nystagmus (reduced gain of ipsilaterally directed slow phases) and an ipsilateral deviation in line bisection test. 3 others had a contralateral bias in this test, furthermore a spontaneous nystagmus (SPN, slow phases drifting ipsilaterally) and - only 1 of them -a direction-specific deviation of goal-directed hand movements (ipsilaterally) and of the SSA (contralaterally). We assume that the multimodal directional bias found in some of the cases might reflect a distorsion of egocentric spatial localization. Others have compensated this bias, maybe by means of an SPN.

443.11

DEPTH-TUNING IN AREA LIP BY DISPARITY AND ACCOMMODATIVE CUES. JW Gnadt and LE Mays. School of Optometry and Vision Science Research Center, University of Alabama at Birmingham, Birmingham AL 35294.

Area LIP in the intraparietal sulcus of rhesus monkeys contains many neurons that carry a presaccadic signal of motor error, representing the metrics of intended eye movements. Their functional properties are similar to those of the QV cells of the superior colliculus, a structure to which LIP projects. We have found that these LIP cells are tuned for depth with a sigmoid, broad-band preference for stimuli either near or far to the point of fixation. Two salient cues for depth, binocular disparity and accommodative demand (blur), were presented independently or in conjunction. Some neurons were found to prefer the combination of cues presented together. Others responded equally well or better to one stimulus alone. In most cases, linear combination of the two monocular response fields could not account for the tuning in depth. The midpoint of the tuning curve usually did not occur at zero disparity and the slopes near midpoint varied between cells. When disparity sensitivity was tested at various levels of vergence, the sensitivity either did not change or it increased in magnitude with no shift in the midpoint. Interestingly, cells identified as projecting to the superior colliculus also exhibited this property. Assuming collicular cells are not tuned for depth, this property may be averaged out at the tectum by convergent projections from near-tuned and far-tuned parietal cells. These data are consistent with the hypothesis that area LIP serves as an output element of convergent sensory inputs for a parietal 3-D visual-spatial system to other oculomotor structures. *These studies were supported by EY08217 to JWG and EY03463 to LEM.*

443.12

COMPARTMENT MODELS OF NEOCORTICAL INTRINSIC BURSTING AND REGULAR SPIKING PYRAMIDAL NEURONS

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Neocortical pyramidal cells have non-linear input-output relationships which may be broadly classified as regular spiking and intrinsic bursting (Connors et al, 1982.) Their distinctive responses to current injection are thought to be due to the interaction of non-linear active conductances distributed in some presently unknown fashion in somatic and dendritic membranes. Any physiologically meaningful model of cortex will likely require model units incorporating physiological input-output characteristics. To explore the specification of such model neurons, a compartment model simulator was built. Dendritic geometry was simplified from filled layer V pyramidals from which intracellular recordings were obtained (Chagnac-Amitai et al, 1990.) Dendritic and somatic morphology was then fine tuned so that input resistance and time constant matched reported values. Ion channels (fast Na, persistant Na, K-DR, K-A, K-AHP, K-Ca, high threshold Ca, low threshold Ca) were modelled with Hodgkin-Huxley kinetics taken in most cases from studies done on cortical neurons. The parameters governing the kinetics of Ca accumulation and decay were preset based upon available data, but were subject to manipulation. Trial specifications of ion channel densities in the soma and in each compartment of the dendritic tree were interatively modified as suggested by comparison between simulated output and current injection results reported in the literature. In this manner, separate compartment models of layer V regular spiking and intrinsic bursting pyramidal neurons were developed which qualitatively matched current injection responses. These models generate detailed predictions which may be testable by techniques capable of applying channel blocker selectively to portions of the dendritic tree.

443.13

STIMULATION-EVOKED CHANGES IN INTRINSIC OPTICAL SIGNALS IN THE GUINEA-PIG ISOLATED WHOLE BRAIN. P. Federico, S.G. Borg*, and B.A. MacVicar. Neuroscience Research Group, The University of Calgary, Calgary, Alberta T2N 4N1 CANADA.

Neuronal activity has been shown to be associated with changes in the intrinsic optical properties of nervous tissue. We have used video imaging of reflectance changes to map patterns of evoked neuronal activity in the guinea-pig isolated whole brain preparation. The lateral entorhinal cortex (LEC) or lateral olfactory tract (LOT) was stimulated with concentric bipolar stimulating electrodes and evoked field potentials were recorded in surrounding cortical areas. Digitized images were averaged during stimulation and were subtracted from averages obtained during control periods. Stimulation of the LOT decreased tissue reflectance in the amygdala, pyriform cortex,intermediate entorhinal cortex, and LEC while stimulation of the LEC decreased tissue reflectance in the LEC, intermediate entorhinal cortex, and pyriform cortex. These intrinsic signals were repeatable, graded with stimulation frequency and intensity, consistently recovered and had an onset of approximately 3 sec. The pattern of the stimulus-evoked changes in optical properties was correlated with maps of the intensities of evoked field potentials in layers I - III of the various cortices. Intrinsic signals were recorded at wavelengths from 500 to 750 nm but the largest signals were observed at 550 and 600 nm which may be due to light adsorption by cytochrome oxidase a and c (550 nm: peak signal ΔR_r = 1.91 %; noise = 0.15%). LEC stimulation occasionally evoked seizure activity in the LEC. A large change in the intrinsic signal was found to spread across the medial entorhinal cortex/subiculum to the amygdala and pyriform cortex during the recorded seizure. Therefore, it is possible to image intrinsic optical signals in the guinea-pig isolated whole brain preparation. This technique may be useful for the examination of neuronal activity patterns and the spread of seizure activity. Supported by MRC (Canada).

POSTSYNAPTIC MECHANISMS IN NEUROTRANSMISSION

444.1

PHOTOLYTIC MANIPULATION OF $[Ca^{2+}]_i$ CONTROLS HYPERPOLARIZATIONS IN HIPPOCAMPAL PYRAMIDAL CELLS. B. Lancaster & R.S. Zucker, Molec. & Cell Biol., Univ. of Calif., Berkeley, CA 94720.

Experiments were performed to assess the reason for the slowness of the AHP in rat CA1 pyramidal cells. This Ca-dependent K current reaches a peak 500 ms following Ca^{2+} entry during spikes (28° C). In order to raise Ca^{2+} instantaneously throughout the cell, conventional intracellular recordings were made with electrodes containing DM-nitrophen loaded with 30% Ca^{2+}. UV light photolyses DM-nitrophen with an attendant reduction in Ca^{2+} affinity, thus releasing Ca^{2+}. For photolysis, a UV flashlamp (flash duration 1.6 ms) was focussed onto the slice surface. A single flash could evoke a 10 mV hyperpolarization from rest (-70 mV). The onset of 30 to 50 ms was limited by the membrane time constant. Durations of initial responses were 20-60 s. DM-nitrophen filled cells displayed small AHPs presumably as a result of Ca^{2+} buffering: these reduced AHPs showed the same slow kinetics as control responses. Flash responses and AHPs showed a common sensitivity to 1 uM isoproterenol, suggesting that both represent activation of the same current. When electrodes contained the caged BAPTA derivative Diazo-4, robust AHPs were observed. A flash (now to release Ca^{2+} buffer) at the peak of an AHP curtailed the response immediately. Subsequently, slow AHPs could not be evoked . These results show that rapid manipulations of $[Ca^{2+}]_i$ can cause similar rapid changes in AHP current. By contrast, this implies that spike induced Ca^{2+} entry causes slow $[Ca^{2+}]_i$ changes over the majority of AHP channels; possibly this is a consequence of spatial localisation.
Supported by NIH grant NS 15114.

444.2

HYPERPOLARIZING AND DEPOLARIZING $GABA_A$ RECEPTOR-MEDIATED DENDRITIC INHIBITION IN AREA CA1 OF RAT HIPPOCAMPUS N.A. Lambert, A.M. Borroni, L.M. Grover and T.J. Teyler Department of Neurobiology, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272.

$GABA_A$ receptor-mediated synaptic inhibition of pyramidal neuron dendrites was studied using intracellular and extracellular recording in the hippocampal slice preparation. In the presence of DNQX and APV stimulation in any layer evoked fast and late monosynaptic IPSPs and fast and late field potentials. Fast IPSPs and field potentials were reversibly blocked by bicuculline methiodide (BMI; 30μM); late IPSPs and field potentials were reversibly blocked by the $GABA_B$ receptor antagonist CGP 35348 (100μM). $GABA_A$ receptor-mediated field potentials (fast pIPSPs) were dependent on the extracellular chloride concentration, and use-dependent depression of fast pIPSPs was antagonized by CGP 35348 (0.8mM). Current source-density analysis of fast pIPSPs revealed current sources (outward current) horizontal to the stimulation site; BMI-sensitive sources could be evoked in all strata of area CA1. In the presence of DNQX, APV and 4-aminopyridine (50μM) BMI-sensitive long-lasting depolarizations (LLDs) could be evoked or occurred spontaneously. Current sinks mediating evoked LLDs and current sources mediating hyperpolarizing fast IPSPs were located in the same dendritic area. These results suggest that pyramidal neuron distal dendrites do not maintain a reversed electrochemical chloride gradient, and that depolarizing responses to dendritic application of GABA or orthodromic activation in area CA1 do not result from inward chloride current (Misgeld et al., *Science* 232:1413). Supported by NS28698.

444.3

SLOWER SPONTANEOUS EXCITATORY SYNAPTIC CURRENTS IN SPINY VS. ASPINY HIPPOCAMPAL HILAR NEURONS C.T. Livsey** and S. Vicini FGIN and *Dept. of Pharmacology, Georgetown Univ., Wash., DC 20007

Spontaneous excitatory postsynaptic currents (sEPSCs) from aspiny hilar interneurons (AHIs) and spiny "mossy cells" (SMCs) were examined using the whole-cell patch-clamp technique in young rat hippocampal slices. AHIs were identified electrophysiologically as neurons with action potentials (APs) followed by large afterhyperpolarizations, whereas SMCs were defined as those with APs followed by small, variably-directed afterpotentials. Confocal microscopy investigation after Lucifer Yellow injection revealed that most AHIs were aspiny, whereas SMCs were spiny and had thorny excrescences on their proximal dendrites. High frequency, large sEPSCs were not abolished by $GABA_A$ receptor antagonists, but were completely eliminated either with a non-NMDA receptor antagonist alone (most instances) or, in a few instances, in combination with an NMDA receptor antagonist. Peak amplitudes and decay time constants were increased by aniracetam (1 mM), a positive allosteric modulator of the non-NMDA receptor. TTX (1 μM) and Mn^{2+} (2 mM) did not abolish sEPSCs in most hilar neurons, suggesting spontaneous release of quanta from single synaptic boutons. The average amplitude of sEPSCs from 44 AHIs was 37 ± 23 pA, and from 34 SMCs was 29 ± 17 pA (mean ± SD). The amplitude distribution of sEPSCs could not be fit by single or multi-Gaussian distributions, reflecting spontaneous synaptic inputs originating from diverse neurons. Rise times of sEPSCs from 44 AHIs averaged 0.5 ± 0.2 ms and decay time constants averaged 3.6 ± 1.5 ms; in 34 SMCs, rise times averaged 1.0 ± 0.3 ms and decay time constants averaged 9.6 ± 4.8 ms (mean ± SD). Slower sEPSCs in spiny neurons result in increased synaptic strength. Spine structure modifications during neural activity may influence hippocampal circuitry and thus be operative in learning and memory. Supported by NIH grant PO1 NS 28130

444.4

Potentiation of Ca^{2+} changes in hippocampal CA3 pyramidal neurons by modulatory neurotransmitters. J.A. Connor, W. Müller and J.J. Petrozzino. Dept. of Neurosciences, Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110

Slow synaptic transmission, mediated by muscarinic, serotonergic, metabotropic quisqualate and β-adrenergic receptors appears to be important for learning in animals as well as for synaptic plasticity in single neurons which might underly learning. Most models of synaptic plasticity involve postsynaptic calcium (Ca^{2+}) changes as a trigger for subsequent processes. We imaged free Ca^{2+} accumulation in CA3 pyramidal neurons in the guinea pig hippocampal slice during synaptic train or direct stimulation and studied the effects of modulatory agonists on these transient Ca^{2+} accumulations. Carbachol, 5-HT, trans ACPD and isoproterenol all reduced a slow Ca^{2+} dependent afterhyperpolarization and were all effective in potentiating postsynaptic Ca^{2+} accumulations by 50 to 400 %. While all agonists enhanced proximal dendritic Ca^{2+} accumulations isoproterenol had a relative strong effect also on the soma. 5-HT could also reduce Ca^{2+} accumulations during an initial hyperpolarizing effect on the membrane. The effect of carbachol could be reproduced by firing cholinergic fibers in the slice. The response to repetitive stimulation of cholinergic fibers was enhanced by the acetylcholine-esterase blocker eserine and blocked by the muscarinic antagonist atropine. We surmise that modulation of postsynaptic Ca^{2+} changes by slow neurotransmission is an important pathway for the induction of synaptic plasticity.
W.M. was supported in part by the DFG.

444.5

GLUTAMATE RECEPTOR DESENSITIZATION GOVERNS THE STRENGTH OF EXCITATORY SYNAPTIC TRANSMISSION IN THE HIPPOCAMPUS. I.S. Isaacson and R. A. Nicoll, Depts. of Physiology and Pharmacology, University of California, San Francisco, CA 94143.

Rapid perfusion experiments with the non-NMDA type of glutamate receptor indicate that this receptor rapidly desensitizes in response to glutamate with a time course similar to that of synaptic responses (Trussell, L & Fishbach, G. *Neuron* 3:209, 1989). However, a physiological role for desensitization in synaptic transmission has not been established. In this study, we have found that aniracetam enhances the responses of neurons to glutamate (Ito, I. et al, *J. Phys.* 424:533, 1990) by reducing receptor desensitization and have examined its effects upon synaptic transmission in the hippocampus.

Outside-out membrane patches and synaptic currents were studied from CA1 and CA3 pyramidal neurons and dentate granule cells using "blind" patch clamp recording techniques in thick (500 μm) slices of adult guinea pig hippocampus. Outside-out patches were rapidly perfused with agonists via a segment of theta tubing attached to a piezoelectric switch. Excitatory post-synaptic currents (EPSCs) were evoked using conventional bipolar stimulating electrodes placed in s. radiatum or the perforant path.

The rapid application of glutamate or quisqualate to outside-out patches yielded responses which desensitized with time constants as short as 4.5 ms. In the presence of aniracetam the rate of desensitization of these responses was dramatically slowed and their amplitude increased, while the responses to kainate, which did not desensitize were unaffected. Aniracetam had two very consistent effects on non-NMDA receptor-mediated synaptic responses: it prolonged the time course and increased the amplitude of evoked EPSCs. To rule out a simple presynaptic site of action, we examined the effects of the drug on "miniature" EPSCs recorded in the presence of TTX. Aniracetam had essentially identical effects on these action potential-independent synaptic events.

The prolongation of synaptic currents by aniracetam provides a physiological role for glutamate receptor desensitization in governing the time course of excitatory synaptic transmission in the brain.

444.6

ELECTROPHYSIOLOGICAL AND PHARMACOLOGICAL CHARACTERISTICS OF A GABA-MEDIATED SYNAPTIC DEPOLARIZATION IN THE RAT HIPPOCAMPUS. Avoli M; Tancredi V, Siniscalchi A and Zona C. McGill University, Canada H3A 2B4; Università di Roma Tor Vergata, Rome, Italy.

Intracellular recordings with K-acetate-filled electrodes were made in slices of the adult rat hippocampus to study the orthodromic inhibitory sequence generated by CA1 pyramidal cells. In 43 of 72 cells studied, stimuli that were delivered in the stratum radiatum induced: (i) an initial EPSP; (ii) an early hyperpolarizing IPSP (peak latency=approx. 20 ms); (iii) an intermediate depolarizing component (peak latency=60-120 ms; duration=60-150 ms) and (iv) a late, long-lasting hyperpolarizing IPSP (peak latency=120-160 ms; duration >400 ms). In the remaining cells this inhibitory response lacked the intermediate depolarizing component. The depolarizing component represented an active, GABA-mediated phenomenon since: (i) it was selectively blocked by local applications of bicuculline or picrotoxin on the apical dendrites (ii) it was enhanced by lowering the temperature or by the GABA-uptake blocker nipecotic acid. Application of the Cl^--pump blocker furosemide reduced and eventually blocked both early IPSP and depolarizing component. These data demonstrate that under physiological conditions rat hippocampal pyramidal cells generate a depolarizing component that is presumably caused by an outward-directed Cl^- movement due to the activation of $GABA_A$ receptors located on the apical dendrites. This novel mechanism might modulate hippocampal excitability under both physiological and pathophysiological conditions.

444.7

INTERACTION BETWEEN CHOLINERGIC AND HISTAMINERGIC CALCIUM SIGNALS IN MOUSE NEUROBLASTOMA CELLS. S. S.-H.Wang and S.Thompson*. Hopkins Marine Station of Stanford University, Pacific Grove, CA 93950.

Muscarinic and histaminergic receptors in N1E-115 cells activate phosphoinositide (PI) hydrolysis and calcium mobilization. Using fura-2 calcium imaging we find that carbachol (CBC) and histamine (HIS) elicit similar Ca "fingerprints" in the same cell, as defined by kinetics and spatial patterns of release from stores. The response to CBC is abolished by phorbol ester, implicating protein kinase C (PKC) in desensitization.

We examined the effects of long exposure to CBC and HIS. A Ca response was defined as ≥33% change in fluorescence at 380 nm after 30 seconds. Analysis was restricted to cells that respond to both agonists. A 5-minute exposure to 1 mM CBC desensitizes the cells by 37% to a second application of CBC, but this did not affect their responsiveness to HIS. Conversely, a 10-minute exposure to 10 μM HIS desensitizes the cells by 41% to a second application of HIS, while preserving the CBC response.

Desensitization is one way that receptor activation can alter synaptic efficacy. The lack of cross-desensitization here indicates that it may take place at a step prior to PI hydrolysis. This does not support a PKC-dependent mechanism.

444.8

SEROTONIN AND NORADRENALINE STRONGLY EXCITE GABAergic NEURONS OF THE THALAMIC RETICULAR NUCLEUS. David A. McCormick and Zhong Wang Section of Neurobiology, Yale Univ. Sch. Med.

The thalamic reticular nucleus (nRt) is a sheet-like formation of GABAergic neurons surrounding the thalamus, is important in the control of thalamocortical activity, and is innervated by noradrenergic and serotoninergic fibers of brainstem origin. Here we investigated the influence of 5-HT and NA on cat and guinea pig thalamic reticular neurons through the use of the *in vitro* slice technique. Application of either 5-HT or NA resulted in a pronounced depolarization of nRt neurons resulting from the reduction of a "leak" current which displayed a reversal potential of -105 mV (2.5 mM $[K^+]_O$), suggesting that it is mediated by K^+ ions. This K^+ current varied in a relatively linear manner with driving force on K^+ ($V-E_K$) and was not blocked by extracellular application of Ba^{++} or Cs^+, suggesting that it is distinct from I_M or I_h. The response to 5-HT was occluded by maximal application of NA, and vice versa, suggesting convergence of the two neurotransmitters onto the same effector mechanism. Application of ketanserin or ritanserin (1-5 μM) blocked the response to 5-HT but not to NA, while the application of prazosin (1 μM) blocked the response to NA but not to 5-HT. These data indicate that 5-HT and NA block a relatively linear K^+ current through activation of $5HT_2$ and α_1 receptors, receptively. Functionally, the slow depolarization of nRt neurons induced by 5-HT and NA resulted in a promotion followed by pronounced inhibition of rhythmic burst firing and the appearance of single spike activity. We suggest that in this manner the timing and prevalence of rhythmic burst firing in thalamocortical systems may be controlled by the ascending serotoninergic, noradrenergic and cholinergic transmitter systems. Supported by NIH and the Klingenstein Fund.

444.9

Excitatory Synaptic Currents Recorded *in-vitro* from Rat Superior Colliculus Neurons. Shaul Hestrin. Department of Physiology, U.C.S.F., S.F., CA. 94143.

The mammalian optical tectum plays an important role in controlling eye movements, integrating retinal and cortical inputs as well as brain stem inputs. The excitatory cortical and retinal inputs have been studied so far with extracellular recording. I have recorded using whole-cell techniques from the stratum griseum superficial (SGS) neurons in brain slices. Excitatory inputs were evoked by stimulating in the optical layer in the presence of picrotoxin.

At membrane potential of -80 mV the EPSC had a rapid decay time of about 2 ms. The fast component was blocked by CNQX was insensitive to APV, had a linear current-voltage relationship and its time course was voltage insensitive.

In the presence of CNQX membrane depolarization revealed a slow component which could be blocked by APV. The decay time of the NMDA component was voltage dependent: doubling by depolarization from -20 to +20 mV. The decay time constant of the NMDA component was quite variable; at -20 mV tau was between 17 ms and 70 ms. This variability could reflect differences in animal age or in cell types.

These results indicate that the EPSC in the SGS are mediated by an NMDA and non-NMDA glutamatergic receptors which are generally similar in their current-voltage relationship and pharmacological properties to those recorded at CA1 pyramidal neurons. The quantitative differences between the EPSCs recorded at SGS neurons compared to those recorded at CA1 may be explained by different receptor subtypes mediating synaptic currents in these two regions.

444.10

NMDA and non-NMDA receptors are not co-localized at excitatory synapses of on-off ganglion cells in salamander retina. W. R. Taylor*, E. P. Chen* and D. R. Copenhagen, Depts. Ophthalmology and Physiology, UCSF, San Francisco 94143-0730.

Previously it has been demonstrated that light evoked excitatory postsynaptic currents (EPSCs) in voltage-clamped salamander retinal ganglion cells are a result of concomitant activation of two types of glutamate receptor, the *N*-methyl-D-aspartate (NMDA) receptor and a non-NMDA receptor, presumably quisqualate or kainate receptors (Mittman, Taylor & Copenhagen, 1990). The non-NMDA input appears to be due to summation of numerous quantal like events. If non-NMDA and NMDA receptors were co-localized within the synapses of these ganglion cells, as supposed, then miniature excitatory postsynaptic currents (MEPCs) produced by spontaneous quantal release should also have a fast non-NMDA component and a slower NMDA component. In this study, analysis of MEPCs recorded during darkness and in light failed to reveal the presence of a slower NMDA component. The decays of MEPCs were well fitted by similar single exponential functionsat -90 mV, where the NMDA component of the light response was blocked, and at -45 mV where the NMDA component of the light response was comparable in magnitude to the non-NMDA component. At -90 and -45 mV MEPCs had average 10-90% rise-times of 1.2 and 1.6 ms respectively and exponential decay time constants of 3.6 and 4.3 ms respectively. CNQX (1μM), a non-NMDA antagonist, blocked the MEPCs and the non-NMDA component of light responses. AP7 (20μM), an NMDA antagonist, had no effect on the MEPCs but blocked the NMDA component of the light response. Furthermore, if co-localization were assumed then the magnitude and time course of the total light response at -45 mV could not be predicted from mathematical simulations of summed MEPC activity. These findings indicate that the non-NMDA and NMDA receptors mediating the light-evoked excitation are not co-localized within the synapses on the ganglion cells.

445.1

IDENTIFICATION OF A DISTINCT POPULATION OF NERVE GROWTH FACTOR LIKE-IMMUNOREACTIVE CELLS WITHIN THE DEVELOPING THYMUS. E.S. Purcell and V.H. Gattone, II. Department of Anatomy and Cell Biology, Univ. of Kansas Med. Center, Kansas City, KS 66103.

We previously described a developmentally increased monoaminergic innervation of thymus in the spontaneously hypertensive rat (SHR), an animal model with a T cell dysfunction (Soc. Neurosci. 16(2):1206). We hypothesized that trophic factors induced this hyperinnervation. To test this hypothesis, we examined NGF and NGF receptor immunoreactivity (-ir) using indirect immunofluorescence. A well defined population of cells with a rounded morphology and strong NGF-ir was identified in the interlobular septae of the developing thymus. These cells were loosely associated with the vasculature along which the innervation initially develops. The SHR thymus had an increased number of these cells which were more intensely labelled as compared to those seen in the Wistar Kyoto rat. Labeling for NGF receptor-ir was minimal, suggesting that the NGF-ir represents cellular production rather than receptor mediated uptake. More diffusely labeled cells were found in thymic cortex at older time points. In conclusion, a specific population of cells is immunoreactive for NGF in the developing thymus. Their location, number and temporal appearance correlate with the development of thymic hyperinnervation in the SHR. Supported by PHS grant MH46511.

445.2

CHARACTERIZATION OF NERVE GROWTH FACTOR RECEPTORS IN THE IMMUNE SYSTEM. C.E. Lomen-Hoerth and E.M. Shooter. Department of Neurobiology, Stanford University School of Medicine, Stanford, CA, 94305.

In addition to its neurotrophic effects, nerve growth factor (NGF) can affect the inflammatory and immune response. The specificity of these effects was investigated by determining NGF receptor expression in the immune system. In the rat spleen and thymus, NGF receptor mRNA is expressed in both the stroma and circulating cells based on northern analysis with the low affinity NGF receptor cDNA probe. To identify specific cell types, fluorescent activated cell sorting (FACS) was used with a low affinity NGF receptor antibody, MC192, and antibodies to cell type specific markers. In the mouse spleen, mature B cells express NGF receptor on their cell surface, while mature T cells, granulocytes, and macrophages do not. Using the same technique, CD4 and/or CD8 positive T cells in the thymus do not express NGF receptors on their cell surface. During development, the NGF receptor protein expression is highest in E18 thymus based on a western blot with MC192 and tapers off into adulthood based on northern analysis. The reverse is true in the spleen. Work in parallel with cell lines supports the above results. NGF binds to the mouse mast cell line BabY5 and the mouse B cell lymphoma CH27 and upregulates the NGF receptor in these cells based on FACS analysis. The adult mouse thymic epithelial line 3D.1 expresses NGF receptor mRNA based on northern analysis. Experiments in progress are aimed at further characterizing these cell lines as well as fetal thymic cell lines to compare the properties of the immune system NGF receptor with the neuronal receptor.

445.3

TRANSFECTION WITH THE *TRK* PROTO-ONCOGENE RESCUES NGF RESPONSIVENESS IN MUTANT NGF-NONRESPONSIVE PC12 CELL LINES. D. M. Loeb*, J. Maragos*, K. A. Phelan, M. V. Chao, L. F. Parada*, and L. A. Greene*. Columbia Univ. Dept. Path., New York, NY 10032, Dept. Cell Bio. and Anat., Cornell Univ. Med. Coll., New York, NY 10021, and Molec. Embryology Sect., ABL-Basic Res. Prog., NCI-Frederick Cancer Res. and Dev. Ctr., Frederick, MD 21701.

The *trk* proto-oncogene product ($p140^{prototrk}$) binds NGF and undergoes rapid autophosphorylation in response to this neurotrophic factor. EMS mutagenized PC12 cell lines deficient in high affinity NGF binding and unresponsive to NGF (PC12nnr; Green et al., J. Cell Biol. 102:830-843, 1986) were used to determine if $p140^{prototrk}$ is involved in transducing a functional NGF signal. Northern blot analysis using a *trk*-specific probe revealed that PC12nnr lines express significantly less *trk* mRNA than do wild type PC12 cells. Expression vectors encoding full length $p140^{prototrk}$ were transiently transfected into PC12nnr cells by the lipofectin method. After 4 days of exposure to NGF, approximately 10% of the cells displayed long neurites ending in growth cones; without NGF, no neurites were observed. Control cultures either mock transfected or transfected with a plasmid lacking $p140^{prototrk}$ did not grow neurites when exposed to NGF. Treatment of the cultures with 200 nM K252a, a drug that specifically blocks NGF responses, effectively suppressed NGF induction of neurite outgrowth. NGF is capable of promoting survival of PC12 cells, but not PC12nnr cells, in serum-free medium. PC12nnr cells transfected with *trk* cDNA were maintained in serum free medium, while untransfected and control transfected cells died. These results indicate that $p140^{prototrk}$ is necessary for PC12 cell responsiveness to NGF; they do not, however, address the issue of whether $p140^{prototrk}$ is sufficient for NGF responsiveness since PC12nnr cells also express the low affinity NGF receptor, p75. Generation of permanent PC12nnr-trk transfected cell lines will allow a more complete evaluation of the responses to NGF that are rescued by *trk* expression.

445.4

Two independent signaling pathways lead to the activation of the nerve growth factor gene. F. Jehan*, D. Wion*, I. Neveu*, Ph. Brachet. INSERM U.298, CHR, 49033 Angers cedex 01, France.

Increased expression of the nerve growth factor gene (NGF) may be obtained by treating L929 fibroblasts with serum or 1,25-dihydroxyvitamin D3. The action of serum is largely prevented by Pertussis toxin suggesting that it is mediated by GTP-binding protein(s). It requires protein synthesis and is associated with a transient activation of c-fos proto-oncogene. However, the expression of c-fos is not sufficient in itself to activate NGF gene, since cAMP also causes a transient increase of c-fos transcripts, which is not followed by an accumulation of NGF mRNAs. In contrast, 1,25-dihydroxyvitamin D3 promotes the synthesis of NGF in the presence of cycloheximide or Pertussis toxin, and does not induce c-fos gene. These data indicate that NGF gene may be controlled by two different signaling pathways.

445.5

THE SCHWANN CELL-DERIVED TRUNCATED FORM OF THE NGF RECEPTOR ARISES FROM POST-TRANSLATIONAL PROCESSING **P.A. Barker, F.D. Miller, T.H. Large, and R.A. Murphy Dept. of Anatomy and Cell Biology. Univ. of Alberta, Edmonton, Alberta, Canada T6G 2H7 and Dept. of Neurosci., Case Western researve Univ., Cleveland, Ohio 44106.**

Low affinity NGF receptors have been identified on neurons in the peripheral and central nervous system and on several non-neuronal cell types. The low affinity receptor is produced both in a membrane bound and soluble truncated form. We have undertaken to determine whether the truncated form is generated as a result of post translational proteolytic processing of intact membrane-bound receptor or from an alternatively spliced mRNA lacking a transmembrane domain.

Pulse chase analysis of primary rat Schwann cells coupled with immunoprecipitations using antibodies to the intracellular and extracellular domains of the receptor were used to monitor receptor production. Three forms of the NGF receptor (Mr 80, 82, 85kd) displaying a precursor-product relationship were detected over a two hour chase period in cell extracts with only the 85 kd species appearing on the cell surface. Truncated receptor (Mr 50 and 52 kd) was detected in conditioned medium five hours after labelling but not intracellularly. PCR and RNAse protection analyses detected no splice variants that could generate receptor and media conditioned by fibroblasts transfected with rat receptor cDNA, in which splicing cannot occur, produced truncated receptor as well. Therefore, the truncated form of the receptor arises from post translational processing of the intact receptor rather than as a distinct translation product.

445.6

NGF REGULATES NEURONAL GENE EXPRESSION IN A CONCENTRATION DEPENDENT FASHION. Y. Ma, R.B. Campenot, and F.D. Miller. Dept. of Anat. and Cell Biol., Univ. of Alberta, Edmonton, Alberta, CANADA.

Developing sympathetic neurons respond to systemic administration of NGF by alterations in gene expression (Mathew and Miller, Dev. Biol., 141, 84, 1990; Miller et al., JCB, 112, 303, 1991). To determine the direct effects of different concentrations of NGF on sympathetic neurons, we studied the regulation of mRNA's encoding the low-affinity NGF receptor (LNGFR), Tα1 α-tubulin (Tα1), and tyrosine hydroxylase (TH) in cultured rat sympathetic neurons from the postnatal day 1 SCG. Northern blot analysis of total cytoplasmic RNA isolated from sympathetic neurons cultured for 5 days in 2-200 ng/ml 2.5S NGF revealed that, as the NGF concentration increased, neurons expressed correspondingly increased levels of all three mRNAs. Both LNGFR and TH mRNAs were increased 7-fold, and Tα1 mRNA 4-fold in neurons cultured in 200 ng/ml versus 10 ng/ml NGF. Furthermore, when neurons were cultured in 10 ng/ml 2.5S NGF for 5 days, and the concentration was subsequently increased to 200 ng/ml, LNGFR, TH, and Tα1 mRNAs all increased within 12 hours. LNGFR and TH mRNA levels continued to increase for 48 hours following the NGF increase (latest timepoint examined) while Tα1 mRNA reached a plateau at 5 hours which was maintained. These experiments show that NGF directly modulates the expression of these neuronal genes in a concentration-dependent fashion. Since tubulin and tyrosine hydroxylase are important components of the axon and nerve terminal the NGF concentration dependency of their genes likely reflects a step in the regulation of neuronal growth by NGF. These data also raise the possibility that the effects of NGF on gene expression may be mediated through multiple signal transduction pathways that are activated at different concentrations.

445.7

A c-fos-NGF TRANSGENIC MICE. NEW PERSPECTIVES IN THE STUDY OF THE FACTOR. B. Onténiente[1], P. Horellou[2*], I. Makeh[3*], F. Lisovoski[1*], G. Grimber[3*], P. Briand[3*], J. Mallet[2*], M. Peschanski[1] 1)INSERM U161, Paris 2)CNRS UPR11, Gif 3)ICGM, Paris, France.

The lack of an appropriate way of delivery is an ongoing impediment to the *in vivo* study of NGF functions in the CNS. Transplantation studies suggest that embryonic neurons could be suitable vectors for the delivery of substances which do not cross the blood brain barrier, provided that the neurons develop and integrate with the host parenchyma definitively and without major damage.

To address this issue, the mouse prepro-NGF gene (gift from P. Brachet, INSERM U298) was inserted within a plasmid containing the *c-fos* promoter and the transcription termination and polyadenylation signals of the *ß-globin* gene (NAR 17:6,1989, gift from P. Fort). The vector-free construct was introduced into C57Bl6xDBA mice genome by micro-injection into fertilized eggs. Southern blot analysis of the offspring of three transgenic founder mice has revealed a stable transmission of the gene over several generations. *c-fos* was chosen as a promoter because of its sensitivity to several *in vitro* and *in vivo* stimuli which unable it to be used as a modulator of the production of NGF in transgenic cells. *In vitro* stimulation by 24h serum deprivation of fibroblasts transfected with the construct induced a clear differentiation of PC12 incubated with the supernatant. Preliminary *in vivo* attempts to modulate the production of NGF by external stimuli revealed an increase in the content of NGF-RNA in sub-maxillary glands of animals treated with pentylenetetrazole, a potent seizure-inducing agent.

445.8

INDUCTION OF NGF OCCURS BY MULTIPLE MECHANISMS. E.K. Hoffman*, M.E. Lewis, K. Clopton*, and S. Carswell. Cephalon, Inc., 145 Brandywine Parkway, West Chester, PA 19380.

NGF has been shown to be induced in various systems by seemingly unrelated compounds, among which are catecholamines, catechol derivatives, agonists of beta adrenergic receptors, serum, retinoic acid, IL-1 beta and prostaglandin E1. We are performing comparative studies to examine the molecular events involved in induction of NGF by various compounds. It was found that NGF, as measured by ELISA, could be induced, although to varying extents, in mouse L cells using each of the above types of compounds. Further, serum and the catechol derivative, 4-methyl catechol (4-MC), appeared to induce NGF synergistically. Northern blot analyses of NGF mRNA suggest that the inductions of NGF by both 4-MC and serum, as well as their synergistic induction, are reflected at the transcriptional level. 4-MC and the beta receptor agonist, isoproterenol, also appear to induce NGF by different means: the beta receptor agonist propranolol blocked induction by isoproterenol, but not by 4-MC. The molecular basis of these findings is now being investigated, including studies to analyze the DNA sequence elements within the NGF promoter and the proteins that bind to these elements that are required for both basal and induced levels of transcription.

445.9

NGF-REGULATORY REGIONS WITHIN THE TRANSIN/ STROMELYSIN GENE IN PC12 CELLS: INVOLVEMENT OF AN AP1 SITE AND OTHER CIS-ACTING ELEMENTS. G. Ciment, C.Machida*, J.Lochner*, L.Matrisian*,†. Dept. Cell Biology & Anatomy, Oregon Health Sciences Univ., Portland, OR 97223; †Dept. Cell Biology & Anatomy, Vanderbilt Univ., Nashville, TN.

In previous work, we found that NGF induced the mRNA encoding the metalloproteinase transin/stromelysin ("transin") in PC12 rat pheochromocytoma cells. Transin was found to be a late gene, requiring expression of transcriptional activator proteins. Transient transfection studies using the p750TRCAT plasmid, which contains a 750 base pair region of the transin promoter just 5' to the transcription start site, were found to confer NGF-responsive reporter gene activity to PC12 cells, indicating that NGF-regulatory sequences are present in this 750 base pair region.

In this study, we show that an AP1 element located at position -70 to the transcription start site is required for both basal and NGF-induced levels of expression. These studies involved transient transfection assays with a mutated version of the p750TRCAT plasmid in which the AP1 site was altered. Deletion studies, in which the transin promoter was truncated in various ways, were also performed. These studies found that additional NGF-responsive regulatory sequences were also present within this 750 base base region.

445.10

COMPARISON OF NGF RECEPTOR DISTRIBUTION ON PC12 AND SCHWANN CELLS USING ANTIBODIES 217c AND 192-IgG. G. Ferrari, M. Fabris*, A. Bruni*[1], L. Petrelli* and P.E. Spoerri. Fidia Research Labs., Abano T. (PD), Italy, [1] Dept. of Pharmacology, Univ. of Padova, Italy

Recently, we reported that the antibody 217c recognizes a different epitope on the NGF receptor (NGFR) than does 192-IgG (Ferrari et al. Exp. Neurol., 1991). These studies were extended by showing that 217c and 192-IgG recognize epitopes located on the extracellular domain of only the low-affinity NGF receptor (LNGFR) as both immunoprecipitated the LNGFR but not the high-affinity (HNGFR) cross-linked complex (Meakin S.O. and Shooter E.M., Neuron 6: 153, 1991). We have employed immunoelectron microscopy to examine the topographical distribution of antigens recognized by 217c and 192-IgG on NGF-primed PC12 cells (which have both LNGFR and HNGFR) and Schwann cells (which have only LNGFR). The two antibodies exhibited similar immunogold reactivity. However, in NGF-primed PC12 cells, colloidal gold was observed at regular intervals along perikaryal and neuritic surfaces. Prominent immunoreactivity was frequently localized in coated endocytotic vesicles, on SER and in secondary multivesicular lysosomes. In contrast, immunoreactivity in Schwann cells was only present along the plasma membrane. We conclude that these antibodies may be useful to follow the fate of the two forms of NGFR.

445.11

E1A domains binding p105Rb and p300 are required to block NGF-induced differentiation of PC12 cells. D. Kalman, K. Whittaker and P.H. O'Lague. UCLA Dept. of Biology. Los Angeles CA 90024.

Nerve Growth Factor (NGF) causes PC12 cells to cease division and undergo neuron-like differentiation including neurite outgrowth. We are testing whether differentiation and division share overlapping control mechanisms in these cells. To do this we are perturbing the activity of proteins known to participate in cell cycle regulation by introducing the E1A oncogene or its mutant forms via microinjection. E1A acts in part through specific binding domains to titrate putative cell cycle control proteins including, for example, p105Rb (retinoblastoma) and p60 Cyclin A, as well as others of unknown function such as p107 and p300 (Whyte et al. 1989; Cell 56:67). Similar to previous results (Maruyama et al. 1986; Oncogene 1:361), we find that wild type E1A detected immunohistochemically blocks NGF-induced neurite extension. In addition, NGF's effects do occur in mutants known to have greatly reduced binding to either p105Rb or p300. Wild type E1A's block can be partially relieved by an exogenous human Rb gene product concurrently expressed at high levels. Our results suggest that cell cycle regulatory proteins such as p105Rb and p300 might play an overlapping role in the NGF signal transduction pathway.

445.12

NGF ACTIVATES A PROTEIN KINASE WHICH PHOSPHORYLATES THE PROTO-ONCOGENE c-FOS. L.K. TAYLOR AND G.E.LANDRETH. Departments of Neurology and Neurosciences, Case Western Reserve University, Cleveland, OH 44106.

NGF rapidly induces the transcription of the c-fos gene in PC12 cells. The resulting Fos protein is highly phosphorylated. C-fos transcription is also induced by a number of other agents including depolarization, yet the Fos protein is not highly phosphorylated. This observation suggested the existence of an NGF-regulated Fos kinase. A peptide corresponding to the C-terminal region (residues 359-370) containing putative phosphorylation sites was synthe-sized and used to detect an NGF-regulated protein kinase.

NGF treatment of PC12 cells resulted in the rapid activation of a protein kinase which phosphorylated the peptide. Fos kinase was activated 3-5 fold in response to NGF or EGF, while bFGF, cAMP, TPA, or depolarization were without effect. Fos kinase activity was maximal after 5 min of NGF treatment, and remained elevated 2-fold after 1 hr. Fos kinase phosphory-lated authenic c-Fos protein and this phosphorylation was inhibited by the peptide substrate. Moreover, a Fos C-terminal deletion mutant was not phosphorylated. Fos kinase is distinct from other previously characterized protein kinases.

The phosphorylation of c-Fos at these C-terminal sites is likely to be responsible for the transrepressive activity of c-Fos. The identification of Fos kinase provides another element in the NGF signal transduction cascade which may mediate communication between the cytoplasm and nucleus.

445.13

PARTIAL CHARACTERIZATION OF NERVE GROWTH FACTOR-RESPONSIVE PHOSPHORYLATED NUCLEAR PROTEIN IN PC12 CELLS. M.Ikegame*,K.Fujita, H.Sakamoto*,H.Kuzuya and K.Titani*‖. Div. Mol. Biol.,‖Div. Biomed. Polymer Sci., Fujita Health University, Toyoake, Aichi 470-11, Japan.

A nuclear protein designated as SMP(slow migrating protein)** with a MW of approx. 30kDa in PC12 cells is known to be increased in its phosphorylation by NGF. In this study, NH_2-terminal amino acid sequence, production of monoclonal antibody, and immunocyto- and histochemistry of the nuclear protein were investigated. Proteins were extracted with 0.5M HCl from nuclei, precipitated with 25% TCA, and then electrophoresed on acid/urea PAGE (15%). Proteins were transferred to PVDF membrane, stained with CBB, and sequenced dierectly. NH_2-terminal sequence,GRVIRGGAGSVHRAHVKHRKGAARL, has considerable homology with those of ribosomal proteins L2 and KD4 of slime mold(Dictyostelium discoideum) and yeast(Schizosaccharomyces pombe), respectively, ICR mice were immunized against the nuclear protein; the gels on which the protein was separated were homogenized with Freund's adjuvant and injected. After the antibody was generated, spleen cells were fused with NS-1 myeloma cells. The monoclonal antibody stained PC12 cells differently among cells. Some cells were stained on nuclei and others on cytoplasms. Rat tissues such as adrenal medulla, superior cervical ganglia, and dorsal root ganglia were also stained. **M-Y.W.Yu,et al.,J.Biol.Chem.,255,10481(1980).

445.14

ACTIVATION OF THE CATALYTIC SUBUNIT OF cAMP-DEPENDENT PROTEIN KINASE BY PROTEIN KINASE N (PKN).

C. Volonté and L.A. Greene*. *Department of Pathology, Columbia University, College of Physicians and Surgeons, New York, N.Y., 10032.*

Protein kinase N (PKN), a serine protein kinase that is rapidly activated by NGF and other agents in PC12 cells and other cell lines, increases the phosphorylating activity of the catalytic subunit of cAMP-dependent protein kinase (PKA). Preincubation of the two kinases in the presence of ATP leads to potentiated phosphorylation of histone HF1, Kemptide (a specific substrate for PKA, but not for PKN) and several additional substrates. The potentiated activity is insensitive to 6-thioguanine (an apparently specific inhibitor of PKN) but is suppressed by the Walsh PKA inhibitor and by the regulatory subunit of PKA. PKN-activated PKA shows a 30-fold decrease in Km for Kemptide and a substantial increase in Vmax. The presence of 6-TG during preincubation of the two kinases prevents the potentiation of substrate phosphorylation, indicating that the catalytic activity of PKN is required for the activation. This is supported by the observation that the catalytic subunit appears to be a substrate for PKN. PKN-pretreated PKA is more stable to freeze/thawing and PKN can reactivate freeze/thawed PKA that has lost activity. This suggests that PKN may activate PKA by promoting a conformation that is more enzymatically active. PKN and PKA appear to be widely expressed, and past studies have shown that PKN can be activated in cells via a PKA-dependent pathway. The occurrence of PKN-PKA interactions within cells thus would have interesting biological implications for signal transduction.

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445.15

DISSOCIATION OF CHANGES IN GTP CYCLOHYDROLASE, TETRAHYDROBIOPTERIN AND TYROSINE HYDROXYLASE INDUCED BY NGF IN SUPERIOR CERVICAL GANGLIA. K. Hirayama and G. Kapatos. Dept. of Psychiatry, Wayne State Univ., Detroit, MI 48235

GTP cyclohydrolase (GTPCH) is the first and rate-limiting enzyme in the tetrahydrobiopterin (BH4) biosynthetic pathway. BH4 is known to serve as a specific cofactor for tyrosine hydroxylase (TH), the rate-limiting enzyme in norepinephrine (NE) biosynthesis. To investigate the regulation of BH4 biosynthesis by NGF in NE-contalning neurons, we have used monolayer cultures of sympathetic neurons derived from the neonatal rat superior cervical ganglia (SCG). SCG maintained in the presence of 100 ng/ml NGF (7S) for 4 or 11 days in vitro (DIV) were treated with different concentrations of NGF (50 ng/ml to 1 μg/ml) for 7 days. When NGF treatment was begun at 4 DIV, GTPCH activity, BH4 levels and TH activity were increased in accordance with the concentration of NGF. In contrast, when treatment was begun at 11 DIV, GTPCH activity and BH4 levels were unchanged while TH activity was increased. These data suggest that in 4 DIV cultures the NGF-induced increase in BH4 is due to an induction of GTPCH and that the regulation of GTPCH and TH may be coordinated regulated. However, in 11 DIV cultures this coordinate regulation was no longer apparent. It is suggested that under certain conditions the induction of TH and of its BH4 cofactor can be dissociated and that changes in cell-free TH activity do not necessarily equate with an alteration in the capacity of the intact neuron to synthesis NE. (Supported by NIH NS26081).

445.16

PHOSPHORYLATION AND ACTIVATION OF SER/THR KINASE OF B-RAF BY NERVE GROWTH FACTOR IN PC12 CELLS. M. Oshima*, U. R. Rapp*, and G. Guroff. Section on Growth Factors, National Institute of Child Health and Human Development, Bethesda, MD 20892 and Laboratory of Viral Carcinogenesis, National Cancer Institute, Frederick Cancer Center, Frederick, MD 21701

The raf family oncogene, B-raf, is expressed in neuronal and urogenital tissues, which suggests a functional role for the product of this gene in these tissues. Treatment of PC12 cells with nerve growth factor (NGF), which causes these cells to differentiate into a neuron-like phenotype, does not alter B-raf mRNA (3.8 kb and 10 kb), but does induce rapid phosphorylation of 67 kD and 95 kD B-raf proteins. Phosphorylation was observed after 1.5 min and reached a maximum by 10-15 min. Phosphorylation was seen mostly on serine, but not on tyrosine. NGF-induced phosphorylation was not affected by the depletion of protein kinase C nor by the removal of extracellular calcium, but was inhibited by K-252a. NGF treatment also activated the ser/thr kinase activity of B-raf within 1-2 min. Preliminary data suggest that B-raf plays a role in sending the NGF signal to the nucleus.

445.17

THE CHARACTERISTICS OF THE NERVE GROWTH FACTOR (NGF)-INDUCED PHOSPHORYLATION OF PHOSPHOLIPASE C-γ (PLC-γ) IN PC12 CELLS. D. W. Fink, Jr.*, U-H. Kim, H. S. Kim, S. G. Rhee, and G. Guroff*. Section on Growth Factors, NICHD, NIH and Signal Transduction Section, NHLBI, NIH Bethesda, MD 20892.

Increased hydrolysis of phosphatidylinositol (PI) occurs within seconds of the addition of NGF to PC12 cells in culture. Receptor-mediated, NGF-stimulated PI turnover is presumed to be the consequence of a calcium-dependent activation of PLC. Treatment of PC12 cells for 5 min with NGF leads to a 2-3-fold increase in the phosphorylation of PLC-γ. Removal of extracellular Ca^{2+} blocks the action of NGF. Phosphoamino acid analysis indicates an increase in both phosphoserine and phosphotyrosine. Time-course studies show that the phosphorylation of tyrosine (30 sec) precedes that of serine (2.5-5 min). Pretreatment with the tyrosine kinase inhibitor genistein (150 uM) blocks both NGF-induced PI turnover and NGF-stimulated tyrosine phosphorylation. These data suggest that NGF-stimulated PI hydrolysis results from increased tyrosine phosphorylation of PLC-mediated by a receptor-linked tyrosine kinase.

445.18

NERVE GROWTH FACTOR (NGF)-STIMULATED RELEASE OF ADRENERGIC NEUROTRANSMITTERS AND UPTAKE OF CALCIUM BY PC12 CELLS B. Nikodijevic, C. R. Creveling*, and G. Guroff*. Section on Growth Factors, NICHD and Laboratory of Bioorganic Chemistry, NIDDKD, NIH, Bethesda, MD 20892.

It has been shown that NGF stimulates a calcium-dependent release of catecholamines from PC12 cells. NGF also stimulates $^{45}Ca^{2+}$ uptake into cells attached to collagen-polylysine coated plates by 60-80% within 3 min, but this effect is transient, being much lower or absent after 15 min of NGF treatment. The effect of NGF was blocked or significantly reduced by EGTA, K-252b, staurosporin, calmidazolium, fluphenazine, or suramine. The effect of NGF was not blocked by inhibitors of N- or T-type calcium channels, nor by calcineurin, nickel, or inhibitors of protein kinases C, A, or N, and only partially by high concentrations of inhibitors of L-type channels. The NGF effect on $^{45}Ca^{2+}$ uptake was much less in cell suspension, in very dense cultures, or in cells pretreated with calcium ionophores. The data indicate that the NGF-stimulated increase in calcium uptake relies on channels different than the well-known voltage- or receptor-operated calcium channels.

445.19

ACTIVATION AND DISTRIBUTION OF PROTEIN KINASE C (PKC) ISOZYMES IN PC12 CELLS AFTER TREATMENT WITH PKC STIMULATORS. J. I. Morris*, R. K. Singh*, A. P. Fields*, and K.E. Neet. Depts. of Biochemistry and Pharmacology, Case Western Reserve University, Cleveland, OH, and Dept. of Biological Chemistry, The Chicago Medical School, N. Chicago, IL.

PKC is activated by nerve growth factor (NGF), phorbol esters (PMA), and the macrocylic lactone bryostatin (BRY) but is not required for the generation of neurites in PC12 cells. We have characterized the response of PKC isozymes to various agents using isotype specific antibodies directed against peptide determinants of PKC α, β_{II}, and γ. Western blots have shown that all three isozymes are present in significant amounts in the cytosol of control cells with α the most abundunt. After acute treatment with NGF, PKC α and β_{II} levels remain unchanged but immunoreactive PKC γ rapidly disappears from all cellular fractions and then recovers by 1-2 h. We are currently investigating the mechanism of this loss. Chronic down regulation by PMA or BRY leads to rapid loss of PKC α and β_{II}, but PKC γ persists even at 24 h. Activity measurements have shown that the cytosolic PKC activity increases after NGF treatment and that PMA or BRY shift PKC activity toward the membrane fraction. The activities for individual PKC isozymes are being measured. We conclude that isozymes of PKC respond quite differently to NGF and that modulation of PKC γ may be important in regulating cellular response to extracellular stimulation. A basis for the differential effect of PMA and BRY on NGF-induced neuritogenesis has not yet been established. (Supported by NIH grants NS24380, GM43186, and AM07319)

445.20

REGULATION OF NERVE GROWTH FACTOR BY ENDOGENOUS OPIOID PEPTIDES. J.P.Schwartz and K.Mitsuo*. Clin.Neurosci.Br., NINDS, NIH, Bethesda MD 20892.

Daily treatment of newborn rats with 50 mg/kg naltrexone (NLT) for 21 days results in increased brain weight and numbers of neurons, neuronal processes, and glia in cortex, cerebellum(CB) and hippocampus(HC) [Zagon & McLaughlin, Science 221: 1179(1983), Dev.Br.Res. 28:233(1986)]. Astrocytes express the proenkephalin gene with processing of the precursor to free enkephalins high from embryonic day 20 to postnatal day 3 and then decreasing significantly, suggesting the possibility that astrocyte-derived enkephalins might play a trophic role early in CNS development. We have used the NLT model as a way to manipulate the effects of endogenous opioids on CNS development in vivo. Since nerve growth factor (NGF) is involved in CNS development, NGF was assayed in 3 brain regions after 1 or 2 wks of NLT. NLT led to a decrease of NGF content, measured by a 2-site ELISA, in striatum(ST) and HC, with no effect on CB NGF. In contrast, astrocyte cultures from CB or ST of NLT-treated rats contained increased levels of NGF, while HC astrocytes were unchanged. Opiate receptor binding assays are underway to determine whether NLT acts directly on astrocytes or indirectly via another mediator.

GROWTH FACTORS AND TROPHIC AGENTS V

446.1

RETROGRADE TRANSPORT OF BDNF AND NT-3 BY SPINAL MOTONEURONS Q.Yan, K.Gray*, J.Sun*, C.Farrell, D.Patel*, R.Rosenfeld*, J.Talvenheimo*, G.Pierce*, J.Miller* Amgen, Inc., Amgen Center, Thousand Oaks, CA 91320

It has been shown that spinal motoneurons express NGF receptors, which can bind, internalize and retrogradely transport NGF (Yan et al., Neuron 1:335-343). Here we examined whether these spinal motoneurons could also specifically retrograde transport BDNF and NT-3, two new members of NGF family.

Recombinant BDNF, NT-3 and cytochrome c were iodinated by lactoperoxidase. ^{125}I-BDNF and ^{125}I-NT-3 retained their full biological activities on the chick DRG explant assay. Foot pads of postnatal day-3 rats were injected with 2.2 x 10^6 cpm in 2-3 μl PBS (n=4) and animals were perfusion fixed after 14 hrs. The lumbosacral spinal vertebral columns were dissected out, processed for either cross or horizontal paraffin sections and emulsion autoradiography. Heavy labeling was found in ipsilateral but not contralateral L4-6 DRG neurons and L4-6 spinal ventrolateral motoneurons of animals injected with either ^{125}I-BDNF or ^{125}I-NT-3. The labeling was on neuronal cell bodies and could be completely blocked with co-injection of excess unlabeled homologous ligand. ^{125}I-cytochrome c with same amount radioactivity was not transported. It is clear that the spinal motoneurons express BDNF and NT-3 receptors which are capable of mediating the retrograde transport of the ligands. These results suggest that BDNF and NT-3 should be evaluated for their potential neurotrophic activities on spinal motoneurons.

446.2

CILIARY NEURONOTROPHIC FACTOR (CNTF) EFFECTS ON RAT SPINAL CORD NEURONS IN VITRO: SURVIVAL AND EXPRESSION OF CHOLINE ACETYLTRANSFERASE AND LOW-AFFINITY NGF RECEPTORS. E. Magal, P. Burnham, S. Varon. Dept. Biol., Univ. Calif. San Diego, La Jolla, CA 92093.

CNTF promotes the survival of a variety of neuronal cell types, induces cholinergic properties in sympathetic neurons and differentiation of O-2A progenitor cells into type-2 astrocytes. We have studied the effects of CNTF and NGF on E14 rat spinal cord cultures. After 7 days of treatments with CNTF (human recombinant or purified from rat sciatic nerve; 100 TU/ml) an increase was seen in: i) the number of neurons not stained with choline acetyltransferase (ChAT) or low affinity nerve growth factor receptor (LNGFR) antibodies (identified by immunostaining for neurofilament and neuron-specific enolase); ii) the number of α-motoneurons (0.5% of the total neuronal population), identified by their size (>25 μm), morphology and ChAT and LNGFR-immunoreactivity, and iii) a population of small- to medium- sized (< 25 μm), ChAT- and LNGFR-positive neurons, representing 5-10% of the total neuronal population. Delayed administration of CNTF revealed that the ChAT/LNGFR-negative neurons and α-motoneurons were dependent on CNTF for their survival. In contrast, the small ChAT/LNGFR-positive neurons were not dependent on CNTF for survival, but were induced by CNTF to express these two markers. These observations suggest that CNTF is a neuronotrophic factor for α-motoneurons and a large unidentified population of spinal cord neurons, as well as a regulator of ChAT and LNGFR expression for some neurons of the fetal spinal cord. This latter regulatory role was also observed for a small number of neurons cultured from various other brain areas. Supported by NINCDS grants NS16349 and NS27047.

446.3

EFFECTS OF CNTF ON RAT SENSORY AND MOTOR NEURONS IN-VIVO. S. C. Apfel, J. C. Arezzo, M. Litwak, M. E. Moran*, and J. A. Kessler, Albert Einstein College of Medicine, Bronx, N.Y. 10461

We investigated the effects of daily administration of human recombinant ciliary neuronotrophic factor (CNTF) on rat motor and sensory neurons. CNTF has previously been shown to promote survival and physiological differentiation of a variety of neuronal populations in-vitro. We administered CNTF subcutaneously to adult Sprague Dawley rats in doses of 0.25 μg/g and 0.12 μg/g daily for two consecutive weeks and the buffer vehicle to control animals using the same schedule. We obtained baseline recordings from the caudal nerve of motor and sensory action potential amplitudes and conduction velocities in all our groups prior to initiating treatment with CNTF or placebo. After the fourteenth day of treatment, we repeated the electrophysiological measurements in all three groups. There were no significant changes of any electrophysiological measurements in the control group from baseline measurements. Administration of low dose CNTF significantly increased sensory conduction velocity ($p < 0.0001$), sensory amplitude ($p < 0.0001$), and motor conduction velocity ($p = 0.001$) as compared to baseline values. It did not significantly effect motor amplitudes. High dose CNTF significantly increased sensory conduction velocity ($p = 0.0001$) and sensory amplitude ($p = 0.01$). High dose CNTF did not significantly effect motor conduction velocity of amplitude. We also examined levels of endogenous neuropeptides in the dorsal root ganglia after the animals were sacrificed. Administration of CNTF at both high and low doses significantly increased levels of substance P ($p = 0.002$ and $p = 0.02$ respectively). Levels of calcitonin gene related peptide and met enkephalin were not changed significantly from control values. This data suggests that CNTF might improve peripheral nerve function in adult rats.

446.4

DOWN-REGULATION OF CNTF mRNA DURING PERIPHERAL NERVE DEGENERATION. N. Seniuk, M. Altares, R.J. Dunn, and P.M. Richardson. McGill University & Montreal General Hospital, Montreal, Canada.

The mRNA encoding CNTF (ciliary neurotrophic factor) has been assayed in rat sciatic nerves by an RNase protection assay at various time intervals after nerve transection or crush. Within one week of sciatic nerve transection, the concentration of CNTF mRNA in the distal nerve stump has fallen at least eightfold. Four weeks after injury, the concentration of CNTF mRNA remains low in cut nerves, but has recovered towards normal in crushed nerves. These results are compatible with earlier observations of a decrease in ciliary neurotrophic bioactivity during Wallerian degeneration (Brain Res 246, 57, 1982). CNTF mRNA has been localized by in situ hybridization to non-neuronal cells in the peripheral nerve. Expression of the gene for CNTF, like those of several myelin proteins, is regulated directly or indirectly by axonal signals.

446.5

DEVELOPMENTAL REGULATION OF KAINIC ACID-INDUCED ELEVATIONS OF BDNF mRNA LEVELS. M. Dugich-Djordjevic, P.A. Lapchak, G. Tocco, I. Najm, M. Baudry, R.F. Thompson, and F. Hefti. Andrus Gerontology Center and Dept. of Biological Sciences, U.S.C., Los Angeles, CA.

Recent studies have shown that hippocampal and cortical BDNF mRNA levels are increased in response to electrolytically induced limbic seizures and kainic acid (KA)-induced seizures in adult rats. We examined the regional and cellular distributions and the time course of BDNF mRNA expression in the hippocampus during postnatal development after KA-induced seizure activity using quantitative in situ hybridization techniques with a 460bp rat BDNF cDNA. KA-induced seizures resulted in large increases in BDNF mRNA levels in all hippocampal layers and in the cortex only in 21d, 25d old and adult rats. No increases were evident at P8, despite massive behavioral seizures of these rats. At P13, a modest elevation occurred only in the dentate gyrus and the CA3 field, whereas the other hippocampal fields and the cortical areas remained unchanged. There were pronounced variations in the time course and extent of BDNF mRNA elevations among the hippocampal and cortical areas at various developmental stages. Furthermore, labeling patterns visualized with emulsion autoradiography differed among regions and developmental stages. In particular, while KA induced a uniform elevation of grain density in the dentate gyrus at P13, a subset of cells within the granular layer showed a much higher grain density than adjacent cells in this layer at P21 to P60. These findings indicate that the regulation of BDNF mRNA expression in the hippocampus after KA-induced seizures is dependent upon developmental stage and cell type. Furthermore, the absence of BDNF mRNA induction during early postnatal development despite massive seizures indicates that elevation of neuronal activity is not sufficient for BDNF mRNA induction.

446.6

MOLECULAR CLONING OF NERVE GROWTH FACTORS FROM THE CHICKEN EMBRYO. A. Bäckström*, F. Hallböök, A. Kylberg*, C. Bark , D. Larhammar (§), H. Persson (#) and T.Ebendal. Department of Developmental Biology, and Department of Medical Genetics (§), Uppsala University, Biomedical Center, S-751 23 Uppsala, and Department of Medical Chemistry, Laboratory of Molecular Neurobiology (#), Karolinska Institute, Stockholm, Sweden.

The chicken embryo offers an accessible system for examining early developmental effects by neurotrophic factors. Hitherto, only the βNGF gene has been cloned from the chicken (Ebendal et al., 1986, EMBO J. 5: 1483-1487) and its appearance described during development. We now present approaches for molecular characterization also of chicken NT-3, BDNF and CNTF in the chicken.

Representative cDNA libraries from different stages and organs of the chicken embryo were prepared. DNA fragments corresponding to part of the chicken BDNF and NT-3 obtained by the PCR technique using genomic chicken DNA as template and degenerate primers covering highly conserved regions of the NGF family, were used to isolate cDNA from the λZAPII libraries. A clone encoding chicken NT-3 was obtained from an E3 embryo library and is presently sequenced in order to extend the sequence outside the PCR fragment used for screening. Specific antisense oligonucleotides were also used for *in situ* hybridization of NT-3 and BDNF in the chicken embryo.

In order to isolate the chicken CNTF we have synthesized both PCR primers and long oligonucleotides suggested by the available sequence data from mammalian CNTFs. A cDNA library prepared from the E18 chicken choroid, a rich source for the CNTF, was screened. Positive clones are now being sequenced in order to verify their identity.

446.7

REGULATION OF CNTF mRNA LEVELS IN RAT BRAIN CULTURES. P. Carroll*, M. Sendtner and H. Thoenen. Dept. Neurochemistry, Max Planck Inst. for Psychiatry, Am Klopferspitz 18a, 8033 Planegg, Germany.

CNTF (ciliary neurotrophic factor) is a 22 kDa molecule which has been shown to have survival and differentiation activities for specific populations of nerve cells in vitro as well as being involved in the in vitro differentiation of O-2A glial precursors to type 2 astrocytes in the rat optic nerve. Application of purified CNTF to axotomised newborn rat facial nerve rescues neurons which would normally die under these conditions. CNTF mRNA and protein are found post-natally in the developing rat optic and sciatic nerves where the protein is found in a sub-population of type I astrocytes and in Schwann cells, respectively. In order to identify factors which may be involved in the regulation of CNTF expression in vivo we have examined CNTF mRNA expression in 2-3 week-old cultures of newborn rat brain cells which contained predominantly astrocytes.

Northern blots were performed on total RNA isolated form primary cultures of rat brain cells. CNTF mRNA is found in relatively high amounts (1 pg/μg total RNA) in these cultures. Variations in culture conditions and treatment with a variety of cytokines and growth factors were used in attempts to alter CNTF mRNA levels. Rat IFN-gamma caused a 2-3 fold increase after 24 hrs. The adenylate cyclase agonist forskolin (10 μM) and the beta-adrenergic agonist isoproterenol (10 μM) down-regulated CNTF mRNA to almost undetectable levels after 24 hours suggesting an involvement of the cAMP second messenger system in the regulation of the CNTF gene. bFGF (20ng/ml) also down-regulated CNTF mRNA over the same time-scale. The effect of bFGF on CNTF mRNA levels is interesting in view of the proliferative and anti-differentiating effects of bFGF on O-2A precursor cells in optic nerve cultures.

446.8

CILIARY NEURONOTROPHIC FACTOR (CNTF) PREVENTS NEURONAL DEGENERATION AND INDUCES NGF RECEPTORS IN THE INJURED ADULT RAT BRAIN; COMPARISON WITH NGF. T. Hagg and S. Varon Dept. of Biology 0601, University of California San Diego, La Jolla, CA 92093

Recombinant human CNTF or purified mouse nerve growth factor (NGF) were infused intraventricularly for 2 weeks in unilaterally fimbria-fornix transected adult rats. NGF prevented the axotomy-induced disappearance of ipsilateral medial septum neurons immunoreactive for choline acetyltransferase (ChAT) and low affinity NGF receptor (LNGFR) and induced their sprouting, maintained their cell body size but had no effect on the substantial loss of the more numerous non-cholinergic neurons. CNTF prevented the lesion-induced disappearance of almost all (cholinergic and non-cholinergic) medial septum neurons. CNTF prevented the reductions of LNGFR and size of cholinergic medial septum neurons, but not the reductions of ChAT. CNTF also induced the appearance or increase of LNGFR-staining in cholinergic neostriatum neurons, lateral septum neurons and non-neuronal cells of the lesioned corpus callosum. In contrast to NGF, CNTF did not cause hypertrophy of septal or neostriatal cholinergic neurons or sprouting of LNGFR-positive fibers in the septum. Thus, CNTF: i) has a general trophic action, i.e. can prevent degeneration of several types of injured adult medial septum neurons, ii) is involved in regulation of LNGFR expression in several (but not all) types of neurons and at least one type of non-neuronal cell, iii) does not stimulate ChAT in the neurons (suggesting also different mechanisms for the regulation of ChAT and NGFR), and iv) does not appear to have neurite-promoting activities. Support: NINCDS grant NS-16349 and 27047.

446.9

TROPHIC ACTIONS OF BDNF ON DOPAMINERGIC NEURONS IN VITRO: EXPRESSION OF CELL-SPECIFIC PROTEINS AND ACCELERATED RECOVERY FROM MPP^+ TOXICITY. Klaus D. Beck, Beat Knüsel and Franz Hefti. Andrus Gerontology Center, University of Southern California, Los Angeles, CA 90089-0191.

Trophic effects of BDNF on nigral dopaminergic neurons in vitro were reported recently (Knüsel et al. 1991, PNAS 88:961; Hyman et al. 1991, Nature 350:230). To further characterize these effects we used our cell culture system of fetal rat ventral mesencephalic cells. Exposure to 100ng/ml BDNF for 7 days in vitro increased the number of tyrosine hydroxylase (TH)-positive cells by 35%. Dopamine (DA) uptake was increased by 100%, whereas TH activity showed only a slight increase, indicating differential responses of parameters reflecting dopaminergic functions. Further experiments will determine whether BDNF stimulates TH mRNA expression.

The highly specific toxin for dopaminergic neurons, MPP^+ (Michel et al. 1989, J. Pharmacol. Exp. Ther. 248:842) was used to study possible protective effects of BDNF. Immediately after termination of the MPP+ treatment, DA uptake in BDNF-treated cultures was reduced to the same level as in MPP+-treated cultures not exposed to BDNF. However, after a recovery period of 3 additional days without growth factors or toxin, DA uptake was higher in cultures pretreated with BDNF. This protection was maximal at MPP^+ concentrations which led to a 30-50% decrease of DA uptake under control conditions. These findings suggest that BDNF accelerates the recovery of dopaminergic neurons from MPP^+ toxicity.

446.10

EXPRESSION OF BDNF AND TYPE II CALCIUM-CALMODULIN DEPENDENT PROTEIN KINASE mRNA IN THE HIPPOCAMPUS OF POSTMORTEM ALZHEIMER'S AND CONTROL TISSUE. K.D. Murray*, C.M. Gall, E.G. Jones and P.J. Isackson. Department of Anatomy and Neurobiology, University of California, Irvine, CA 92717.

We have used in situ hybridization with ^{35}S-labeled cRNA probes to examine the expression of mRNAs for brain derived neurotrophic factor (BDNF) and type II calcium-calmodulin dependent protein kinase (CAM II kinase) in the hippocampus of control human and Alzheimer's disease tissue. Like nerve growth factor, BDNF, has been shown to be expressed in the hippocampus and neocortex and may provide trophic support for projecting cholinergic neurons of the basal forebrain which degenerate in Alzheimer's disease. In hippocampus, BDNF mRNA was predominantly localized in the dentate gyrus granule cell layer and the pyramidal cell layer of region CA3. In a comparison of tissue sections from 4 Alzheimer's and 4 age-matched control cases, there were no differences in the localization or level of expression of BDNF mRNA in hippocampus. CAM II kinase mRNA is normally present in the same cell layers as BDNF mRNA but at much higher levels. CAM II kinase mRNA was reproducibly increased in the pyramidal cell layer of region CA3 in Alzheimer's disease tissue. In view of previous results demonstrating increased CAM II kinase mRNA expression in monocularly deprived columns of adult monkey visual cortex (Benson et al., *J. Neurosci.* 11,31), these results suggest that the CA3 pyramidal cells receive reduced afferent input or decreased trophic support in Alzheimer's disease. (Supported by NIH grants AG00538 and MH 44188)

446.11

AUTORADIOGRAPHIC ANALYSIS OF NEUROTROPHIN-3 (NT-3) RECEPTOR BINDING IN ADULT RAT BRAIN Ai Shih*, E. Escandon, L E. Burton, H.S. Phillips, J. W. Winslow Departments of Developmental Biology and Process Development, Genentech, Inc., S.S.F., CA 94080.

Neurotrophin-3 (NT-3) is a structural homolog of NGF which displays a restricted pattern of mRNA expression in adult rat brain, confined mainly to hippocampus. To reveal possible targets of NT-3 trophic activity within mammalian brain, receptor binding autoradiography was performed with purified, recombinant human ^{125}I NT-3 labelled by the lactoperoxidase method. Approximately 2 moles of ^{125}I was incorporated per mole NT-3 homodimer, and ^{125}I NT-3 retained full biological activity as measured by chick embryonic nodose ganglion and sympathetic ganglion neuron survival assays. The pattern of specific binding of ^{125}I NT-3 was compared with that of ^{125}I NGF and displayed a broader distribution within one month old rat brain. Specific binding of 30 pM ^{125}I NT-3 was observed in cerebellum and telencephalic sites, particularly hippocampus. Binding was blocked by increasing concentrations of unlabelled NT-3 but not by equivalent concentrations of NGF. These results suggest possible targets of NT-3 trophic activity within mammalian brain.

446.12

AXONAL TRANSPORT OF [^{125}I]-LABELED NEUROTROPHINS IN THE CENTRAL NERVOUS SYSTEM. S.J. Wiegand, C. Alexander*, R.M. Lindsay and P.S. DiStefano. Regeneron Pharmaceuticals, Inc., Tarrytown, NY 10591.

The mRNA's for the three known neurotrophins (NGF, BDNF and NT-3) are differentially distributed within the mammalian CNS. Therefore, it is expected that at least some neuronal populations responsive to BDNF and NT-3 will be found to be distinct from those which respond to NGF. The prototypic neurotrophin, NGF, is a survival factor for the cholinergic neurons of the basal forebrain and it is retrogradely transported by these cells. This suggests that studies of axonal transport of BDNF and NT-3 might prove useful in identifying neuronal populations potentially responsive to these related trophic molecules. [^{125}I]-BDNF, -NGF and -NT-3 (~0.6-1.5 μCi in 0.2-0.5 μl) were injected into the hippocampus or striatum of adult, male rats. Sections processed for autoradiography showed robust transport of NGF from the hippocampus to the medial septum and the diagonal band; transport of BDNF and NT-3 to these cholinergic nuclei was also evident, though less marked. All 3 neurotrophins were transported to the ventral mesecephalon from the striatum. However, the distribution for BDNF and NT-3 was distinct from that seen for NGF. Furthermore, BDNF and, to a lesser extent, NT-3 were transported within several additional telencephalic and diencephalic cell groups which did not transport NGF. Neuronal populations which selectively transport BDNF and/or NT-3 may respond specifically to these members of the neurotrophin family under normal or pathological conditions.

446.13

RETROGRADE AXONAL TRANSPORT OF [^{125}I]-LABELED NEUROTROPHINS IN PERIPHERAL NEURONS. P.S. DiStefano, B. Friedman, S.J. Wiegand and R.M. Lindsay. Regeneron Pharmaceuticals, Inc., Tarrytown, NY 10591.

NGF is the prototype of target-derived neurotrophic factors. Endogenous, or exogenously administered NGF is internalized by responsive neurons and is retrogradely transported to the cell body where it exerts its actions by unknown mechanisms. Two recently described members of the neurotrophin family, BDNF and NT-3, were examined for their ability to be retrogradely transported in sensory, sympathetic and motor neurons. ^{125}I-NGF, -BDNF and -NT-3 used in transport studies exhibited activities in chick DRG bioassays that were equivalent to those of unlabeled neurotrophins. When injected into the crushed sciatic nerve in adult rats, ^{125}I-BDNF, -NT-3 and -NGF were specifically transported to the ipsilateral L4 and L5 DRG. Transport was blocked by the addition of excess, unlabeled homologous neurotrophin; however, transport was not completely blocked by the co-injection of heterologous neurotrophin. When injected into the anterior chamber of the eye, ^{125}I-NGF transport was evident in sympathetic neurons which supply the iris muscle, but negligible transport to sympathetic neurons was seen with radioiodinated BDNF or NT-3. These experiments provide a framework for further studies of subsets of peripheral neurons which may respond to various neurotrophins by selectively transporting these factors in a retrograde fashion.

446.14

DISTRIBUTION OF CNTF AND ITS RECEPTOR IN THE CNS AND PNS. N.Y. Ip, B. Friedman, J. McClain*, P. Masiakowski, S. Davis, M.E. Furth, S. Wiegand and G.D. Yancopoulos. Regeneron Pharmaceuticals, 777 Old Saw Mill River Road, Tarrytown, New York, 10591.

Initially identified as a neurotrophic activity that supports survival of parasympathetic neurons *in vitro*, ciliary neurotrophic factor (CNTF) has recently been shown to promote survival of motor neurons *in vivo* (Sendtner et al., 1990. Nature 345: 440-441; Oppenheim et al., 1991. Science 251:1616-1618). We have examined the distribution of CNTF message and protein *in vivo* in adult rats. The abundant CNTF message found in sciatic nerve by northern analysis was localized to Schwann cells by *in situ* hybridization. Immunocytochemical staining of CNTF with a polyclonal antibody showed that the CNTF protein was also restricted to Schwann cells in the peripheral nerve. In the CNS, northern analysis has demonstrated significant CNTF message in spinal cord, midbrain, hindbrain, brainstem and olfactory bulb. Preliminary immunocytochemical studies showed that CNTF can be localized to neuronal elements, as subsets of olfactory receptor axons are densely stained. We have recently cloned the rat CNTF receptor (CNTFR) by screening the rat brain cDNA library with a human CNTFR probe (Davis et al., in preparation). The rat CNTFR is extremely well conserved compared to its human counterpart. Studies with northern analysis showed that CNTFR mRNA is widely distributed throughout the brain. This result is corroborated by *in situ* analysis which has revealed that significant levels of CNTFR mRNA are found in cerebellum, olfactory bulb, and thalamus.

446.15

INJURY-INDUCED REGULATION OF NEUROTROPHIC FACTOR mRNA IN ADULT RAT BRAIN. J. S. Rudge, J. K. Morse, S. J. Wiegand and N. Y. Ip Regeneron Pharmaceuticals, 777 Old Saw Mill River Road, Tarrytown, New York 10591.

Mechanical injury to the adult rat brain results in the accumulation of neurotrophic activity for cultured parasympathetic, sympathetic and sensory neurons at the lesion site (Nieto-Sampedro et al, 1983. J. Neurosci. 3: 2219-2229). In order to determine which neurotrophic factors are responsible for this activity, we have measured changes in the levels of mRNA by northern analysis for Ciliary Neurotrophic Factor - CNTF, Brain Derived Neurotrophic Factor - BDNF and Neurotrophin 3 - NT3 in tissue samples taken from brain areas both adjacent and distant to the site of an aspirative lesion of the dorsal hippocampus and overlying cortex. We find that in the normal adult rat hippocampus and cortex, CNTF mRNA levels are relatively low but this increases dramatically after lesion by as much as 7 fold compared to sham operated controls. Areas distant from the lesion site show no increase in CNTF mRNA unless they are directly interconnected to the ablated areas, such as the regions of the contralateral cortex and hippocampus where mRNA levels are as high as at the wound site. In contrast, mRNA levels for the neurotrophins BDNF and NT3 were relatively high in normal adult hippocampus but fell by 2-3 fold after lesion both contralaterally and adjacent to the wound.

446.16

CHARACTERIZATION OF RECEPTORS FOR CILIARY NEUROTROPHIC FACTOR ON CULTURED RAT HIPPOCAMPAL ASTROCYTES. R. F. Alderson, Y. You*, E. Pasnikowski*, S. Davis, N. Panayotatos, G. D. Yancopoulos, J. Rudge and R. M. Lindsay. Regeneron Pharmaceuticals Inc., Tarrytown, NY 10591.

Rat ciliary neurotrophic factor (CNTF) has recently been molecularly cloned, purified to homogeneity and biologically characterized (Panayotatos et al,1991). Binding studies with [^{125} I]-CNTF on monolayer cultures of purified rat hippocampal astrocytes demonstrates equilibrium binding to be reached following a 60 min incubation period at room temperature and to remain stable up to at least 120 min. Scatchard analysis suggest that the [^{125} I]-CNTF binding is conferred through two sites. Experiments to biochemically characterize the ligand binding component of the CNTF receptor demonstrate a loss of specific [^{125} I]-CNTF binding following exposure (60 min at 37°C) to purified preparations of phospholipase C suggesting that a glycosyl-phosphatidylinositol anchor is involved in the attachment of the CNTF binding component to the plasma membrane. In addition to investigating potential second messenger mechanisms which may be activated as a consequence of the interaction of CNTF with its receptor, we have observed the induction of the messenger RNA for the immediate early gene c-fos. Time course studies demonstrate that a maximal increase in the c-fos mRNA occurs approximately 1 hour following a challenge with 50 ng/ml CNTF.

446.17

REDUCTION OF CNTF LEVELS IN SCHWANN CELLS IN DEGENERATING SCIATIC NERVE. B. Friedman, N.Y. Ip, M.E. Helgren, J. Rudge, T. Rakowski, L. Fu, D. Morissey, S.J. Wiegand, R.M. Lindsay. Regeneron Pharmaceuticals, Inc., Tarrytown, NY 10591.

The adult rat sciatic nerve is a rich source of CNTF (ciliary neurotrophic factor). Immunocytochemical staining indicates that most of the CNTF is present in Schwann cells. To determine if the integrity of Schwann cell-axon contact is a prerequisite for high levels of CNTF expression, we have examined CNTF levels in degenerating and regenerating peripheral nerve. In adult rats, the sciatic nerve was transected and then examined histologically at a series of survival times from 1 day to two weeks. A second series of rats were prepared where the sciatic was crushed and examined after two week survival times. CNTF levels were assessed immunocytochemically with a polyclonal antibody generated against recombinant rat CNTF. CNTF mRNA was identified with in situ hybridization. Schwann cells in the **proximal stump** of a transected sciatic nerve remain densely stained by anti-CNTF antibody and continue to express message for CNTF. However, by one week post-transection, the Schwann cells in the degenerating **distal stump** lose most of their CNTF immunoreactivity and CNTF message is barely detectable. When axons are allowed to regenerate distal to a nerve crush then Schwann cells re-express CNTF protein. These results suggest that Schwann cell expression of CNTF is maintained by signal(s) from related axons and/or by the maturational state of the Schwann cell.

446.18

MOLECULAR CLONING OF THE RECEPTOR FOR CILIARY NEUROTROPHIC FACTOR. S. Davis, T. H. Aldrich, D. Valenzuela, M. E. Furth, V. Wong, S. P. Squinto, and G. D. Yancopoulos. Regeneron Pharmaceuticals, Inc., 777 Old Saw Mill River Road, Tarrytown, New York 10591.

Although neurotrophic factors were originally discovered based on their ability to support neuronal survival, these molecules are thought to be important in a variety of processes involved in the development and maintenance of the nervous system. Defining the receptors for neurotrophic factors should aid in identifying the cells on which they act, as well as in understanding the mechanisms of their actions. We developed a novel "tagged ligand panning" procedure to clone a receptor for ciliary neurotrophic factor (CNTF). Cross-linking studies reveal that CNTF-responsive neurons and neuronal cell lines express a CNTF-binding protein with the same molecular weight as the cloned receptor expressed in heterologous cells. In vivo, this receptor is expressed almost exclusively within the nervous system. This receptor has a structure unrelated to the receptors utilized by the NGF-family of neurotrophic molecules, but instead displays intriguing analogies with the receptor for a cytokine, interleukin-6 (IL-6). Structural as well as other similarities suggest that the CNTF receptor, like the IL-6 receptor, requires a second, signal transducing component. In contrast with the IL-6 receptor, the CNTF receptor is anchored to cell membranes via a glycosyl-phosphatidylinositol (GPI) linkage, raising important questions concerning the regulation and release of the membrane bound form of this receptor.

446.19

EXPRESSION OF NEUROTROPHIC FACTORS IN THE AXOTOMIZED FACIAL NERVE SYSTEM. W. Tetzlaff and K. C. Harrington*, Neuroscience Research Group, University of Calgary, Calgary, Alberta, Canada, T2N 4N1.

Following axotomy CNS neurons undergo significant cellular atrophy and cell death, whereas PNS neurons usually survive disconnection from their targets and do not show atrophy until later stages post lesion. It is generally believed that this is due to differences in the availability of trophic factors. For motoneurons trophic support may come from the peripheral nerve and/or from the neuronal microenvironment with its reactive glial cells. We have tested these possibilities by using Reverse Transcription followed by Polymerase Chain Reaction amplification to study the expression of CNTF, bFGF and several other trophic factors in i) the normal facial nerve ii) the distal nerve stump after transection iii) the normal and iv) axotomized facial nucleus. Our preliminary results indicate increased expression of CNTF mRNA in the axotomized facial nucleus while the expression is reduced in the distal nerve stump (compared to normal nerve). bFGF mRNA was increased in the axotomized facial nucleus, but not detected in the peripheral nerve (both normal and distal stump).

These data suggest that the neuronal microenvironment may provide trophic support to the axotomized facial motoneurons. *In situ* hybridization analysis is under way to determine the cellular source within the facial nucleus.

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446.20

A NEUROBLASTOMA CELL LINE REGULATES VIP GENE EXPRESSION IN RESPONSE TO CNTF Aviva J. Symes, Steven E. Hyman and J. Stephen Fink *Molecular Neurobiology Laboratory, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA 02129.*

Ciliary neurotrophic factor (CNTF) was originally identified as a survival factor for embryonic neurons of the chick ciliary ganglion. CNTF has also been shown to promote the differentiation of cultured sympathetic neurons towards the cholinergic phenotype, inducing choline acetyltransferase activity and peptides including vasoactive intestinal peptide (VIP). We sought to determine whether CNTF would regulate expression of VIP in a neuronal cell line. CNTF treatment of the VIPergic human neuroblastoma cell line NBFL increased VIP mRNA levels 30-fold at 3 days. CNTF induction of VIP mRNA was detectable after 1 day of treatment and was sustained for at least 11 days. NGF treatment has no independent effect on VIP mRNA in NBFL cells but may enhance the effect of CNTF. Using VIP promoter-luciferase fusion genes we demonstrated that the effect of CNTF on VIP mRNA in NBFL cells is at least partially transcriptional. Two kb of 5' flanking region of the human VIP gene was sufficient to confer CNTF responsiveness to these fusion genes, increasing luciferase activity 20-fold in CNTF-treated, transfected NBFL cells. However, a deletion which yielded a still active promoter was unresponsive to CNTF. The CNTF-responsive NBFL cell line will enable further investigation of the molecular mechanisms by which CNTF, through alterations in gene expression, promotes neuronal differentiation.

446.21

CLONING AND EXPRESSION OF GROWTH-PROMOTING ACTIVITY (GPA), A CILIARY NEUROTROPHIC FACTOR ISOLATED FROM FROM ADULT CHICK SCIATIC NERVES AND EMBRYONIC CHICK EYES. Parent, A.S., Cachianes, G.*, Lee, A.*, Leung, D.W.*, Nikolics, K., Eckenstein, F. P., and Nishi, R., Dept. of Cell Biol. & Anat., Oregon Health Sciences University, Portland, OR 97210 and Dept. of Mol. Biol., Genentech, Inc., 460 Point San Bruno, So. San Francisco, CA 94080

Neurons in the chick ciliary ganglion (CG) undergo a defined period of cell death, and it has been postulated that the survival and development of CG neurons is regulated by a target-derived trophic factor. We have previously purified and characterized the biological activity of growth promoting activity (GPA) from adult chicken sciatic nerves. We have also found that a similar protein can be purified from embryonic chick eyes, the source of all target tissues for CG neurons. Based upon partial amino acid sequence obtained from 3 peptide fragments of the purified protein we designed three oligonucleotide probes. We used these probes to screen a cDNA library derived from embryonic day 15 chick eyes. A 1453 bp GPA cDNA clone was isolated which shows approximately 45% homology with mammalian sciatic nerve ciliary neurotrophic factor (CNTF). This relatively low degree of homology for a potent neurotrophic factor (ED50= 50pg/ml) suggests that GPA may be a homologue of CNTF, and not merely the chicken version of CNTF. GPA, like CNTF, does not seem to contain a signal sequence; however, evidence from our laboratory indicates that GPA is secreted by cells in culture derived from the choroid layer of the eye, which is one of the targets of CG neurons *in vivo*(see Coulombe et al, this meeting). In order to test whether GPA is a neurotrophic factor *in vivo* for CG neurons, and to determine the relationship between GPA and CNTF, we are examining the developmental- and cell-specific gene expression of the GPA gene, using northern analysis and *in situ* hybridization. We are also expressing a recombinant GPA to examine the *in vitro* and the *in vivo* activity of the protein. **Supported by NS25767 (RN) and AG07424 (FPE).**

446.22

Evidence for Target Cell Secretion of Survival and Differentiation Factors for Ciliary Ganglion Neurons.
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The recent purification and cloning of several factors which influence the survival and differentiation of embryonic neurons in culture has provided the curious observation that some of these factors lack the signal sequence normally associated with secretion. This observation has initiated controversy over the potential roles of these factors in vivo. We have established long-term cultures of cells from the choroid layer, a target tissue innervated by chick ciliary ganglion neurons. Conditioned medium (CM) from these cultures contains at least two biochemically separable factors: one which supports the survival and growth of ciliary ganglion neurons in culture (Growth Promoting Activity, GPA), and another which supports expression of the neuropeptide somatostatin (Somatostatin Stimulating Activity, SSA). To examine whether these factors are secreted into the culture medium or are instead released by cell lysis, we allowed choroid cells to condition medium for 24 hours before removing the CM and lysing the choroid cells. We then compared the amounts of GPA and SSA present in CM and cell lysate. As a measure of cell lysis we compared the amounts of activity of a ubiquitous cytoplasmic enzyme lactate dehydrogenase (LDH) present in CM versus cell lysate. CM contained less than 5% of the LDH activity present in the cell lysate indicating that little lysis occurred during the conditioning period. GPA was distributed about equally between the CM and cell lysate, suggesting that it is a secreted factor. The GPA present in CM was not due to a fibroblast growth factor since very little heparin-binding mitogenic activity was present in the CM. SSA in contrast was only found in CM and was not detectable in the cell lysate, suggesting that it is a secreted factor which may require activation upon release. These observations suggest that both GPA and SSA are secreted from target cells and therefore may serve as neurotrophic factors during development. *Supported by EY06178 (JNC), NS25767 (RN), AG07424 (FE).*

446.23

STRUCTURE-ACTIVITY STUDIES OF HUMAN CILIARY NEURONOTROPHIC FACTOR (CNTF) USING MUTAGENESIS. L. Callegaro*, V. Corsa*, G. Corona*, S.D. Skaper# and A. Negro. Advanced Technology Division and #Fidia Research Laboratories - FIDIA S.p.A., 35031 Abano Terme, Italy.

CNTF promotes the survival and/or differentiation of a variety of neuronal cell populations in vitro, and reportedly prevents lesion-induced degeneration of motoneurons within the facial nucleus of the neonatal rat. We have recently described the production and purification of recombinant human CNTF (Negro et al., 1991). A structural analysis of the CNTF molecule is critical to an understanding of its mode of action. Here we have utilized the Bal31 system and polymerase chain reaction to obtain mutant human CNTFs. Mutant CNTF DNA constructs were expressed in E.coli. The resultant CNTF proteins were isolated by SDS-PAGE, eluted and assayed for trophic activity on CNTF responsive ganglionic neurons. Several mutations directed at the hydrophobic core region of the protein yielded marked losses in biological activity. These findings are consistent with the observed alpha-helical nature of CNTF, and indicate that retention of this conformation is necessary for activity. Purification of these mutant human CNTFs now in progress will provide the material needed to accurately determine the 3-D structure of this trophic factor.

446.24

NUTRITIONAL CONTROL OF THE EXPRESSION OF EXERCISE-INDUCED GROWTH IN MATURE HAMSTERS. K.T.Borer, J.-O.Jansson*, C.A. CONN, and O.P.G.ISAKSSON.* Dept.of Movement Sci.,Univ. of Michigan,Ann Arbor,MI 48109 and Dept.of Physiology,Univ.of Göteborg,Göteborg,Sweden.

Voluntary running accelerates somatic growth in mature hamsters. When food restricted,exercising hamsters fail to grow but retain the capacity for delayed growth.To assess how food restriction affects the expression of growth, we measured the transcription of insulin-like growth factor-I (IGF-I) gene in liver and muscle and the concentration of circulating IGF-I in hamsters that were either exercising or sedentary and fed ad libitum or restricted to 70% of ad libitum food for 40 days. Expression of IGF-I gene was measured by solution hybridization of a radiolabeled mouse cRNA antisense probe with RNA from the hamster liver and quadriceps, IGF-I concentration,by RIA in acid-ethanol extracted and cryoprecipitated sera. Exercise accelerated ponderal growth by 8.5% and food restriction reduced it by 41%. There was a significant ($p<0.05$): (1)46% stimulatory effect of exercise and a 45% inhibitory effect of food restriction on hepatic IGF-I mRNA concentration; (2) 37% inhibitory effect of food restriction on the concentration of circulating IGF-I ,and (3)22% inhibitory effect of food restriction on muscle IGF-I mRNA. Hepatic IGF-I gene transcription constitutes the primary growth response to exercise, and a 50% reduction in this response in deprivation is accompanied by lesser reductions in circulating IGF-I and transcription of IGF-I gene in muscle. Support:NSF.

446.25

FACTORS AFFECTING SURVIVAL OF MOTONEURONS PURIFIED BY PANNING. C.E.Henderson, E. Bloch-Gallego*, W. Camu*, A. Charnet*, C. Mettling*, H. El M'hamdi*, F. Rassendren*, and F.K. Xie*. CNRS-INSERM, 34033 Montpellier, France.

Existing methods for enrichment of motoneurons (MN) from embryonic spinal cord have provided useful results but are often costly or insufficiently selective. This has hindered the molecular characterization of motoneuron growth factors. Recently, we showed (Bloch-Gallego *et al.*, Development (1991) **111**, 221) that monoclonal antibody SC1 could be used to identify chick MN in mixed cultures, and to purify them by "panning" on SC1-coated Petri dishes. MNs thus obtained are >98% pure by immunological criteria; more than 10^6 may be produced in 3 hrs and cultured on laminin in Terasaki wells (80 cells in 10 µl). We are developing an analogous method for rat MN. In serum-free conditions, chicken MN survival is considerably enhanced by muscle extract, but not by other factors tested, including NGF, TGFβ, human bFGF and chicken CNTF (gift of K.Wewetzer & K.Unsicker). However, in the presence of 10% horse serum, bFGF (100 ng/ml) was as effective as muscle extract in supporting MN survival; bFGF action may therefore require a cofactor.

Xenopus oocytes injected with poly-A^+ RNA from denervated chick muscle secreted a survival-promoting activity for MN. Only low levels of activity were seen using oocytes injected with RNA from control innervated muscle, or sham-injected. Levels of mRNA for such neurotrophic factors may therefore be regulated in muscle or nerve by the motoneuron itself.

This work was supported by A.F.M. and I.R.M.E.

446.26

BRAIN EXTRACT AND GLIAL-DERIVED TROPHIC AGENTS PREVENT DEAFFERENTATION-INDUCED MOTONEURON DEATH IN THE CHICK EMBRYO. Y. Qin-Wei, D. Prevette*, R.W. Oppenheim, and L.J. Van Eldik[1]. Dept. of Neurobiology & Anatomy, Bowman Gray Sch. of Med., Wake Forest Univ., Winston-Salem, NC 27103 and [1]Dept. of Pharmacology, Vanderbilt Univ., Nashville, TN 37232.

Removal of spinal and supraspinal descending input to the lumbar spinal cord by a thoracic spinal transection performed on embryonic day (E) 2 (deafferentation) results in a significant loss of lumbar motoneurons (MN) between E10 and E16. A small amount of naturally occurring MN loss (1500-2000 cells) also occurs between E10-E12. To examine whether these losses reflect the deprivation of CNS-derived trophic agents, brain extract (BEX), astrocyte conditioned medium (ACM), and several purified proteins (bFGF, CNTF & S100) were administrated daily to embryos in ovo between E10 and E15. MN numbers were assayed histologically on E16. Crude and partially purified BEX, ACM and S100 prevented both the late naturally occurring (E10-12), as well as the additional deafferentation-induced MN death. By contrast, bFGF and CNTF prevented the deafferentation-induced MN death, but were without effect on naturally occurring MN death between E10-E12. Surprisingly, partially purified muscle extract also prevented both types of cell death between E10 and E16. Although these data demonstrate that deafferentation-induced MN death can be prevented by CNS-derived proteins, the specific agents involved, their mode of regulation by afferents and their relation to muscle-derived trophic factors remain to be clarified.

NEURONAL DEATH II

447.1

A THEORETICAL MODEL OF NATURALLY OCCURRING NEURONAL DEATH IN DEVELOPMENT. Lucia Galli-Resta and Giovanni Resta*, Istituto di Neurofisiologia, CNR 56127, Pisa, Italy and *Istituto di Elaborazione dell'Informazione, CNR 56100 Pisa, Italy.

Throughout the animal kingdom, the formation of the nervous system involves the elimination of many cells. This naturally occurring cell death is observed in the majority of nervous structures and causes the loss of 30-75% of the neurones initially generated.

Elimination of erroneous projections, as well as proper size matching between connecting structures can be achieved through cell death. However many more cells die than it would seem necessary to eliminate erroneous projections and conflicting data exist as to the issue of size matching between target and input structures. It is believed therefore that no unitary model can be presented to account for the phenomenon of neuronal death in the different cases.

We have developed a simple theoretical model which accounts for naturally occurring neuronal death in development. The model quantitatively predicts the outcome of many of the experiments tried to investigate cell death and it finally reconciles in a single framework experiments until now viewed as contrasting.

447.2

INTENSE SOUND RELEASES AN AMINE PRESENT IN PERILYMPH AND CSF. R.P. Bobbin and M. Fallon*, Kresge Hearing Research Laboratory, LSU Medical Center, New Orleans, LA 70112.

Unk 2.5 is an amine released by 50 mM K^+ into effluent from the perilymph compartment of the cochlea (Bobbin et al., Hear. Res. 46: 83, 1990). We examined the effect of sound on levels of Unk 2.5 in cochlear effluent. Using anesthetized guinea pigs, the perilymph compartment of the cochlea was perfused with an artificial perilymph containing low [Na^+]. Effluent was collected before, during and after exposure of the ear to intense sound. Amines in the effluent were measured utilizing precolumn derivatization with o-phthalaldehyde/2-mercaptoethanol and HPLC. Broad band sound (10 min at 118, 124 and 130 dB SPL) increased the levels of Unk 2.5 without affecting the levels of glutamate and 15 other amines in the effluent. We call Unk 2.5 "noise induced release amine," or "niramine". Niramine eluted almost at the same time as carboxyglutamate, but before Asp, phosphoserine, cysteic acid, cysteine sulfinate, homocysteine sulphinate, and homocysteic acid. In CSF, niramine was found in higher concentrations than in plasma (5:1) and at about the same relative fluorescence as Asp. The chemical identity and role of niramine are unknown. (Supported by NIH grant DC-00379).

447.3

PC12 AND SYMPATHETIC NEURONAL APOPTOSIS DO NOT INVOLVE DNA FRAGMENTATION. P.W. Messner*, T.R. Winters* and S.H. Green. Dept. of Biology, Univ. of Iowa, Iowa City, IA 52242

A common feature of apoptosis is the cleavage of cellular DNA into fragments with sizes that are integral multiples of 180 bp (nucleosome repeat). DNA fragmentation is believed to be causal to apoptosis because apoptosis is inhibited by aurintricarboxylic acid (AT) — a drug that, among its many other effects, also inhibits nucleases. We have directly assayed DNA fragmentation during cell death in rat sympathetic neurons, in neuronal PC12 (nPC12) cells, and in chromaffin-like PC12 (cPC12) cells by observation of purified cellular DNA on agarose gels. The DNA was visualized by ethidium bromide staining or, if greater sensitivity was required, by transfer to nylon filters which were probed with [^{32}P]-labeled rat genomic DNA. Cell death was initiated by transfer of PC12 cells to serum-free, NGF-free medium or transfer of sympathetic neurons to NGF-free medium. No evidence of DNA fragmentation could be observed in these cells at any time up to 72 h after initiation of cell death (by 72 h most of the cells are dead). Electrophoresis of the DNA on denaturing gels also showed no detectable increase in single-stranded breaks. Similar assays performed using glucocorticoid-treated rat thymocytes showed typical DNA fragmentation, indicating that our assay system is capable of detecting neuronal DNA fragmentation were it to be present. Nevertheless, AT strongly inhibited cPC12 cell death and had a similar, albeit weaker, effect in nPC12 cells and neurons. Another difference between nPC12 and cPC12 cells is that cell death in the former, but not in the latter, was partially inhibited by protein synthesis inhibitors. We conclude that DNA fragmentation, whatever its role in nonneuronal cells, is not required for neuronal apoptosis. Furthermore, inhibition of apoptosis by AT, a very general inhibitor of protein-nucleic acid interactions, can't be taken to imply that nuclease activity is necessary for apoptosis. Finally, we suggest that apoptosis in nPC12 cells and cPC12 cells does not proceed by identical mechanisms, with a transcription-dependent process playing a role in nPC12 cell death as one does in neuronal death.

447.4

ABLATION OF THE THORCULUMBAR NEURAL TUBE IN CHICK EMBRYOS PROMOTES SURVIVAL OF CERVICAL PREGANGLIONIC NEURONS. P. Cauwenbergs. Anat.Dept., CMCC,Toronto,Ont. M4G 3E6.

In chick embryos two distinct columns of preganglionic neurons develop from a common primordium and establish structural contact with sympathetic trunk ganglia (STG);(1) a thoracolumbar column of Terni (CT) normally forms permanent synaptic connections with STG cells and (2) a primitive cervical column (CC) develops initial contact with STG early in development but degenerates and disappears by day 4.5E. The inability of CC neurons to form permanent contacts with STG neurons during normal development appears to be a characteristic intrinsic to CC cells alone, since neurons of the thoracolumbar CT transplanted into the cervical neural tube region do survive, contact the STG and maintain contact to at least day 16E (Soc. Neurosci. Absts. 14(2):867).

To test the possibility that CC neurons have the potential to establish stable structural contacts with STG the thoracolumbar neural tube was removed surgically at day 2.5E prior to neurite outgrowth. In these experimental (TL-Rem) embryos the cervical neural tube was left intact, while postganglionic STG neurons developed in the absence of their normal innervation. Analysis of urea silver nitrate stained serial cross-sections of day 8E experimental (TL-Rem) embryos revealed that after neural tube removal STG develop normally and primitive CC neurons now survive and maintain contact with STG. Thus, CC neurons have the potential to form stable contacts with STG neurons.Current analysis will determine if these connections are maintained beyond day 8E and define the pattern of neurite outgrowth into STG in experimental (TL-Rem) embryos.

447.5

DECREASE IN THALAMIC AND NEOCORTICAL VOLUMES AFTER A SMALL PRENATAL CORTEX LESION IN THE CAT. L. D. Loopuijt, J. R. Villablanca, R. Gayek* and D.A. Hovda, Mental Retardation Research Center and Division Lab Animal Medicine, UCLA, Los Angeles, CA 90024.

In monkeys, prenatal frontal cortex lesions have little effect on behavior or neuronal cell packing density (CPD) in thalamic mediodorsal nucleus [Brain Res. 152(1978)451]. In cats, lesion-induced degeneration is less in neonatal- than in adult-lesioned animals. Thus, degeneration in fetally lesioned cats might be minimal; yet, our pilot data indicated the opposite [Soc. Neurosci. Abstr. 13 (1987) 1116]. In the current study, 6 fetal cats (E43-48) received a small unilateral frontal or parietal cortex lesion and 6 controls were intact. After reaching adulthood, brain neocortical and thalamic volumes were determined. In thalamic ventrolateral (VL), principal ventromedial (VMP), external ventrobasal (VBX) and basal ventromedial (VMB) nuclei neuronal and glial CPD and neuronal soma size were measured. Thalamic and neocortical mean volumes were decreased compared to intact animals (thalamus: ipsil. 238.4, intacts 359.8 mm3; cortex: ipsil. 1,861.9, int. 2,175.3 mm3, $P<0.05$). Thalamic mean neuronal CPD did not differ between lesioned and intact (VL ipsil. 2.3, int. 2.2; VM ipsil. 2.1, int. 3.0, VBX ipsil. 2.2, int. 2.9, VMB ipsil. 2.9, int. 3.0 neurons/0.0003 mm3), while glial CPD tended to be lower ($P<0.05$ for VL, VMP). Preliminary analysis of neuron size did not reveal marked differences between lesioned and intact cats. Decreases in volume indicate atrophy in cortex and thalamus. Thalamic shrinkage without increase in CPD is probably due to increased developmental cell death. These findings fit well with behavioral impairments in fetal lesioned cats (Villablanca et al., this meeting). Grants USPHS R01 NS- 25780; P01 HD-05958.

447.6

RETROGRADE RESPONSE IN THE DORSAL LATERAL GENICULATE NUCLEUS (LGN) OF THE RAT TO DAMAGE OF VISUAL CORTEX. D.R. Diaz and R.E. Kalil. Neuroscience Training Program and Dept of Ophthalmology, Univ of Wisconsin, Madison WI 53706.

While varying in swiftness, severity, and extent among different species, the retrograde reaction of neurons in the LGN to a lesion of visual cortex is usually pronounced. In general, the reaction begins with the simple pallor of affected cell somas and leads subsequently to atrophy and often cell death. Retrograde degeneration in the LGN has been well-studied in some animals, but surprising there is little systematic information available in the rat.

To clarify the retrograde reaction of the LGN in the adult rat, we made a unilateral lesion of visual cortex in a series of animals, and studied the LGN after post-operative survivals that ranged from 24 hours to 6 weeks. At 24 hours, LGN neurons ipsilateral to the cortical lesion display a noticeable pallor, which becomes prominent by 3 days. By 7 days survival, affected LGN cells are atrophied and some are degenerating. At longer survivals, LGN cell atrophy and loss increase in magnitude, and thus after 6 weeks the LGN ipsilateral to the cortical lesion is markedly shrunken in comparison to the normal nucleus.

We also have studied the early stages of the astroglial response in the LGN that accompanies the retrograde neuronal reaction by staining for glial fibrillary acidic protein (GFAP) with a monoclonal antibody. An increase in GFAP immunoreactivity is detectable at 3 days survival, prominent at 7 days, and intense at 2 weeks and thereafter.

447.7

DEVELOPMENTAL CHANGES IN THE ARRANGEMENT OF MOTOR POOLS IN THE CHICK SPINAL CORD MAPPED USING FLUORESCENT BEADS R.V.Stirling and D.Summerbell. Department of Biology, Open University, Milton Keynes MK7 6AA, UK and National Institute for Medical Research, Mill Hill, London NW7 1AA, UK.

Each muscle of the vertebrate limb is innervated by a cluster of motor neurons in the spinal cord, its motor pool. These are usually mapped using retrograde filling techniques in which single muscles are injected with tracers, but such methods do not reveal the overlap between adjacent pools.

In the present experiments fluorescent latex microspheres (Lumafluor Inc) were used to retrogradely fill motor neurons. The position of filled motor neurons within the cord was plotted in three-dimensions using a computer-aided drawing system (InterActions Company). The advantages of using these beads is that they are available in several colours, do not fade, and all colours are transported in an identical manner. The advantages of the computer-aided system is that the position of the filled cells can be mapped in the intact spinal cord, thus avoiding problems related to sectioning.

Initially the pools are diffuse and overlapping but gradually become more discrete. We suggest that selective cell death is responsible for this change. This could not have been detected using single tracers. The method of filling also offers the opportunity of injecting motor neurons with dyes to study the relationship between morphology and position within the motor pool.

447.8

Expression of Ubiquitin in the Chick Embryo Spinal Cord. **S.E.McKay, L.M.Schwartz+ & R.W.Oppenheim, Dept.of Neurobiology & Anatomy, Wake Forest Univ.,Winston-Salem, NC & +Dept.of Zoology, Univ. of Mass.,Amherst, MA.**

We have been examining the expression of ubiquitin, a protein associated with cell death in insects,during the death of motoneurons and neural crest derived neurons in the developing chick embryo. Northern analysis has demonstrated that polyubiquitin transcripts increase in the spinal cord coincident with neuronal death. In situ hybridization is being used to determine the source of this mRNA. Western blotting experiments showed that ubiquitin-protein conjugates comprise a greater percentage of total spinal cord protein at early vs. later stages of cell death. Immunohistochemistry has revealed possible sites of expression: increased ubiquitin immunoreactivity (IR) was present over the entire lumbar lateral motor column at early stages of motoneuron death followed by more punctate staining at later stages. IR was also present in peripheral ganglia and nerves mainly associated with nerve fibers in the dorsal and ventral roots and in the marginal zone of the neural tube. These data suggest that polyubiquitin may play a role in developmental cell death in the vertebrate nervous system.

447.9

MOTONEURON CELL DEATH IN THE NUCLEUS AMBIGUUS AND HYPOGLOSSAL NUCLEUS OF RATS: CRITICAL PERIODS IN THE DEVELOPMENT OF UPPER RESPIRATORY TRACT CONTROL.
D.R. Friedland*, P.J. Gannon, A.R. Eden, J.T. Laitman*, Depts. Otolaryngol. & Cell Biology/Anatomy. Mt. Sinai Sch. Med., NY 10029.

Early neuromuscular development is characterized by a dramatic decrease in neuronal populations during the process of motoneuron cell death (MCD). Parameters of MCD in brainstem nuclei subserving upper respiratory tract (URT) function may, therefore, indicate critical periods in the development of URT motor control.

The nucleus ambiguus (NA) and hypoglossal nucleus (XII) of fetal rats at embryonic days 14 (E14), E15, E16, E17, E19 and adults (for NA) were examined (n=2/age). Fetal rats were removed from the mother, brainstems dissected out, processed for wax embedding, sectioned at 8μm, and stained with hematoxylin and eosin. Counts were made of large neuroblasts (>10μm) exhibiting distinct nucleoli.

Results show little organization of NA and XII at E14 and E15. At E16 NA is not yet discernible, however, XII is distinct and contains clusters of immature neuroblasts. By E17 motoneurons are clearly seen within both NA and XII. E19 brainstems are similarly organized with a more distinct appearance of motor nuclei. Counts were made of motoneurons in NA at E17, E19 and adult. There was a 53% decline in motoneuron numbers between E17 and adult, with 66% of this loss occurring prior to E19. Counts of motoneurons in XII showed an 18% loss between E17 and E19. The observed disparity in degree of cell death between NA and XII may reflect earlier maturation of XII, with a significant portion of cell death occurring prior to E17. Further investigation will clarify these observations.

These results establish late gestation as a period of intense modification of brainstem nuclei subserving URT function. As such, insults occurring during this period may interfere with the normal development of URT neuromuscular control and underlie neonatal pathologies such as SIDS.

447.10

NEURONAL BIRTH AND LOSS IN DEVELOPING RABBIT RETINAE.
S.R. Robinson[1*], F.C. Dangerfield[2*], B. Dreher[2*] and D.H. Rapaport[2]
[1]Vision, Touch and Hearing Research Centre, Department of Physiology, University of Queensland, St. Lucia, QLD, Australia. 4072.
[2]Department of Anatomy, University of Sydney, Sydney, NSW, Australia. 2006.

We have investigated the spatio-temporal patterns of cell birth and loss in the retinae of developing albino rabbits. Under Halothane and nitrous oxide anaesthesia, ^{3}H-thymidine was injected into foetuses on either the 13th post-conceptional day (13PCD), 18PCD or 22PCD. The mother and babies were sacrificed with an overdose of barbituate between 20PCD and 34PCD. The young retinae were embedded as wholemounts in plastic and cut into 3μm sections passing from superior retina through the optic disc to inferior retina. The sections were processed for autoradiography and the distributions of well labelled (i.e. born on the day of ^{3}H-thymidine administration) amacrine and horizontal cells mapped.

Cells born on 13PCD were concentrated in the visual streak and mid-superior retina, while those born on 18PCD or 22PCD tended to be uniformly distributed or more frequent towards the retinal periphery. These findings are consistent with the idea that the visual streak is developmentally advanced.

Three of our findings challenge accepted views of cell death in the developing retina. First, in all cohorts, the main period of cell loss (indicated by a reduction in the number of labelled cells and an increase in the number of pyknotic profiles) occurred between 30PCD and 34PCD. Thus, contrary to the predictions of the "Temporal Matching Hypothesis" *(Dreher and Robinson, 1988, Brain, Behav. Evol., 31: 369-390)*, cell birth arfd death are not temporally correlated. Second, much of the loss is unlikely to be target related, since most horizontal cells die before synaptogenesis begins in the outer plexiform layer. Third, the magnitude of cell loss varied substantially between cohorts: the proportions of labelled amacrine and horizontal cells lost from the 18PCD cohort (24% and 38%, respectively) were considerably less than the proportions lost from the 13PCD (73% and 88%) and 22PCD (64% and 55%) cohorts. Thus, a neurons' chances of survival are very much dependent on a timely birth.

MOLECULAR AND PHARMACOLOGICAL CORRELATES OF DEVELOPMENT: PEPTIDES

448.1

EXPRESSION OF PEPTIDE mRNA IN THE CAUDAL RAPHE COMPLEX DURING EARLY POSTNATAL DEVELOPMENT IN RAT.
R.K. Kanter, J.T. Erickson, C.L. Szymeczek and D.E. Millhorn.
University of North Carolina at Chapel Hill

Neurons of medullary caudal raphe complex (n. raphe obscurus and pallidus) contain substance P (SP), thyrotropin releasing hormone (TRH), and 5-hydroxy-tryptamine in the adult rat. We recently reported that TRH mRNA was not present in these neurons at birth but appeared during the first postnatal month (FASEB J. 4: A1109, 1990). In the present study we analyzed preprotachykinin A (PPT_A) mRNA (which encodes SP and neurokinin A) in 0-28 day old rats to determine if its expression is also developmentally regulated in raphe neurons. *In situ* hybridization showed very low levels of PPT_A mRNA in the raphe complex at 0d, rising to a maximum at 21d and 28d. Results from immunohistochemical studies revealed that fibers originating in the raphe complex demonstrated increasing levels of SP and TRH from 0-28 days consistent with the hypothesis that developmental regulation of these peptides occurs at the level of their mRNA. Maturation of the chemical phenotype of caudal raphe cells involves the parallel regulation of expression of coexisting neuropeptides SP and TRH which both increase progressively during the first postnatal month. The raphe neurons of the medulla provide an excellent model for studying regulation of peptide gene expression in the nervous system. (HL33831, HL34919, AHA, ALA)

448.2

PROENKEPHALIN TRANSGENIC MICE — INTEGRATION DEPENDENT PATTERNS OF TRANSGENE EXPRESSION IN EMBRYONIC CNS.
H. Dressler*, K. Herrup, & S. Hyman. E.K. Shriver Center, Waltham, MA. and Mass. General Hospital, Boston, MA

To study the regulation of proenkephalin (PE) gene expression, we joined 3.0 kb of human PE 5' flanking sequences to *E. Coli lacZ*, followed by 1.0 kb of human 3' PE sequences including the poly-adenylation signal. Three lines of transgenic mice (ENKL/1, 2, and 3) have been produced from this construct. In the adult CNS, a variable pattern of expression is found. To describe the developmental onset of this expression, X-gal-stained embryos were examined. In ENKL/1, two broad bands of staining appear in the prosencephalon as early as E10. By E12, cells in the nasal placode area are stained as is a broad patch of cells in the diencephalon. At E15, cells in the nasal area remain and a group of migratory cells (reminiscent of LHRH neurons) also appear; the CNS cells coalesce in the developing hypothalamic anlage. In ENKL/2, the staining of nasal placode cells is similar to ENKL/1, but neither migratory cells nor CNS cells are stained. Embryos of the ENKL/3 line exhibit the highest levels of adult transgene staining, but at midgestation only the trigeminal ganglion is X-gal positive. Our findings suggest that the PE sequences included in the transgene contain only part of the information needed for correct embryonic PE expression. The spatial variability of expression is thus likely to be a function of both the included PE sequences plus position effects conferred by the site of chromosomal integration.

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448.3

EXPRESSION OF PRE-PROENKEPHALIN MESSENGER RNA IN DEVELOPING RAT SUPERIOR CERVICAL GANGLION.
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Previous studies on enkephalin expression in sympathetic ganglia have reported that only a limited number of neurons express either leu- or met-enkephalin, using immunohistochemical staining methods. We have examined the expression of pre-proenkephalin (PPENK) mRNA in developing rat superior cervical ganglion (SCG) by in situ hybridization histochemistry, using RNA probes. In adult ganglia, 55% of large principal neurons express PPENK mRNA. The amount of labeling varied between individual neurons; 14% were heavily labeled. PPENK mRNA can first be detected in developing SCG by in situ hybridization on postnatal day 4 (P4), when a few sparsely labeled cells are seen. At P7, 11% of principal neurons are labeled, increasing to 30% by P21. A greater percentage of cells fall into the heavily-labeled category in young SCG; the percentage decreases with age as the total number of labeled cells increases.

Our results suggest that expression of PPENK mRNA is more widespread than expression of peptides derived from it. The enhanced sensitivity of in situ hybridization reveals that different subpopulations of neurons express different amounts of mRNA.

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448.4

PROENKEPHALIN AND PREPROTACHYKININ mRNA EXPRESSION IN THE DEVELOPING CHICK BRAIN.
T.L. Overbeck*J.L. Bradley*M.S. Randolph,*M.S. Kindy, K.M. Albers* and B.M. Davis. Dept. of Anatomy & Neurobiology, Univ. of Kentucky, Med. Ctr., Lexington, KY 40536.

Our lab is interested in the relationship between the mechanisms underlying regeneration and development. Enkephalin-like immunoreactivity is seen in the accessory occulomotor nucleus (AON) by stage 25, shortly after cell division ceases. Substance P (SP) coexists with enkephalin, but does not appear until late in development. We have initiated experiments to test whether the expression of proenkephalin mRNA (PRNA) is correlated with synaptogenesis during both phenomena. We have screened a chick E13 cDNA library (in λgt10, generously provided by L. Baizer) using rat random primed cDNAs. One of four positive clones for PRNA has been isolated and sequenced. This clone contains a sequence for Met-Enk and has 100% amino acid identity for the rat sequence (Rosen et al. JBC 259:14309) for amino acids # 121-143 and from 150-163. We have also isolated two positive clones for the preprotachykinin mRNA, which codes for SP. This clone is homologous for the non-tachykinin portion of the mRNA and contains a string of 45 nucleotides that has a 75% identity for the rat sequence. These clones have been inserted into pGEM and will be use to make cRNA probes to examine regeneration and development of the chick AON-ciliary ganglion system. Supported by NS25617 to BMD.

448.5

THE DEVELOPMENT OF VIP mRNA EXPRESSION AND VIP RECEPTOR BINDING IN THE RAT CNS. J.M. Hill, A.C. Tanner*, D. V. Agoston and S.K. McCune. Laboratory of Developmental Neurobiology. NICHD, NIH, Bethesda, MD 20892.

VIP has neurotrophic properties (PNAS:83:1159,1986) and a role in neurobehavioral development (Peptides 12:187,1991). The present work traces the development of VIP with in situ hybridization and receptor binding to both GTP-sensitive and insensitive sites with in vitro autoradiography in rat embryos from E14 to P14 and adult CNS.

The mRNA for VIP was expressed at very low levels until P8, however, by E14 it was seen in the cortex. By P8, message increased and was additionally found in the thalamus, superchiasmatic nucleus (SCN), striatum, superior colliculus and dorsal raphe. By P14, message was greater in the cortex than the thalamus, but in the adult, the SCN and thalamus had the greatest levels.

VIP binding was uniform and dense in the brain stem and spinal cord by E14, but localized to specific cortical and hippocampal layers. By E16, VIP binding was also seen in the SCN, thalamus, pineal and dorsal raphe. By E19, VIP binding was seen in the diagonal band, septum, cerebellar peduncles and hypothalamus. In D8-D14 VIP binding remained uniform and dense in the brain stem and basal forebrain and midbrain structures but in the cortex and hippocampus was limited to specific layers. In the adult, VIP binding was localized to specific cytoarchitectural sites throughout the brain. As in the adult, most VIP binding sites were sensitive to displacement by GTP, however, as early as E19 GTP-insensitive binding sites were observed in the spinal cord and thalamus.

VIP receptor binding develops earlier and is more widespread than VIP mRNA. Although changes in relative abundance occur in both throughout development, by P8 VIP and its receptors are colocalized in several brain regions and remain so in the adult.

448.6

REGULATION OF IGF-II GENE EXPRESSION DURING NEUROBLASTOMA PROLIFERATION AND DIFFERENTIATION. D.M. Martin, R.O. Carlson* and E.L. Feldman, Department of Neurology and Mental Health Research Institute*, University of Michigan Medical School, Ann Arbor, MI 48109.

Insulin-like growth factor-II (IGF-II) belongs to the insulin family of polypeptides and has been implicated in neuronal mitogenesis. Both primary neuroblastomas and some cell lines have been shown to produce large amounts of IGF-II, which can act as an autocrine growth factor (El-Badry, O. et al., J Clin Invest 84:829-839, 1989). Similarly, reduction of IGF-II production may be a means for inhibiting cellular growth. We speculated that IGF-II gene expression is regulated during neuronal proliferation and differentiation. In this study, we report decreased IGF-II gene expression in differentiated neuroblastoma cells.

We have found by Northern analysis that the clonal neuroblastoma line SH-SY5Y expresses high amounts of IGF-II mRNA. Neuroblastoma cells can differentiate in response to interferon-γ (IFN-γ) (Watanabe, H. et al., Jpn J Cancer Res 80:1072-1076, 1989). We observed growth arrest and gross morphological changes in SH-SY5Y following addition of IFN-γ. We analyzed IGF-II mRNA levels in SH-SY5Y cells maintained in the presence or absence of IFN-γ. Under these conditions, IGF-II mRNA levels decreased over a period of 3 or 6 days. These data suggest that decreased IGF-II gene expression is correlated with neuronal differentiation.

Supported by grant #NS07222-09 to D.M.M. and #NS01380 to E.L.F.

448.7

NMDA ACCELERATES AND AMPLIFIES THE ONTOGENY OF SOMATOSTATIN IN MONOLAYER CULTURES OF FETAL RAT TELENCEPHALON. R.J. Robbins, and S. Welsh*. Neuroendocrinology Program, Departments of Medicine and OB/GYN, Yale Univ. School of Medicine, New Haven, CT 06510.

The survival and differentiation of mammalian neurons depends in part on the stimulation which they receive during a critical developmental period. We have previously reported that telencephalic SS interneurons *in vitro* display enhanced susceptibility to excitatory amino acids (EAA) only after they become mature. For the first 11 days in vitro cortical SS neurons obtained from 16 day old embryos are not killed by NMDA. We hypothesized that EAAs would still bind to immature SS neurons and alter their in vitro development. Fetal rat telencephalic cultures were grown for 20 days in the presence of various concentrations of EAAs. The SS content of the cells was measured by RIA. NMDA (100 μM) added at the time of plating increased cellular SS content by 130% at day 5, by 180% at day 10, and by 50% on day 20. Equimolar doses of Quisqualic acid resulted in a greater than 80% decrease in SS at all time points. AMPA (10-100 μM) significantly increased SS content at days 10,15, and 20. The NMDA blocker APV, retards early SS development but results in increased SS content by days 20 and 27. In summary, EAAs can exert dramatic effects on the development of somatostatinergic neurons in vitro. We conclude: (1) that the normal development of SS interneurons in vivo may depend in part on their exposure to glutamate and, (2) that functional EAA receptors exist on neurons prior to their full maturation.

DEVELOPMENT OF SENSORY SYSTEMS II

449.1

NEUROMAGNETIC ASSESSMENT OF SOMATOSENSORY CORTICAL ORGANIZATION FOLLOWING PERIPHERAL NERVE INJURY AND RECONSTRUCTION A. Mogilner, L. Lopez, U. Ribary, F. Lado, J.A.I. Grossman*, and R. R. Llinás Department of Physiology and Biophysics, NYU School of Medicine, NY, NY 10016.

A 14-channel neuromagnetic recording system is being used to evaluate changes in cortical organization following peripheral nerve injury and surgical repair in a number of subjects. Studies in primates (Wall et al, J Neurosci 1986 6(1)218-233) have shown that following peripheral nerve transection and repair, the somatosensory cortical maps reorganize over a period of time during the repair process. The high spatial resolution of the MEG, on the order of a few millimeters, supports the idea that cortical plasticity in human subjects may be detected via recording and source localization of neuromagnetic evoked potentials. Two groups of patients are being studied: 1)Patients who have undergone traumatic nerve section of the upper extremity followed by surgical reconstruction of the nerve, 2)Patients who have undergone skin grafts with intact innervation ("neurovascular island transplant") from one region of skin to another. Both groups of patients are studied before the surgery, and after the surgery at regular intervals. Magnetic responses to vibratory stimulation of the digits are recorded at each session, and a single dipole model is used to localize the cortical sources of the evoked activity, resulting in a map of the hand area of somatosensory cortex SI. The MEG studies are accompanied by a neurological examination which delineates the area and the nature of the sensory pathology, and which will be used to correlate the MEG findings with the subjective sensory state of the subject during the process of recovery. For example, patients in the second group being studied who have undergone skin/nerve relocation initially cannot distinguish between tactile stimulation of the graft and stimulation of the finger which supplied the graft skin, but over time they become able to discriminate between the different fingers. MEG studies have confirmed that immediately following graft surgery, the dipole locations of the graft source and the graft are nearly identical. Further MEG studies during the process of sensory reeducation might elucidate the cortical processes associated with recovery following peripheral nerve injury and reconstruction.

449.2

A PERIPHERY-RELATED PATTERN IS EVIDENT IN RAT SOMATOSENSORY CORTEX AT BIRTH. B.L. Schlaggar and D.D.M. O'Leary Molecular Neurobiology Laboratory, The Salk Institute, La Jolla CA 92037.

The "barrels" of rat somatosensory cortex (SI) are arranged in a pattern isomorphic to the distribution of vibrissae on the body surface. A host of techniques have shown that barrels emerge postnatally and are distinct by the end of the first postnatal week. The timing and sequence of events that lead to the differentiation of barrels are crucial to defining the agents that instruct their formation. Ventrobasal thalamocortical (VB) afferents are hypothesized to control barrel differentiation and the somatotopic patterning characteristic of SI. Here, we investigate the emergence of barrel patterning of VB afferents in SI in rats at ages E16 to P4. (E0 is the day of insemination. P0 is the first 24 hrs after birth). We assayed for AChE, an early marker of VB afferents (Kristt JCN '79; Neurosci '89), and directly labeled VB afferents with DiI in fixed brains. AChE histochemistry reveals a periphery-related pattern in SI as early as 1 hr postnatal, 3 days earlier than previously shown with this method and 2 days earlier than with any other marker, including DiI labeling (Erzurumlu & Jhaveri Dev Br Res '90). The trigeminal (face) representation develops several hours earlier than that of the medial lemniscus (body) - the latter is apparent by late P0. At birth, AChE-positive rows are apparent from which the barrels of the PMBSF later emerge; the complete barrel pattern can be discerned by 24 hrs postnatal. DiI labeling renders a similar sequence of barrel emergence, but delayed by roughly a day. AChE histochemistry is a more precise indicator of developing barrels than DiI because it is specifically expressed by VB afferents, resulting in a higher "signal to noise" than DiI which backfills cortical neurons and their intracortical processes, masking the early differentiation of a barrel-related patterning. These findings provide the earliest evidence for the emergence of barrels and support the hypothesis that VB afferents govern the development of somatotopic patterning in SI. (Support: NIH NS17763.)

449.3

DEVELOPMENT OF THALAMOCORTICAL TERMINAL ARBORS IN LAYER IV OF MOUSE BARREL CORTEX. A. Agmon and E.G. Jones. Dept. of Anatomy and Neurobiology, College of Medicine, Irvine, CA 92717.

Thalamocortical axon terminals in the adult mouse somatosensory cortex are highly ordered. Radially they are segregated into a diffuse tier in the deep layers and a dense tier spanning layers IV and lower III. Tangentially the upper tier is parcellated into discrete patches, each at the core of a cluster of cells or "barrel". In the newborn mouse layers II-IV are still undifferentiated and thalamocortical terminals are restricted to layers V/VI. The formation (around postnatal days 3-4) of the barrels with their associated thalamocortical arbors is a complex morphogenetic event that is not yet fully understood. Of particular interest is whether the axonal clusters in layer IV arise from an initially diffuse projection by elimination of surplus branches or whether they are formed *ab initio* as discrete entities. We studied this question by placing several particles of Di-I in the thalamus in fixed 400 μm thick thalamocortical slices (Agmon and Connors, Neurosc. 41, 365-379, 1991) prepared from animals at different ages during the first two postnatal weeks. The dye was allowed to diffuse for 1-4 weeks at 37° and the slices imaged as whole mounts using a laser confocal microscope. At postnatal day 2 (P2) almost all labeled branches were in layers VI and lower V, with an occasional unbranched axon coursing up through the cortical plate (presumptive layers II-IV). By P4 individual axons were seen to course radially upwards through upper layer V without branching and upon entering lower layer IV abruptly fanned out into several branches; arbors of several axons overlapped to form a cluster, and adjacent clusters were clearly separated. The number of layer IV branches per axon increased dramatically over the next 10 days but the clusters remained well-defined. Our data are consistent with the hypothesis that the terminal arbors in layer IV are segregated from their very beginning and that new branches are added within their "correct" cluster.

449.4

THE CRITICAL PERIOD FOR SENSORY DEPRIVATION IN RAT VIBRISSA CORTEX. By K. Fox, and F.F. Ebner Center for Neural Science, Brown University, R.I. 02912.

Rats were raised with just the D1 vibrissa intact and the other vibrissae carefully pulled out. Deprivation was started at P0, P2 or P4 and continued until recording at 30-60 days. The latency and magnitude of responses to the D1 vibrissa were measured at histologically recovered locations within the cytochrome oxidase "barrel map". Small enlargements of the cytochrome oxidase D1-barrel were noted in 1/3rd of P0-onset animals but never in P2 or P4 animals. Short latency responses (5-10ms) to D1 stimulation were found in 30% of cells in barrels neighboring D1 in layer IV (22/73) compared with none in controls (0/82). Short latency responses fell to 18% at P2 and 13% at P4. Layer IV cells responding with greater numbers of spikes per stimulus to D1 than to the vibrissa anatomically related to their barrel location (shifted cells) fell from 37% at P0, to 23% at P2, to 8% at P4 (0% for controls). Urethane anesthesia was controlled by maintaining layer V burst rate between 0.7 and 2Hz. As a further control for anesthetic effects, responses to the deprived D2 vibrissa were tested in the same animals, but no enlargement of the D2 domain occurred, unlike for D1. Plasticity was greater in layers II/III than in IV at all ages, and showed a minor decline from 62% shifted cells at P0 to 49% at P4. Deprivation induced plasticity appears to end within the first post-natal week subcortically and/or in layer IV, but continues for an, as yet, undetermined period in supragranular layers. (supported by NIH grant #27759).

449.5

AGE-RELATED DIFFERENCES IN SI CORTEX RESPONSE LATENCIES AFTER SOMATIC STIMULATION OF THE FACE, FOREPAW, AND HINDPAW IN POSTNATAL DAY 1 THROUGH POSTNATAL DAY 14 NEONATAL RATS. C.A. McCandlish, C.X. Li*, and R.S. Waters. Dept. of Anatomy and Neurobiology, Univ. of Tennessee, Memphis, Col. of Medicine, Memphis, TN 38163.

Using natural somatic and electrical stimulation, we reported that evoked responses could be elicited from SI cortex of newborn rats prior to PND-7 and that the functional development of the barrel field map follows a lateral to medial gradient. We extend those findings to include a description of the latencies of evoked responses over the first 14 postnatal days.

Neonatal rats, ranging in age from PND-1 through PND-14, were anesthetized with Nembutal (40 mg/kg ip) and placed on a heating pad. A midsagittal incision was made overlying the skull, the skin retracted, and the cisterna magna opened. The animal's head was held rigid by a custom designed head holder. The area of skull overlying SI was removed and the dura opened. Carbon-fiber electrodes (1-2 MΩ) were used to record mechanically and/or electrically evoked responses from SI after stimulation of regions of the contralateral face, forepaw, and hindpaw. At the end of the experiment, electrolytic lesions (3 μA/5 sec) were made at selected sites, and animals sacrificed. Hemispheres were removed, sectioned coronally (40 μm), and processed using cresyl violet stain.

Beginning on PND-1, response latencies were measured and compared between each SI cortical subfield area showed that latency periods are shortest after stimulation of face, followed by forepaw, and then hindpaw region. Furthermore, latencies measured from each subfield region decreased over each successive postnatal day. (Supported by NSF Grant BNS 88-02766)

449.6

LECTIN BINDING PATTERNS IN WHISKER PATHWAYS OF PERINATAL RATS AFTER LESIONS TO SOMATOSENSORY THALAMUS OR CORTEX. J.J.Christensen, N.L. Chiaia, C.A. Bennett-Clarke, R.W.Rhoades and T.A. Woolsey. Dept. Neurosurgery, Washington University, St. Louis MO 63110 and Dept. Anatomy, Medical College of Ohio, Toledo, OH 43699

The plant lectin peanut agglutinin (PNA) transiently reveals patterns in the rat trigeminal (V) pathway that are isomorphic to the vibrissae on the face. It is not known whether the spatial distribution of PNA binding is determined primarily by afferents, neurons, glia, or some combination of these elements. Neonatal destruction of either the somatosensory cortex or VB results in a smaller principal nucleus of V (PrV) and an absence of the normal, vibrissa-related pattern of V-thalamic projection neurons, but does not qualitatively change the distribution of V primary afferents in this nucleus. We made lesions of either somatosensory cortex or thalamus on P0 and assessed patterns of PNA binding in the V neuraxis on P6. The vibrissa-related pattern of PNA binding was absent in PrV contralateral to the damaged cortex or thalamus. The pattern of PNA binding in PrV ipsilateral to the lesion and in spinal subnucleus interpolaris and spinal subnucleus caudalis on both sides of the brainstem was normal. Neonatal cortical lesions led to substantial retrograde degeneration and an absence of any vibrissa-related pattern in ipsilateral ventrobasal thalamus. Thalamic lesions resulted in the absence of a vibrissa-related pattern of PNA binding in the somatosensory cortex on the same side. These results indicate that segregated afferents are necessary, but not sufficient, for whisker-like PNA binding patterns to develop in PrV. Specifically, the patterned distribution of PNA requires rather than directs the organization of the neuropil resulting from the interactions between afferent axons and their target neurons.

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449.7

VIBRISSECTOMY-INDUCED EXPRESSION OF GAP-43 mRNA IN ADULT RAT TRIGEMINAL NUCLEUS AND BARREL CORTEX. B.E. Levin, R.L. Neve and A. Dunn-Meynell. Neurol. Serv., DVA Med. Ctr., E.Orange, NJ 07019 and Dept. of Neurosci., NJ Med. Sch., Newark, NJ 07103.

Vibrissectomy induces plastic change in adult rat barrel cortex associated with a persistent increase in GAP-43 immunoreactive protein beginning 1wk post-surgery. Here we examined GAP-43 mRNA expression in barrel system relays from 1-7d after total unilateral vibrissectomy with sparing of C3. Autoradiograms were generated from brain sections hybridized with labeled mRNA transcribed from a cDNA clone for human GAP-43 which cross-reacts with rat GAP-43 mRNA. As compared to analagous areas in the control side of each section, densities representing GAP-43 mRNA transiently increased by 15% from 1-3d in the ipsilateral principal trigeminal n. (P=0.01), followed at 6-7d by a 12% increase (P=0.025) in layer IV of the contralateral barrel cortex. Levels were unchanged elsewhere. Thus, there is a sequential, vibrissectomy-induced, transsynaptic induction of GAP-43 mRNA in specific barrel system neural relays associated with plastic change in lamina IV of the barrel cortex.

499.8

POST CRITICAL PERIOD PHENOMENA IN CORTICAL BARREL FIELDS IN ORGANOTYPIC SLICE CULTURES FROM RAT SOMATOSENSORY CORTEX. M. Behan[1], A. Kroker, and J. Bolz; Friedrich-Miescher Labor der Max-Planck Gesellschaft, 7400 Tübingen, Germany. [1]Present address: Department of Comparative Biosciences, University of Wisconsin, Madison, WI 53706, USA.

During early postnatal development of the barrel field in rodent somatosensory cortex there is a critical period when alterations of the sensory periphery disturb barrel formation. However, peripheral manipulations after the critical period appear not to affect the barrel architecture. The mechanisms by which barrels are formed and maintained are, as yet, unknown. Although there is evidence that thalamocortical afferents play a role in shaping barrel fields, inputs from other subcortical and cortical sources may be equally important. We have begun to study barrel fields in slice cultures, where all of these inputs can be eliminated. First, we determined whether barrels could be maintained in the absence of afferent input, and secondly, in the absence of input from other cortical layers. Slice cultures of the somatosensory cortex were prepared from 5-8 day-old rats, by which time barrels are formed. Cytochrome oxidase histochemistry and Nissl staining were used to identify the presence of barrels in slice cultures. In sagittally cut slices, barrels were observed in cortical layer 4 after up to 10 days *in vitro* (DIV), the longest period examined. In tangentially cut cortical slices, after 1 DIV, barrels could usually be identified. However, after 2-4 DIV cells in the barrels reorganized, and cells from the barrel wall moved into the barrel hollow. After another 1-2 DIV, the remaining barrel septa disappeared and the cells were homogeneously distributed in the slice culture. Thus, intact connections within cortical columns appear to be necessary to sustain the functional architecture of barrel cortex *in vitro*. These results suggest that interactions between cortical layers may play a role in the termination of the critical period.

449.9

THE PRODUCTION OF CRYPTOTIA IN OPOSSUM PUPS FOLLOWING LESIONS OF THE TRIGEMINAL GANGLION. D.M. Feinberg* and B.L. Munger, Dept. of Neuroscience & Anatomy, Penn. State Univ., M.S. Hershey Med. Ctr., Hershey, PA 17033

During the course of studies on the impact of lesions of the trigeminal ganglion in opossum pups, we observed that the auricles on the side of the lesion were small and misshapen, i.e. evidence cryptotia. The auricle develops as a condensation of mesenchyme separated from the skull by a plate of stratified epithelium that is directly continuous with the meatal plug. This auricular plate is present at birth, progressively enlarges & undergoes keratinization in the center dividing the posterior surface of the auricle from the skin overlying the temporal bone. The separation of the auricular plate typically begins at 12-15 days of age. We created lesions of the trigeminal ganglion using a microcautery on pups attached to their mother at 1 day of age with halothane anesthesia. The condensing mesenchyme of the auricle and the auricular plate were consistently smaller and keratinization delayed on the side of the trigeminal lesions. By 21 days, grossly smaller auricles could be recognized in all experimental cases. We conclude that afferent nerves have a critical role in the differentiation of the auricular plate as other cutaneous appendages, and trigeminal lesions produce cryptotia that resembles its human counterpart. Supported in part by USPHS Research Grant NS 19462.

449.10

THALAMIC LESIONS AT BIRTH ALTER DENDRITIC MORPHOLOGY IN TRIGEMINAL NUCLEUS PRINCIPALIS (PrV). I.Z. Rana*, N.H. Hobart*, M.H. Cooper, N.L. Chiaia, R.W. Rhoades, & M.F. Jacquin. Dept. Anat. & Neurobiol., St. Louis University School of Med., St. Louis, MO 63104; Dept. of Anat., Medical College of Ohio, Toledo, OH 43699.

PrV cells undergo a dramatic postnatal regressive process (Hobart et al., Neurosci. Abstr. 15, '89). Dendritic #, length, area of influence, branching, and appendages are reduced to produce a simple, truncated, and polarized tree that is restricted to one "barrelette". We asked whether target destruction alters this process. Four newborn rats received electrocautery lesions of the right thalamus, therein "depopulating" PrV of 59±7% (mean ± SD) of its cells (Jacquin et al., Neurosci. Abstr. 16, '90), yet sparing primary afferent projection patterns (Chiaia et al., JCN 303, '91). In adulthood, standard Golgi staining and computer-assisted neuron tracing/morphometric methods were used to compare dendritic trees of 50 experimental (E, contralateral to lesion) and 50 control (C, ipsilateral) cells. Soma diameters did not differ (E: 15.5±3.9, C: 16.9±5.3 um), indicating that the E sample was not biased towards the small % of normally large PrV cells with widespread and heavily branched trees. Main effects (t-tests, $p < .05$) were significant increases in total dendritic length (E: 988±649, C: 774±599 um), # of dendritic branch points (E: 16.1±12.9, C: 9.8±7.6), and # of spines or swellings (E: 23.1± 35.6, C: 13.1±17.6). Important measures that did not differ were transverse area of influence (E: 22,498, C: 20,805 um2) and # of primary dendrites (E: 4.5, C: 4.4). These data suggest that near-normal patterning of afferent inputs is insufficient for normal dendritic development of PrV cells. Other factors, such as interactions among dendrites and retrograde signals from the target, may also play important roles. DE07662, DE07734, DE08941,

449.11

MECHANISMS UNDERLYING RAPID ENLARGEMENT OF SPARED-WHISKER PROJECTIONS AFTER NEONATAL INFRAORBITAL NERVE SECTION. N.H. Hobart*, T.A. Henderson, W.E. Renehan, N.A. Connors, & M.F. Jacquin. Anat. & Neurobiol., St. Louis Univ. Sch. Med., St. Louis, MO 63104; Dept. Med., Henry Ford Hospital, Detroit, MI 48202.

Waite & de Permentier (in press) have shown that the central projections of non-infraorbital whiskers occupy supranormal transverse areas in the rat brainstem after infraorbital injury at birth. We (Neurosci. Abstr. 16, '90) reported that such plasticity is not due to peripheral sprouting, regeneration errors, or preservation of an immature projection pattern. Anatomical and physiological tools were used to study the time course and mechanisms underlying this phenomenon. Cytochrome oxidase patches representing the 2 **supraorbital** whiskers in trigeminal subnucleus interpolaris were reliably larger than normal by postnatal day (P) 1.5 (1.15 ± .11 X control), but had not achieved adult-like disparity by P3 (1.81 ± .36 X control). To determine whether patch enlargement is due to relative changes in impulse activity produced by nerve section, tetrodotoxin (N=3) or bupivicaine (N=4) were applied from birth to block infraorbital nerve impulses during the first 5-7 Ps. As previously reported, infraorbital maps developed normally. Supraorbital patch areas were also unaffected (1.05 ± .28 X control). Thus, injury-induced patch enlargement does not reflect an activity-based competitive advantage conferred upon spared collaterals. To determine whether patch enlargement reflects collateral reorganization, intra-axonal recording and HRP staining methods were used to compare 10 control and 10 experimental spared-whisker primary afferents. Bouton or collateral numbers did not differ in interpolaris; however, experimental arbor areas were reliably larger ($p < .05$). Thus, patch enlargement reflects arbor expansion, but not collateral or terminal sprouting, in spared whisker collaterals. DE077734,DE07662.

449.12

DAILY WHISKER TRIMMING FROM BIRTH MIMICS NERVE INJURY-INDUCED RESPONSE ALTERATIONS IN TRIGEMINAL SECOND-ORDER CELLS. M.F. Jacquin & N.H. Hobart*. Anatomy & Neurobiology, St. Louis Univ. Sch. Med., St. Louis, MO 63104.

To assess experiential contributions to developing response profiles in subnucleus interpolaris, 343 single units were studied in 10 rats after 6-9 weeks of left mystacial whisker trimming. Data were compared to 385 control cells. Encounter rates and whisker maps were normal. However, receptive field (RF) size, character and higher-order inputs were abnormal (neonatal infraorbital nerve cut data also shown, N=478; from JCN 282:63, '89):

Significant Effects	Control	Nerve Cut	Deprived
% local circuit with > 1 whisker RF	3.4	41.0	35.3
% with split RF	0	14.2	4.9
% interdivisional RF: local circuit	0.5	5.1	4.0
" thalamic-project	18.0	45.1	33.3
" cerebellar-project	3.7	13.1	7.3
% unresponsive to mechanical stimuli	2.8	7.2	7.6
% with intermodality convergence	1.4	23.1	12.6
% with directional sensitivity	3.9	9.8	13.2
% with high velocity sensitivity	13.9	24.9	27.1
% responsive to cortical shocks	8.7	14.5	23.9

These data indicate that deprivation alters development of response profiles in some trigeminal brainstem cells. These changes mimic those induced by nerve section. Thus, deprivation may underly nerve injury-induced effects on trigeminal brainstem RF size and character. Inasmuch as deprived primary afferents had normal RFs (N=59) and maps, second-order RF changes likely reflect subtle alterations in brainstem circuits. Support: DE07734, DE07662.

449.13

THE MATURATION OF THE LATERAL SPINOTHALAMIC AND TRIGEMINOTHALAMIC TRACTS IN RATS. D.Y.Miya & G.A. Barr. Biopsychology Doctoral Program, Dept. of Psychology, Hunter College-CUNY, NY, NY 10021 and Dept. Developmental Psychobiology, New York State Psychiatric Institute, NY, NY 10032.

In order to identify the neurons of origin and to study the ontogeny of both the lateral spinothalamic tract (LSTT) and the trigeminothalamic tract, two retrograde labels, WGA-HRP (5%) or green fluorescent latex microspheres were used. Rat fetuses of 17, 18, 19 (FD-17, FD-18, FD-19) days of gestation were injected with either tracer into the ventral posterior lateral nucleus (VPL) or the ventrobasal complex of the thalamus unilaterally. All injections were made through the use of the suture landmarks of the skull and penetrated the uterine and amniotic membranes. After a 24 hour survival period, a caesarian section was performed and all animals were perfused intracardially. Last year at this meeting it was reported that spinal cord cells were labeled in the contralateral dorsal horn of postnatal day 1 and day 10 rat pups. In the FD-19 rat, both methods yielded labeled cells in the same pattern as postnatal rats with the exception that the neurons of the lateral superficial lamina (lamina III) were absent and only neurons of the contralateral ventromedial dorsal horn were labeled. The ipsilateral medial lemniscus and most lateral aspects of the contralateral principal sensory trigeminal nucleus were also labeled following VPL injections. Results in the younger animals so far demonstrate that both sensory systems, the trigeminothalamic and LSTT are early developing tracts that are present prenatally in the rat.

449.14

STEREOLOGICAL ANALYSIS OF SYNAPSE NUMBER IN MEDULLARY DORSAL HORN LAYERS I AND II AFTER INFRAORBITAL NERVE SECTION AT BIRTH. I. Golden, D.S. Zahm, & M.F. Jacquin. Dept. Anat. & Neurobiol., St. Louis Univ. Sch. Med., St. Louis MO 63104.

A large # of trigeminal (V) ganglion and brainstem cells die after neonatal infraorbital (IO) injury, producing shrinkage and permanant deafferentation of the V brainstem complex. Yet, the cytoarchitecture of layers I and II of the medullary dorsal horn is selectively preserved. As a first step in characterizing the anatomical reorganization subserving such "plasticity" in the dorsal horn, random systematic sampling and EM stereological methods were applied to layers I and II 90 days after cutting the left IO nerve at birth in 2 rats. To count synapses and terminals, as well as to compute a terminal areal fraction, the double dissector, profile counting, and point counting methods were used, respectively. In each case the # of IO nerve axons proximal to the point of neonatal injury were also determined.

	LEFT	RIGHT
	Case 1 2	Case 1 2
# of infraorbital axons (2 cases listed)	8,281, 9,737	36,236, -
# of synapses/100 um3	27.5, 28.9	22.6, 25.2
# of terminals/100 um2	36.0, 37.5	32.9, 28.3
# of terminals with dense core vesicles/100 um2	14.8, 15.4	13.5, 15.6
# of terminals with round vesicles/100 um2	18.1, 18.8	13.8, 10.8
# of terminals with flat vesicles/100 um2	2.6, 3.3	5.4, 1.5
terminal areal fraction (%)	14.9, 17.1	16.7. 14.9

These data suggest that at least normal #s of terminals and synapses exist in layers I and II, despite the fact that only 25% of IO axons survive axotomy at birth. The selective preservation of inputs to layers I and II may reflect a higher % survival of ganglion cells that normally project here, maintenance of immature projections, or sprouting. DE07734, DE07662, NS23805.

449.15

NF-L AND PERIPHERIN IMMUNOREACTIVITIES DEFINE DISTINCT CLASSES OF RAT SENSORY GANGLION CELLS. M.E.Goldstein, S.B. House* and H. Gainer. Lab of Neurochemistry, NINDS, NIH, Bethesda, MD 20892.

We have used double immunofluorescence techniques with antibodies against NF-L and peripherin to identify peripherin positive, NF-L positive, and peripherin/NF-L coexistent subpopulations in adult and neonatal (PN2) DRG *in vivo*. In addition, we have determined which of the subpopulations contain the neuropeptides, substance P and calcitonin gene-related peptide (CGRP). Finally, we have determined which of these subpopulations are stably maintained in tissue culture. These studies have revealed three distinct subpopulations of neurons in rat dorsal root ganglia (DRG). In the adult rat, 46% of the DRG neurons were small and peripherin positive (NF-L negative), and 48% were large and NF-L positive (peripherin negative). About 6% were both peripherin and NF-L positive. All of the DRG neurons reacted with antibodies to NF-M and nonphosphorylation-dependent or phosphorylation-independent antibodies to NF-H. The neuropeptides were predominantly found in the small peripherin positive cell population. Eighty-seven percent of the peripherin positive small cell population contained substance P immunoreactivity, while 43% of this cell population contained CGRP. In contrast, only 18-24% of the NF-L positive, large cell population contained neuropeptides and these were primarily in a smaller sized subpopulation. Similar patterns of antigen representation were observed in neonatal (PN2) DRG cell populations. Tissue cultures of sensory ganglion cells from PN2 DRG, in serum free medium, stably maintained exclusively peripherin positive neurons, with about five percent of these containing coexistent NF-L immunoreactivity. Very high levels of neuropeptide gene expression were exhibited by these postnatal neurons in culture. In future experiments we hope to confirm these results using *in situ* hybridization histochemistry and to examine the effect of growth factors on the phenotypes of DRG neurons in culture.

449.16

THE STRUCTURAL, FUNCTIONAL AND NEUROCHEMICAL DEVELOPMENT OF SOME OF THE CUTANEOUS RECEPTORS AND THEIR AFFERENT FIBRES IN FETAL SHEEP. S.Rees, I.Nitsos* and J. Rawson*. Dept. of Physiology, Monash Univ., Clayton, Victoria, 3168, Australia.

The age at which a fetus is capable of receiving and experiencing sensations delivered by noxious or tactile stimuli remains uncertain. In this study we have used electrophysiological and anatomical techniques to examine the development of some of the cutaneous receptors and their afferent fibres in fetal sheep (n=28) from 83 to 143 days of gestation (term=146). At each age, recordings were made in the dorsal root ganglia to assess the response properties of these afferents to brushing, touching and pinching the skin of the hindlimb. At the conclusion of the experiment, the fetuses were perfused and the tissue taken from the skin, dorsal root ganglia and spinal cord and stained for immunoreactivity for substance P (SP) and calcitonin-gene related peptide (CGRP). Samples of skin were also processed for silver staining to demonstrate nerve fibres. The earliest responses, recorded at 87 days resulted from activation of high threshold, rapidly adapting mechanoreceptor afferents. By 97 days, afferents responded to all of the above stimuli and had well defined peripheral receptive fields. Immunoreactivity for SP and CGRP was detected in the dorsal horn as early as 83 days. Wool follicle innervation and free nerve endings in the skin were present in silver-stained sections at 87 days. With increasing gestational age, cutaneous innervation increased in density and complexity and the responses to stimulation were more readily elicited. These results suggest that cutaneous receptors are functional and afferent input is reaching the spinal cord as early as sixty percent of the way through gestation in the sheep.

449.17

Fos-Protein-Like-Immunoreactivity in the Suprachiasmatic Nucleus of Developing Rats. Myra P. Joyce and Gordon A. Barr. Dept. of Psychology, Hunter College-CUNY, New York, NY 10021.

Visual system projections from the retina (RHT) and the lateral geniculate nucleus terminate in the ventrolateral portion of the suprachiasmatic nucleus (SCN) and entrain the circadian activity of SCN neurons. In the 3 day old rat pup synapses arising from the RHT are not observed in the SCN, but by 4 days of age, RHT synaptic contacts are present. By 10 days of age, the number of SCN synapses is similar to that of the adult. In the present study we examined the appearance of Fos in the SCN of rats, 3 to 35 days of age. The avidin-biotin-HRP method was used with the primary antibody, rabbit-anti-Fos (Oncogene Sciences) diluted 1:10,000. In the ventrolateral SCN, developmental changes in activity, as indicated by the presence of Fos-LI, were observed that coincide with the synaptogenesis of the visual system projections. Three day old pups showed essentially no Fos-positive nuclei in the ventrolateral SCN. By 4 days of age, a few Fos-positive nuclei were observed and the number of labeled nuclei increased as the animal matured. At all ages, large numbers of Fos-positive nuclei were present in the dorsomedial SCN and this number also increased as the animal matured. Neither naturally occurring eye opening at 14 days of age nor surgical eye opening at eight days of age changed the activity of SCN neurons significantly. The results suggest that Fos appearance coincides with synaptogenesis in this system.

DEVELOPMENT OF CEREBRAL CORTEX AND LIMBIC SYSTEM II

450.1

PARALLEL DEVELOPMENTAL CHANGES IN HIPPOCAMPAL PKC AND ELECTROPHYSIOLOGICAL PROPERTIES OF CA1 PYRAMIDAL CELLS. J.V. Sanchez-Andres, D.L. Alkon, J.L. Olds. Neural Systems Section, NINDS, NIH, Bethesda MD 20892.

Electrophysiological and autoradiographic experiments were performed on rabbit littermates in order to elucidate the effect of activated protein kinase C (PKC) on hippocampal physiology in ontogeny. Brains were rapidly removed from littermates (Day 0 to Day 15 post partum) and bisected. Half of the brain was used for electrophysiology. The other half of the brain was processed for quantitative 3H-PDBU autoradiography.

CA1 impulse patterns recorded from littermates prior to the day when neonates opened the eyes (NEO) showed marked adaptation and few impulses (1-3) in response to a depolarizing pulse. After NEO, all cells showed an adult firing pattern, i.e. with much less adaptation, as has been extensively characterized (Disterhoft et al., 1986).

The pattern of PKC distribution seen before NEO was qualitatively different from that seen either after that point in development or in adults. This was manifested by extremely dense bands of 3H-PDBU binding on either side of the stratum pyramidale both in CA1 and in CA3. These bands dramatically delineated the cell body layer and were localized to the most proximate apical and basilar dendritic zones. After NEO, this pattern shifted to one reminiscent of that seen in an adult classically conditioned animal 24 h into retention (i.e. stratum oriens and pyramidale showing together the greatest binding).

Taken together, the above data support the hypothesis that PKC plays an important role in controlling the excitability of CA1 pyramidal cells both in development and in learning. Thus, this enzyme may control functional connectivity both intrinsic and extrinsic to the hippocampus during and after behavioral and developmental modification.

450.2

CALCIUM CURRENTS IN FETAL HIPPOCAMPAL NEURONS IN PRIMARY CULTURE FROM THE TRISOMY 16 MOUSE, A MODEL FOR DOWN SYNDROME. **Z.Galdzicki, E.J.Coan & S.I.Rapoport. LNS,NIA,NIH,Bethesda, MD 20892**

The entry of calcium through voltage-gated membrane channels plays a crucial role in a variety of hippocampal neuronal activity. The trisomy 16 mouse is considered to be a model of human trisomy 21 (Down Syndrome). It has been reported that both low voltage activated (LVA) and high voltage activated (HVA) calcium currents can be detected in embryonic hippocampal neurons in primary culture. Hippocampal neurons were cultured from fetal trisomy 16 and normal mice at gestation day 14-16 using standard techniques. Whole cell inward currents were recorded from the somatic region of 10-21 day old cells, with 120 mM choline chloride, 20 mM 4AP, 10 mM $CaCl_2$, and 10 mM TEA in the bath solution and 130mM CsCl, 10 mM BAPTA, and 20 mM TEA in the pipette solution. Currents were evoked with 10 mV, 150 ms steps, from holding potentials of -90, -40, and -60 mV. Both HVA and LVA calcium currents were identified. In trisomy neurons the peak amplitude of the inward current reached a maximum at a membrane potential (Vm) of 6.7 mV (n = 12) for currents evoked from a holding potential of -40 mV and at 0.4 mV (n = 19) for currents evoked from a holding potential of -90 mV. The latter currents showed a statistically significant hyperpolarizing shift for Vm of 7.5 mV compared to control neurons. During the recording time the I-V response of the trisomy 16 cells showed a significant shift of 7.1 mV (n = 15) in a depolarizing direction which was not observed in control neurons. These observations suggest that calcium currents are abnormal in trisomy 16 fetal hippocampal neurons.

450.3

ACTION POTENTIALS IN CULTURED FETAL HIPPOCAMPAL NEURONS FROM MOUSE TRISOMY 16, A MODEL OF DOWN SYNDROME. E.J.Coan, Z.Galdzicki & S.I. Rapoport. LNS, NIA, NIH, Bethesda, MD 20892.

The trisomy 16 (Ts16) mouse is considered to be a model of human trisomy 21 (Ts21) (Down syndrome). This laboratory has reported that action potentials in Ts16 and Ts21 cultured dorsal root ganglion (DRG) neurons are different from those in control neurons. The aim of these experiments was to investigate action potentials in Ts16 hippocampal neurons. Primary cultures of hippocampal neurons were prepared from Ts16 embryos and littermate controls at gestation day 14-16, whole cell patch clamp recordings were made from 2-4 week old neurons. Extracellular medium included 140 mM NaCl and 5 mM KCl, intracellular solution 5 mM NaCl and 130 mM KCl. 5 mV 10 ms or 50 pA 1 s pulses were used to measure passive membrane properties. Action potentials were evoked by 0.1 to 0.8 nA, 5 ms depolarizing pulses. All measurements were done at -60 mV. Data were taken from 10 control and 14 Ts16 cells. Resting membrane potentials were -49 mV and -42 mV for control and Ts16 neurons respectively. In control cells the mean action potential amplitude ranged from 87 mV (0.1 nA pulse) to 114 mV (0.8 nA pulse), the mean time to peak amplitude from 5.3 to 3.9 ms, the mean width from 3.5 to 3.6 ms, the mean depolarization rate from 100 to 117 V/s and the mean repolarization rate from -31 to -54 V/s. None of the mean passive or active membrane properties measured in Ts16 neurons was significantly different from control values. This result is different to that reported in Ts16 and Ts21 DRG neurons, and might be explained by differences in maturation and/or the presence of growth factors.

450.4

MORPHOLOGICAL AND PHYSIOLOGICAL DEVELOPMENT OF NEURONS AND AFFERENTS IN ORGANOTYPIC SLICE CULTURES OF PARIETAL CORTEX. C.M. Annis, R.T. Robertson, A. Agmon, and D.K. O'Dowd Depts. of Anatomy and Neurobiology & Developmental and Cell Biology UC Irvine, Irvine, CA 92717

Previous studies have demonstrated extensive changes in the rodent neocortex at both the anatomical and electrophysiological levels during early postnatal development. We are utilizing an organotypic slice culture system (Gähwiler, 1981) to facilitate examination of cellular mechanisms that underlie postnatal development of cortical neurons and their afferent connections. Transverse slices of rat parietal cortex or oblique thalamocortical slices from mouse brain (Agmon and Connors, 1991) were taken from P0 animals and maintained in defined medium (Annis et al., 1990). The whole cell recording technique was used to examine cortical neurons between 1 day and 3 weeks in culture. Inclusion of Lucifer Yellow in the recording electrode permitted correlation of electrophysiological and morphological properties of individual cells.

Electrophysiological recordings were obtained from three morphologically distinct classes of neurons in cultures from rat parietal cortex: pyramidal, multipolar and bipolar. During the first five days in culture the majority of intracellularly labeled neurons exhibited cell bodies with short unbranched processes. By 8-14 days most of the cells encountered had much longer processes with the pyramidal cells exhibiting basal dendrites, elongated apical dendrites and axons with several collaterals. Physiological experiments revealed an increase in the action potential amplitude, decrease in the duration and an increase in the probability of eliciting multiple spikes in response to depolarizing current pulse, as a function of age in culture. The role of afferents in cortical neuron development is being studied in mouse thalamocortical slices. Morphological data, using DiI, demonstrate growth of thalamocortical projections in culture. These two slice preparations and the baseline information on their development in culture provide a unique system for identifying the factors that may influence cortical development. This work was supported by grants from Alzheimers Fnd. 90-082, NS25674 (RTR) and NS27501 (DOD).

450.5

CHOLINERGIC RESPONSES OF DEVELOPING RAT NEOCORTICAL CELLS IN TISSUE SLICES, A LAMINAR STUDY. Finlayson, P.G. and Cynader, M.S., Dept. of Ophthalmology, Univ. of British Columbia, Vancouver, B.C. Canada, V5Z-3N9

Enhanced plasticity of neonatal systems are paralleled by developmental changes in the cortical laminar distribution of many receptors, as observed in autoradiographic studies. Muscarinic cholinergic receptors, M1 and M2, redistribute in the developing kitten cortex, and are transiently high in layer IV at young ages but predominantly concentrated in layers I, II and III in adult cats. Nicotine receptors exhibit a transient density in layer IV of rat pup visual and auditory cortices during the second postnatal week. In this study, physiological correlates of receptor redistributions during development were examined. Responses of neurons in layers 2/3, 4, and 5 in the visual areas of rat neocortex tissue slices from P9 to P25 day rat pups were evaluated. Membrane properties, spike properties, afterhyperpolarizations and responses to electrical stimulation of either supra or infragranular layers were recorded. One or two cells per slice were also labelled with Lucifer yellow and imaged using a confocal microscope to determine the type, size and location of neurons recorded. Responses to carbachol and acetylcholine, applied by pressure ejection, were depolarizations, decreases in after-hyperpolarizations, and changes in input resistance and evoked psp's. Developmental changes in the laminar distribution of these response were compared to changes in binding distributions of pirenzipine, oxotremorine, and nicotine in developing rat neocortex.

450.6

DEVELOPMENT OF CHOLINERGIC AND SEROTONERGIC RECEPTORS IN THE SOMATOSENSORY CORTEX OF THE RAT. M.I. Davila-Garcia, A.K. Chan*, P. Tran* and F.M. Leslie. Dept. of Pharmacology, University of California, Irvine, College of Medicine, Irvine, CA 92717.

There is now considerable evidence that neurotransmitters may regulate many developmental processes. In particular, cholinergic and serotonergic systems have been found to appear early in the development of somatosensory cortex, and have been implicated in control of morphogenesis. The receptors through which these neurotransmitters may act are currently unknown. In the present study we have used radioligand binding and autoradiography to examine the distribution of both cholinergic and serotonergic binding sites at postnatal day 10 (P10), and have compared this with the adult binding pattern. Radioligands used to label muscarinic receptors included [^{3}H]pirenzipine for M1 sites, [^{3}H]AFDX-384 for M2 sites and [^{3}H]NMS for non-M1/non-M2 sites, while [^{125}]α-BTX was used to label a subpopulation of nicotinic binding sites. For the serotonergic system, we have examined the distribution of [^{3}H]8-OH-DPAT-labeled S1A binding sites. While muscarinic M1 and non-M1/non-M2 binding sites were found to exhibit a distribution in the neonate that is similar to that in the adult, with the highest density of sites concentrated in superficial layers, M2 sites at P10 are patchy, bilaminar and arrayed in dense columns. [^{125}I]α-BTX sites in neonatal somatosensory cortex are distributed in radially oriented columns that have various thin, dense bands in the developing laminae, in contrast to the distribution in the adult which is mostly concentrated in lower layers. Similarly, the distribution of S1A binding sites at P10 is found to be patchy in this area and exhibits a columnar arrangement which is distinct from that of the adult. These data suggest that cholinergic and serotonergic receptor ontogeny within somatosensory cortex is complex, and that some receptor types exhibit developmental changes consistent with a role in the modulation of cortical architecture and function. *Supported by USPHS grant DC 00450.*

450.7

DEVELOPMENT OF ACHE-POSITIVE FIBER CONNECTIONS BETWEEN BASAL FOREBRAIN AND CEREBRAL CORTEX IN ORGANOTYPIC TISSUE SLICE CULTURES P.G. Distler* & R.T. Robertson. Dept. of Anatomy and Neurobiology, Univ. of Calif., Irvine, CA 92717

Projections of the cholinergic basal forebrain nuclei into the cerebral cortex are part of a complex neuronal network of the brain especially involved in learning and memory. To study the development of these projections *in vitro*, slices of basal forebrain and lateral cortex tissue from early postnatal rats were co-cultured for up to 4 weeks using the roller tube technique (Gähwiler, 1981). During this time period the development of the cholinergic innervation of the cortex by basal forebrain neurons was studied by means of AChE-histochemistry and ChAT-immunostaining. Tissue cultures were maintained either with serum-based medium or with chemically defined medium (Annis et al., 1990), in some cases supplemented with nerve growth factor (NGF).

Basal forebrain explants show numerous outgrowing AChE-positive neurites. Although AChE-positive neurons are observed in cerebral cortex, these neurons never gave rise to AChE-positive axons. ChAT-immunostaining reveals positively labelled neurons only in the basal forebrain tissue. Within one week of culturing, AChE-stained fiber processes are visible growing into the adjacent cortex slice. Many labeled axons travel tangentially beneath the cerebral cortex and then turn to grow into the cortex, in some cases extending to the pial surface. Ramifications are visible in the subplate region as well as in the more superficial cortical layers. Cortex slices placed with the pial surface to the basal forebrain reveal a slightly different innervation pattern; AChE-positive axons enter the cortical tissue through the pial surface and show dense ramifications within the superficial layers. Co-cultures with the different culture media exhibit similar innervation patterns of the cortex. Control experiments with co-cultures of basal forebrain and cerebellum slices show no AChE-positive fiber ingrowth into the cerebellum tissue and provide evidence for the specificity of the basal forebrain-cortical connections *in vitro*.

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450.8

DEVELOPMENTAL ANATOMY OF BASAL FOREBRAIN CHOLINERGIC PROJECTIONS IN THE RAT. G.R. Stewart and B. Stanfield, Lab. of Neurophysiology, NIMH, NIH Animal Center, Poolesville, MD 20837.

The basal forebrain cholinergic (BFC) system provides the major source of cholinergic innervation to the neocortex, hippocampus, and amygdala. In the present study, the postnatal development of these projections was examined following injections of the retrograde tracers fast blue and diamidino yellow into various cortical and subcortical regions in the rat (Sprague-Dawley). By postnatal day 5 (P5), the distribution and proportion of labeled neurons within the various subsectors of the basal forebrain (medial septum, diagonal band, etc) that project to a given target are similar to that seen in adult rats. Non-overlapping cortical injections of both tracers into the same hemisphere or bilaterally results in a low incidence (0-5%) of double labeled cells as has also been noted in adults. These observations suggest that BFC projections are established with the appropriate target early in postnatal development, and display even at this stage, an adult-like degree of topographic specificity.

The development of cholinergic innervation within BFC targets was studied quantitatively using densitometric measurement of acetylcholinesterase (AChE) histochemistry and qualitatively with cholineacetyltransferase (ChAT) immunohistochemistry. Within most cholinoceptive regions, there is a rapid increase in staining for both enzymes between P2 and P20 with adult levels present by P40. One notable exception is the delayed development of staining within the nucleus of the lateral olfactory tract (NLOT). In adults, the NLOT has one of the highest concentrations of ChAT and AChE activity in the forebrain, but through P9 there is virtually no staining of the nucleus even though neighboring regions are well-stained. In addition, placement of the carbocyanine dye, DiI, into the basal forebrain prior to P9, fails to label fibers within the NLOT, but between P10 and P20 there is a rapid invasion of DiI-positive fibers in conjunction with increased staining for ChAT and AChE. It is unclear why the cholinergic innervation of such a prominent, and proximal, target of the BFC system lags behind all other regions during this critical phase of development.

450.9

EARLY FETAL DEVELOPMENT OF CALCIUM-BINDING PROTEINS, PEPTIDES, DARPP-32 AND MONOAMINERGIC INNERVATION IN THE HIPPOCAMPAL REGION OF THE RHESUS MONKEY.

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Immunocytochemical detection of calbindin (CaBP), parvalbumin, neurotensin (NT), somatostatin (SST), DARPP-32 as well as tyrosine hydroxylase (TH), dopamine- -hydroxylase (DBH) and serotonin (5-HT) was carried out in 5 rhesus monkey fetuses from E64 to birth (gestation 165 days). At E64, the already well defined entorhinal cortex displayed all aforementioned types of innervation but in different density and laminar and topographical distribution: CaBP-immunoreactive (IR) plexus in layer I and numerous IR cells in all layers and subplate (SP) contrasted with the few parvalbumin-IR neurons in layer 6 and DARPP-32-IR perikarya in layers 2-3; a dense SST-IR plexus was present in layers 1 and 5-6 as well as IR-neurons in layer 6 and SP. NT innervation mainly developed from E83 on, in layers 1 and 5-6. Aminergic afferents predominated in the lateral entorhinal cortex, 5-HT afferents being denser than TH-IR ones. At E64, the hippocampal formation was much less differentiated; the relatively low density of aminergic afferents contrasted with an area-specific expression of the other markers: DARPP-32 -IR neurons, NT-IR neurons and terminals in both the ventral and dorsal subiculum and cingulate cortex; CaBP-IR neurons and terminals in Ammon's horn, dentate g., dorsal sub. and cingulate cx; dense SST innervation restricted to the ventral hippocampal formation. The density of neuroactive substances in the main association and output areas of the hippocampal region (subiculum and entorhinal cortex) in early fetal life in primates, is of special interest in view of the hypothesis that some forms of schizophrenia could be related to developmental abnormalities of this region.

450.10

DOPAMINE BETA-HYDROXYLASE (DBH) AND TYROSINE HYDROXYLASE (TH) IMMUNOREACTIVITY IN HIPPOCAMPUS-LOCUS COERULEUS CO-CULTURES.

A.M. Moudy, D.D. Kunkel, C.A. Robbins*, M.E. Gross* and P.A. Schwartzkroin. Dept. of Neurological Surgery, Univ. of Washington, Seattle, WA 98195.

Co-cultures of hippocampus (HC) and locus coeruleus (LC) were studied immunocytochemically to examine noradrenergic innervation of hippocampus. Vibratome slices (350 μm) of P4 rat HC and E17-P1 LC were co-cultured on glass coverslips for 2-6 weeks. TH and DBH positivities were then evaluated in fibers and cells. Large, multipolar cells were labelled in LC, and an occasional cell appeared near or within HC and in fiber tracts. DBH-positive cells tended to concentrate near the edges of LC tissue, apparently having migrated out from more central regions. LC slices cultured alone showed little sign of fiber outgrowth in any direction. TH- and DBH-positive fiber tracts were traced from LC to HC. Cresyl violet counterstaining, used to distinguish hippocampal cell layers, revealed that immunopositive fibers entered the HC at the hilus and near CA3. Beaded fibers formed extensive collateral branches upon reaching the HC. Light microscopy suggests that DBH-positive fiber growth is densest at or near the pyramidal cell layer in CA3b and CA3c and the infragranular region of the dentate hilus. Electron microscopic studies are currently underway to determine the postsynaptic targets of these fibers.

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450.11

IMMUNOCYTOCHEMICAL CHARACTERIZATION OF PUTATIVE NEUROTRANSMITTERS IN RAT HIPPOCAMPAL SLICE CULTURES.

D.D. Kunkel, D.L. Schmiege, A.T. Malouf and P.A. Schwartzkroin. Dept. of Neurological Surgery, Univ. of Washington, Seattle, WA 98195.

Hippocampal slice cultures retain their basic structural organization when "grown" by the roller-tube technique. Typical neuronal morphology and much of the intrinsic connectivity is maintained. However, since extrinsic afferents are lost in cultured slices, one would expect at least subtle re-organization of local circuitry. We have been particularly interested in local inhibitory function and structure, and therefore initiated a morphological study of two major putative inhibitory neurotransmitter systems - gamma-aminobutyric acid (GABA) and somatostatin (SRIF) - using immunocytochemical staining techniques and electron microscopy. GABA immunoreactivity was seen in numerous somata distributed throughout different strata of the slice cultures. GABA-positive cells were often clustered near major pyramidal and granule cell layers. A very extensive GABA immunoreactive axonal plexus was seen; a dense positive fiber arborization surrounded principal cell somata, and numerous positive terminals were found making synaptic contacts with pyramidal and granule cells. SRIF immunoreactivity was observed mainly in cell populations localized in stratum oriens and within the hilar region. Numerous SRIF-positive axonal fibers extended throughout all regions of the culture, with synaptic connections found on principal cell somata and dendrites. These characteristics of the GABA and SRIF systems in culture were grossly similar to GABA and SRIF patterns previously defined in rat brain, but differed in a number of important aspects which require further elucidation.

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450.12

INTRACELLULAR INJECTION OF TWO DYES INTO DIFFERENT NEURONS IN THE RAT HIPPOCAMPAL SLICE PREPARATION: DUAL RECOGNITION FOR LIGHT AND ELECTRON MICROSCOPY.

D.L. Schmiege, D.D. Kunkel, H.E. Scharfman and P.A. Schwartzkroin. Dept. of Neurological Surgery, Univ. of Washington, Seattle, WA 98195.

Intracellular recordings and labeling techniques have become extremely important in the physiological and morphological identification of neurons in the central nervous system. Intracellular dyes, such as biocytin/Neurobiotin (BC/NB) and Lucifer Yellow (LY) have proven useful for morphological analysis of neurons at the light (LM) and electron microscopic (EM) levels. We now report the physiological and morphological identification of two different hippocampal neurons in the same slice with electrodes containing two different intracellular markers - LY in one pipette and biocytin (or neurobiotin) in the other. BC/NB was injected with positive current, and the filled neuron was recognized using avidin-HRP; LY was injected with hyperpolarizing current and the labeled cell identified with a LY antibody (a generous gift from Molecular Probes), followed by a secondary IgG-HRP. HRP histochemical processing then proceeded with different chromagens (e.g., diaminobenzedine (DAB), DAB/nickel, benzenedihydrochloride (BDHC), chloronapthol, or carbazole) associated with the LY and BC/NB label. Cells could be easily distinguished, and processes clearly visualized by the different colored chromagens. We have also successfully differentiated labeled neurons at the EM level, using different electron-dense markers (e.g., DAB, BDHC, ferritin, and gold) associated with the LY and biocytin labels. Simultaneous use of these intracellular dyes and recognition markers allows for a more detailed examination of cell-to-cell circuitry in the hippocampus.

Supported by NIH grants NS18895 and NS20482.

450.13

ANATOMICAL STUDIES OF CA3 HIPPOCAMPAL NEURONS AND NETWORKS DURING POSTNATAL DEVELOPMENT.

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Age-dependent alterations in local circuit collaterals from individual CA3 hippocampal pyramidal cells were examined. These results were related to CA3 neuronal density at each age.

Individual CA3 hippocampal neurons from postnatal day (PND) 4 to 70 rats were characterized physiologically in slice preparations. After labelling with biocytin, the morphology of the cells was quantified by a computer-assisted neuron reconstruction system. The perikaryon number was also assessed in Schiff stained sections by confocal microscopy.

An extensive pyramidal cell axonal arborization occurs during the second postnatal week, concomitant with a transient period of hippocampal hyperexcitability. Total arbors were about three times longer than those observed in mature neurons and about thirty times longer than those observed from rats under one week of age. Density of varicosities along the axons was similar between week two and adulthood.

Qualitatively, CA3 cell number per 500um slice decreases significantly from PND 5 to PND 50. Quantitative estimations are in progress.

Clearly, individual cells from week two make many more synapses per 500um slice than their adult counterparts. Whether the overabundance in total varicosity number translates to a potential excess of synapses onto neighboring neurons is currently under study.

450.14

STAINING FOR ZINC REVEALS COLUMNAR PATCHES IN THE HIPPOCAMPUS OF THE NEWBORN RAT.

C.J. Frederickson, G.Danscher, K.J. Cravens, L. Slomianka, and L.B. Sylvan, Laboratory for Neurobiology, UT-Dallas, Box 688 Richardson TX 75083

In the striatum of the newborn rat, staining for zinc-containing axonal boutons is an early marker that distinguishes the patches from the matrix. (Dev. Brain Res., 45 (1989) 155). We now report that staining for zinc-containing axonal boutons also reveals a patchy, "columnar" pattern of organization in the hippocampus of the newborn rat.

Pup brains were stained for zinc-containing boutons by the selenium method. Na2SeO3 was injected i.p. 1 hr before sacrifice, then brains were removed, cut frozen, and developed by silver-enhancement.

In newborns (P0), the staining in hippocampal zone s. oriens was organized into distinct bands or patches, each about 50 - 75 um wide, alternating with unstained patches of comparable width. Often the patches continued from s. oriens through s. pyramidale and into the proximal half of s. radiatum, producing radially-oriented bands, or columns, of stain oriented perpendicular to s. pyramidale. So far, the patches have been observed only in CA1 of the mid-body of the hippocampus. In development, they become indistinct by day P2 - P3, and virtually invisible by P5, when uniform staining of zinc-containing boutons fills the neuropil.

The possibility that columnar units endure in some form within the internal circuitry of the adult hippocampus is raised. Supported by MH42798 to CJF.

450.15

DEVELOPMENT OF DIAPHORASE CONTAINING NEURONS IN THE AMYGDALA, HIPPOCAMPUS AND ENTORHINAL CORTEX IN HUMANS. S.B. Schueler, H.K. Le*, E.J. Mufson, and J.H. Kordower, Dept of Anatomy and Cell Biology, Univ. Illinois Sch. Med. Department of Neurological Sciences, Rush Presbyterian Medical Center, Chicago Ill. 60612 USA.

As part of our continuing comparison between normal human developmental patterns and changes seen in neurodegenerative disease, we examined the distribution of diaphorase (NADPH-D)-containing neurons within the medial temporal lobe of the embryonic human brains at gestational (E) points 10-26 weeks. No NADPH-D-containing profiles appeared in the medial temporal lobe prior to week E19. At E19, faint NADPH-D staining was seen within the central nucleus of the amygdala. Staining for Nissl substance and acetylcholinesterase (AChE) at E19 revealed an immature, but existing, cytoarchitecture suggesting that neurons which normally synthesize NADPH-D are present but are not expressing the enzyme. At E21, the accessory basal and lateral basal amygdaloid nuclei contained numerous NADPH-D-positive neurons and a dense neuropil. The lateral amygdaloid nucleus was moderately labeled. The entorhinal cortex displayed a trilaminar pattern with darkly labeled cell islands in layers I-II, a dense neuropil in II-III and V-VI. In the hippocampus, a continuous band of NADPH-D was seen from CA1-CA3. CA4 contained only light NADPH-D staining. NADPH-D-stained profiles were also seen in the dentate granule cell layer. Clouds of NADPH-D stained neuropil were seen in layer II of the subiculum. At E24-26, NADPH staining within the medial temporal lobe resembled the adult. These data will be compared to potential morphological changes seen in normal aging and disease.

450.16

AXONAL STRATA AND FIBRE SYSTEMS IN THE FRONTAL LOBE OF THE HUMAN FETAL AND INFANT BRAIN. I. Kostović, H.B.M. Uylings, C.G. Van Eden and M. Judaš. Croatian Institute for Brain Research, Zagreb, Yugoslavia and The Netherlands Institute for Brain Research, Amsterdam, The Netherlands.

We have analyzed arrangement and sequence of growth of fibre systems in the frontal lobe of postmortem human fetuses and infants (10 weeks of gestation - 1 year) using Di-I, acetylcholinesterase (AChE) and silver techniques. The correlated analysis of Di-I, AChE and silver staining of the fetal brains revealed the stratified, discrete distribution of axons within the cerebral wall. The earliest fiber system demonstrable occupies the outermost position within the white matter and forms external capsule radiation. Thalamocortical radiation and efferent cortical fibres occupy intermediate stratum, while the deepest, subventricular stratum contains growing callosal fibres (in fetuses older than 11 weeks). Cortico-cortical association fibres (including U-fibres) grow in the superficial stratum situated between the subplate zone and external capsule during the perinatal and early postnatal period. The growing axons from all strata penetrate the subplate zone forming a transient plexiform compartment. In conclusion, afferent fibres form axonal strata as a result of the laminar sequential growth. The development of cortico-cortical fibres correlates with the perinatal development of cerebral convolutions.

450.17

NOVEL **ANTENNAPEDIA**-CLASS HOMEOBOX GENES ISOLATED FROM HUMAN 11 WK FETAL BRAIN LIBRARY. J.F. Leckman, X. Lin*, A. Swaroop*, M.T. Murtha*, F.H. Ruddle*. Child Study Ctr., Depart. of Biol., Psychiatry, and Pediatrics, Yale University, New Haven, CT 06510 and W.K. Kellogg Eye Ctr., Depart. Ophthalmol. and Human Genetics, Univ. Michigan, Ann Arbor, MI 48105.

Three novel **Antp.**-class homeobox genes have been isolated from a human 11 wk. fetal brain cDNA library. PCR with two sets of oligonucleotide primers (specific for highly conserved regions of the **Antp.**-class homeobox) was used to amplify portions of homeobox genes present in the fetal brain library. The products were cloned into a modified **pGem** vector and transformed into DH5a cells. Sequencing 100 clones (spanning 77 to 81 bases between primers) identified 11 unique **Antp.**-class genes. The base sequence of one of the novel HOX genes, HOXBA, showed 65% homology with HOX1.1, 89% homology with the CHOX7 gene (isolated from fetal chicken), and 95% homology with the MMOXA gene (isolated from fetal mouse telencephalon). Based on the origin of MMOXA, it appears that HOXBA is among the first **Antp.**-class homeobox genes identified in the developing human telencephalon.

VISUAL SYSTEM: SUBCORTICAL PATHWAYS

451.1

MOLECULAR CLONING OF A TYPE I KERATIN FROM GOLDFISH OPTIC NERVE:INCREASED KERATIN EXPRESSION DURING REGENERATION. R. Druger*, E. Levine*, P. Jones*, N. Schechter and J. McCaughran. Depts. of Biochemistry and Psychiatry, SUNY Stony Brook, Stony Brook, NY 11794.

The goldfish visual pathway displays continuous growth, development, and plasticity throughout life. Furthermore, a remarkable capacity for functional regeneration occurs after optic nerve injury. This laboratory is interested in intermediate filament (IF) proteins and their structural relationship to cellular growth and development.

Glial cells in this pathway express a type II keratin, not the expected GFAP. The presence of a type II keratin predicts the expression of a type I keratin partner. Using a PCR protocol, core regions from type I keratins were amplified from a goldfish optic nerve cDNA library. DNA fragments from this core region were then used to rescreen the cDNA library to isolate larger clones. The largest cDNA clone was translated, compared to known IFs, and classified as a type I keratin. Northern analysis revealed an mRNA of 1.6 kb that is abundantly expressed in the optic nerve. In situ hybridization on regenerating optic nerve shows that both the type I and type II keratins have increased mRNA levels throughout the optic nerve 10 days after nerve crush.

Keratins are known to be expressed in mammalian CNS only in early development. Thus, these IF proteins may have structural attributes which support the growth characteristics of this pathway and therefore may be linked to the capacity for growth in this system. (supported by NIH grant EY05212 to NS)

451.2

FIBER RE-ORGANIZATION IN THE MAMMALIAN OPTIC CHIASM: *DE NOVO* ESTABLISHMENT OF THE DORSO-VENTRAL RETINAL AXIS. B.E. Reese, G.E.Baker*, G. Tassinari* and C.A. Marzi*. Neuroscience Research Institute, University of California at Santa Barbara, U.S.A., Department of Human Anatomy, University of Oxford, U.K. and Istituto di Fisiologia Umana, Università di Verona, Italy.

Optic axons undergo a transformation in their fiber ordering within the chiasmatic region to become segregated by type within the optic tracts. In the tract, these segregated axon classes are themselves roughly retinotopically ordered, particularly with respect to the dorso-ventral retinal axis. The present study has examined the emergence of this retinotopy within the chiasmatic region of adult ferrets and cats. Ferrets were given discrete retinal lesions and were then examined for the distribution of degenerating myelin in semi-thin sections along the course of the fiber pathway. Cats were given implants of Horseradish peroxidase via a transbuccal approach to label the chiasmatic path and retinal origin of the superficially placed axon classes coursing through the lateral or medial halves of the optic tract.

The retinotopic ordering of individual axon classes observed in the tract must be established afresh within the chiasmatic region, since 1) axonal degeneration induced by dorsal vs. ventral retinal lesions is relatively well-segregated in the post-orbital nerve and tract but is widely dispersed along the intracranial and pre-chiasmatic segments of the nerve in adult ferrets, and 2) retrogradely labelled fibers of a given ganglion cell class, labelled from the lateral vs. medial optic tract, are intermingled in the pre-chiasmatic optic nerve of adult cats despite arising from the ventral vs. dorsal retina. Dorsal retinal axons decussate caudal to ventral retinal axons for any axon class, yet prior to crossing the midline they are intermingled.

The degree of retinotopic precision becomes too degraded along the nerve to account for the order found in the optic tracts. An active re-organization must occur within the chiasmatic region, and for the decussating fibers this occurs in the region of the midline.

451.3

THE ARRANGEMENT OF AXONS BY SIZE AND LATERALITY OF PROJECTION IN THE OPOSSUM'S OPTIC TRACT.

L.A. Cavalcante, S. Allodi# and §B.E. Reese, Instituto de Biofisica C. Chagas Filho, UFRJ, 21941 Rio de Janeiro, Brazil and §Dept. of Human Anatomy, University of Oxford, OX1 3QX, U.K.

We have examined the relative positions and size spectra of crossed and uncrossed optic axons in the opossum's optic tract (OT) to compare its regional organization with both the patterns of ganglion cell genesis and the disposition of axonal terminations in target nuclei. Regional histograms of axon size show that (1) the deep parts of the OT contain medium and fine fibers with uncrossed axons displaced towards the tract's pial margin, (2) the intermediate parts contain axons of all diameter classes with coarse crossed elements deeper than uncrossed counterparts, (3) the superficial parts contain primarily crossed, fine fibers and mostly uncrossed, medium and coarse axons. Thus, as in eutherians, the fiber order in the opossum's OT arises from the sequences of genesis of ganglion cells and presages the (partial) segregation of axonal terminations in the thalamus and midbrain. (CNPq, FAPERJ, FINEP, CEPG/UFRJ)

451.4

RETINAL DECUSSATION PATTERN IN THE RHESUS MONKEY EMBRYO. B. Lia. Dept. Psychol., & Calif. Regional Primate Res. Ctr., Univ. Calif., Davis CA 95616-8686

Using DiI in fixed embryos of the rhesus macaque (*Macaca mulatta*), the first optic axons are seen to enter the optic tracts by embryonic stage 20 (E40±2). At stage 21 (E42±2), retrograde labeling from the optic tract reveals that the most advanced axons arise from ganglion cells within a discrete area just dorsal to the optic nerve head and far from the prospective fovea. Given the exceptional hemiretinal specificity evident at later fetal ages (Chalupa & Lia, 1991), it is remarkable that the first ipsilateral projections arise from this portion of the presumptive nasal hemiretina, similar to the case in the mouse (Colello & Guillery, 1990). By the end of the embryonic period (E48±2), the most advanced projections arise from scattered ganglion cells distributed in relatively low density across the entire retina, some with well-developed dendrites. This early projection cohort exhibits the inherent primate decussation pattern, lacking the crossed temporal projection present among nonprimate mammals.

These observations suggest that retinal positional information is present for the decussation of the early projection cohort, but that expression of this information is dependent upon interaction of nasal and temporal axons at the chiasm. Nasal axons alone may not be endowed with explicit instruction to cross the chiasm. Temporal axons seem to avoid a sector of the midline chiasm and grow along crossed fibers from the other eye (Godement, Salaün & Mason, 1990). Additionally, prior to their crossing of the chiasm, nasal axons may avoid growing along the temporal axons filling the ipsilateral optic tract. Such a guidance mechanism for crossed projection from the nasal retina would not be operative until the arrival of temporal axons at the chiasm. (Supported by NIH RR00169)

451.5

TRANSCELLULAR DIFFUSION EVENTS OF THE CARBOCYANINE DYE, DiA, IN THE EMBRYONIC FERRET VISUAL SYSTEM. I. Kelly Johnson[1] & Vivien A. Casagrande.[1,2] Depts. of Cell Biology[1] & Psychology[2], Vanderbilt Univ., Nashville, TN 37232-2175.

Optic axons appear to make a choice at the optic chiasm. It is unclear what mechanisms may signal such a choice. We inoculated DiA into embryonic eyecups between E21-E30 to examine interactions between cross/uncrossed labeled retinal axons at the optic chiasm. Embryos were delivered via cesarean section from anesthetized ferrets and immersion fixed in 4% paraformaldehyde in phosphate buffer. DiA crystals [4-(4-dihexadecylaminostyryl)-N-methylpyridinium iodide; Molecular Probes] were then put into the eyecup. Following incubation at 37°C for 1-2 months, brains were vibratome sectioned and mounted on subbed slides.

Optic axons reach the chiasm by E25 (Guillery & Walsh, 1987, JCN, 265:203-217; Johnson & Casagrande, 1991, IOVS Suppl., 32(4):1035). The chiasm, and optic nerve and retina contralateral to the inoculated eye, become stikingly labeled beginning at this age. Ganglion cells and radial glial cells, putative Müeller cells, also become labeled. Transcellular labeling of thalamic radial glia bordering labeled optic tracts was routinely observed. Transneuronal diffusion into cortex was not observed.

These results suggest that the dye communicates between cells (neuron-neuron, neuron-glia) at and near the optic chiasm, and within the retina, via specialized cell-cell contacts. Alternatively, the strongly labeled projection to the contralateral retina may reflect an interretinal pathway present early in development. Johnson, et al. (1990, Soc. Neurosci. Abst., 16:53) reported similar transcellular diffusion events of carbocyanine dyes in fixed tissues of the horseshoe crab.

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451.6

ALTERATION OF AXON ARBOR COMPENSATES FOR NUMERICALLY MISMATCHED POPULATIONS IN THE HAMSTER RETINOTECTAL SYSTEM. M. Xiong (1), S.L. Pallas (2), and B.L. Finlay (1). (1) Dept. of Psych., Cornell Univ., Ithaca, NY 14853 and (2) Dept. of Brain and Cog. Sci., M.I.T., Cambridge, MA 02139.

After early partial tectal lesions in the hamster, the spatial convergence from the retina to the tectum increases at the population level but is unchanged at the single neuron level, i.e., the visual receptive fields of single neurons are the same size as normal (Pallas and Finlay, 1989). Thus, how developing connections are adjusted in order to meet both functional needs is of interest. In this study, we used an *in vitro* bulk fill technique to visualize individual axon arbors in both normal and early partial lesioned tecta. After a bead of HRP was placed into the brachium, the brain was incubated in a culture media for the transport of HRP (3 or 4 hours) and then processed with DAB histochemistry. Axon arbors were traced and branching pattern and bouton number were quantified for *en fâce* sections.

Axon arbors (n=8) from lesioned tecta were qualitatively less complex: covered a smaller area, had fewer branches, and had significantly decreased bouton numbers compared to normal (n=7) (Normal=113±17, Lesion=63±9; $t=4.995$, $p<.001$). These findings indicate that each retinal axon makes fewer contacts in the lesioned tectum than normal to compensate for the numerical disparity between number of retinal axons and tectal space.

Since prior research has shown that cell death is little changed and synaptic density is unchanged in this preparation, present data indicates the principal means of adjustment of numerically disparate populations is alteration of axonal arborizations. A more extensive morphological examination is under way to describe in more detail the structural changes in axonal arborizations.

Supported by NIH R01 NS 19245.

451.7

CORRELATION BETWEEN CELL PROLIFERATION IN EYE FRAGMENTS AND DUPLICATED RETINOTECTAL PROJECTIONS: INDICATION FOR AN EPIMORPHIC REGULATION OF POSITIONAL MARKERS IN THE RETINA OF XENOPUS LAEVIS. J.Häse*, N.Degen and K.Brändle*, Zool.Inst., Univ.Frankfurt, Siesmayerstr.70, D-6000 Frankfurt 11, FRG

In Xenopus embryos fragmentation of the early eye anlage often results in pattern - duplicated retinotectal projections: i.e. two retinal ganglion cell groups, located at different sites in the retina, project to the same tectal area. Such experiments indicate that these cells carry identical positional markers. Thus, in eye fragments a respecification of retinal ganglion cells seems to occur in relation to their positional information.

We tried to find out whether effects, such as mitosis, caused by eye fragmentation in the retinal tissue could be involved in positional respecification. For this purpose the temporal half of the eye anlage was removed in Xenopus embryos at tail bud stages (St.33/34). The remaining nasal half - eye rounded up within 3 to 5 days. Computer reconstructions of serial sections show that the cut edges enclosed the lens and fused together in the temporal region of the orbit, forming an eye of normal shape. We determined histologically the distribution of mitosis in the eye fragments during the rounding - up process. The results indicate that in most cases mitotic cells outside the normal germinative growth zones appeared during the fusion of the cut edges. These additional mitotic cells were always in the fusion area of the eye fragment, i.e. in the temporal part of the retina. We also examined the optic projection of the nasal half - eyes in Xenopus tadpoles at metamorphosis (St.60) by means of electrophysiological recordings in the tectum. In most cases double projections occured, which originated from a restricted sector of the visual field. Quantitative measurements showed that this area corresponded to the fused area of the retina, where extra mitosis had been found.

Therefore we suggest that the duplication of positional markers in eye fragments is correlated with cell proliferation, which indicates an epimorphic regulation of positional information following partial extirpation of the eye anlage.

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451.8

ANTIBODIES AGAINST NEURITE GROWTH INHIBITORS FROM OLIGODENDROCYTES CHANGE THE COLLICULAR TERMINATION PATTERN OF POSTNATALLY SPROUTING RETINAL FIBERS. Josef P. Kapfhammer,[1,2] Gerald E. Schneider[1] and Martin E. Schwab[2]. Dept. Brain & Cogn. Sci., M.I.T., Cambridge, MA 02139[1] and Brain Res. Inst., Univ. of Zürich, August-Forel-Str. 1, CH-8029 Zürich, Switzerland[2].

After early postnatal unilateral ablation of the superior colliculus (SC) together with the ipsilateral eye in Syrian hamsters, retinal fibers abnormally cross the tectal midline and innervate the remaining SC (Schneider, 1973). The recrossing retinal fibers are mainly confined to the stratum griseum superficiale (SGS), with little ingrowth or termination in the deeper stratum opticum (SO); laterally, most termination is in the superficial part of SGS. Establishment of this pattern, observed at postnatal day 12, is temporally correlated with the appearance of oligodendrocytes in the SO during growth of recrossing fibers (Schneider et al., SN Abstr.,'90; Carman et al., SN Abstr.,'87).

We now show that application of the IN-1 antibody directed against neurite growth inhibitors from oligodendrocytes (Caroni and Schwab, 1988) changes this termination pattern. In the presence of this neutralizing antibody, but not with a control antibody, recrossing retinofugal fibers traverse the SO as well as the SGS, with greater depth of termination in SGS and SO. This pattern resembles that of the normal contralateral retinotectal projection.

The results indicate that neurite growth inhibitors expressed by oligodendrocytes are responsible for restricting the innervation of a target area in postnatal plasticity.

Support: NIH grant EY00126, Swiss National Science Foundation, EMBO.

451.9

VIP- AND SEROTONIN -LIKE IMMUNOREACTIVITY IN THE VISUAL SYSTEM OF *RANA PIPIENS*. Q. Liu and E.A. Debski. Sch. of Bio. Sciences, University of Kentucky, Lexington, KY 40506.

NMDA receptors mediate the topographic mapping of retinal ganglion cell terminals onto the optic tectum of the frog (Cline & Constantine-Paton, Neuron, 3:413-426, 1989). In other systems, serotonin and VIP have been shown to modulate the activity of NMDA receptors (Reynolds et al., Brain Res. 456: 28-292, 1988; Sah, Biophys. J. 53:356a, 1988). We wish to determine if serotonin and VIP modulate topographic map formation by interacting with the NMDA receptor. As a first step we have investigated the distribution of these two substances in the frog visual system using immunocytochemical techniques. VIP-like immunoreactivity was detected in both the cellular and plexiform layers of the optic tectum. VIP-like immunoreactive (VIP-IR) cell bodies constituted 19, 26 and 29% of the cells in layers II, IV and VI, respectively. In addition to being located in layers V and VII, VIP-IR fibers were also found in layer IX where retinal ganglion cells terminate. The nucleus isthmi (NI), a structure reciprocally connected to the optic tectum, also contained a high proportion of VIP-IR cells in the posterior nonrim cortex (53%) and the rim cortex (42%). There were very few VIP-IR cell bodies in the anterior nonrim cortex. The thalamus, which has several projections to the optic tectum, also contained many VIP-IR cells. Serotonin-like immunoreactivity in visual areas was limited to a subset of cells in tectal layer VI and a ventral thalamic region. This project was supported in part by BRSG SO7 RRO7114-22.

451.10

NON-UNIFORM DISTRIBUTION OF SOMATOSTATIN-LIKE IMMUNOREACTIVITY IN THE FROG OPTIC TECTUM. E.A. Debski. Sch. of Biol. Sci., Univ. of Kentucky, Lexington, KY 40506.

Retinal ganglion cell terminals create a topographic map of visual space at the optic tectum, their primary target. The formation of this map is dependent on 1) differences in cell properties that cause the rough alignment of the retinal projection onto the tectum and 2) an activity-dependent process that stabilizes the terminals from neighboring retinal ganglion cells at neighboring synaptic sites.

Staining of the optic tectum in adult *Rana pipiens* with an antibody directed against somatostatin suggests that different regions of the tectum are composed of functionally non-equivalent cells. Somatostatin-like immunoreactive cell bodies were distributed non-uniformly along the rostral to caudal extent of the tectum: 0.6% of the immunoreactive cells were found in the rostral third of the tectum, 8.8% of the cells were present in the middle third of the tectum and 90.6% were found in the caudal third of the tectum. However, these same cells were distributed uniformly in the medial to lateral dimension being present in approximately the same numbers in lateral and medial sagittal sections of the tectum. In the radial dimension, the immunoreactive cells were divided equally between tectal layer IV (36.5%) and layer VI (42.4%). An additional 6.8% of the cells were found in layer II. The remainder of the cells (14.3%) occupied positions where the laminar identities of layers IV and VI began to break down. This project was supported in part by BRSG SO7 RRO7114-22.

451.11

QUANTITATIVE ANALYSIS OF AGONIST-EVOKED CURRENTS IN IDENTIFIED TECTAL NEURONS OF *RANA PIPIENS*. P.W. Hickmott and M. Constantine-Paton, Dept. of Biology, Yale Univ., New Haven, CT 06511

Previous work in our lab has implicated the N-methyl-D-aspartate (NMDA) receptor in the activity-dependent refinement the retinotectal map in *Rana pipiens*. For example, chronic NMDA treatment of the optic tectum of *Rana* tadpoles causes both a sharpening of the retinotopic map, and a decrease in the sensitivity of the tectum to acutely-applied NMDA (E. Debski and M. Constantine-Paton, in preparation) However, since this decrease has only been examined at the level of the electrically-evoked field potential, the precise mechanism responsible for the decrease is unknown. To address this question, we have developed a tectal slice preparation in which we can record from single tectal neurons, using whole-cell voltage clamp. Previously, we have examined postsynaptic currents (PSC) evoked by electical stimulation in the optic tract, and the effects of bath application of various drugs on these PSCs (P. Hickmott and M. Constantine-Paton, Soc. Neurosci Abstr., 16:985 1990).

Instead of using electrical stimulation to evoke the PSC, we have now developed techniques for using iontophoresis of neurotransmitter agonists to evoke PSCs. We have determined that tectal neurons can respond to iontophoresed glutamate, NMDA, AMPA, and GABA; in some cases neurons respond to all these agonists. By using multibarrel iontophoresis we will examine the quantitative relationships between these currents in various morphologically-identified cells in the tectum. Thus, we will be able to examine the question of whether certain tectal cell types respond preferentially to one agonist over another, which will give us further insight into the tectal circuitry. We will also compare these data from normal tadpoles to similar data obtained from tadpoles that have had their tecta chronically treated with NMDA, to determine if the decrease in NMDA effectiveness seen at the level of the field potential is reflected in a selective change in any of the single-cell currents.

451.12

EM ANALYSIS OF SINGLE RETINAL GANGLION CELL TERMINALS IN NMDA-TREATED TECTA OF *RANA PIPIENS*. Lai-Hsing Yen and Martha Constantine-Paton, Dept. of Biology, Yale University, New Haven, CT 06511

Previous work in this lab has shown that chronic NMDA application to the striped tecta of three-eyed frogs causes a dramatic decrease in branching within retinal ganglion cell (RGC) terminals (Cline & Constantine-Paton. 1990. J. Neurosci. 10:1197). To determine whether these alternations are accompanied by changes in retinotectal synaptic contact we have examined the distribution of synapses within single, HRP-filled, RGC terminal in NMDA-treated striped tecta. The tecta of young post-metamorphic frogs were treated with a low dose of NMDA for 4 to 5 weeks and then processed for EM. The morphology of isolated, HRP-labled Type IV RGC terminals were then reconstructed at the light microscope level (LM). Using the centripetal Strahler scheme we labeled all the branches in the reconstructed RGC terminals in a hierarchy of order 1 to 5. Order 1 refers to the most distal branches and order 5 to the major axon cylinder. The ratio of synaptic contact to axon membrane of HRP-filled profiles was sampled at the EM level, and multiplied by the total axonal length measured at the LM level to estimate total synaptic contact for each branch order. These values were normalized to give the percentage of total synaptic contact contributed by each branch order within the arbor.

The analyses showed that, in the NMDA-treated arbor, the distal order 1, 2, 3 branches contained almost all the synaptic contact, 42%, 35%, 17% respectively. This synaptic distribution is similar to that of Type IV arbors in normal tecta even though NMDA-treated arbor are less branched. The decrease in branching and the maintenance of the same proportion of total synaptic contact contributed by each branch order suggests a proximal shift of synaptic distribution toward the main axon in NMDA-treated arbors. In addition, these analyses suggest that total synaptic contact of NMDA-treated arbors decrease relative to that of the arbors in normal tecta. However, this may not indicate a decrease in total synaptic efficacy: Earlier analyses (Yen & Constantine-Paton. 1990 Proc. Soc. Neurosci 16: 532.4) showed an increased synaptic convergence and thickened pre- and post-synaptic density following NMDA treatment of striped tecta. (Supported by NIH grant EY08760).

451.13

RESPONSES OF CULTURED FROG RETINAL AXONS AND DISSOCIATED TECTAL CELLS TO GLUTAMINERGIC AND CHOLINERGIC AGENTS. M. Constantine-Paton and Glen Prusky, Department of Biology, Yale University.

We have examined the cellular sites of action and the immediate structural effects of receptor activity in *Rana pipiens* retinal and tectal cells in culture to identify the mechanisms responsible for intraocular competition and retinotopic map refinement during development. Retinal explants, elaborating immunocytochemically-identified ganglion cell axons, were monitored with timelapse video microscopy. Focal application of nicotine (100 μM) to isolated growth cones rapidly inhibited filapodial growth and movement, and initiated the collapse of the growth cone and retraction of the axon. The noncompetative ganglionic nicotinic receptor antagonist, mecamylamine, blocked this effect. Similar application of glutamate did not produce the same effect. The complete range of neuronal cell types found in the intact tectum appeared to be represented in dissociated tectal cultures; the cultures contained both large and small pyramidal and pear-shaped bipolar and multipolar neurons. We observed the level of intracellular-free Ca^{++} (Ca^{++}) in these cells after loading them with either Fluo-3 or Fura-2. These neurons exhibited considerable heterogeneity in basal somatic Ca^{++} concentration, however, there was no clear correlation between cell morphology and this basal level. Non-uniform subcellular distributions of Ca^{++} were common among these neurons with varicosities along their processes frequently showing elevated levels. Focal application of glutamate to these cells often resulted in a dramatic increase in Ca^{++} in the neurites, possibly indicating a loci for glutamate/Ca^{++} interactions. We are currently investigating the morphological responses of tectal neurons and their processes to a range of applied glutamate concentrations to determine if cell type or cell process-specific differences are present. In addition, we have recently developed reliable methods for co-culturing retinal explants and dissociated tectal cells. We are now in a position to examine possible differences in the responsiveness of retinal axons and tectal cells to applied glutaminergic and cholinergic agents when their processes, which are normal synaptic partners *in vivo*, have an opportunity to recreate these contacts *in vitro*. Support: NEI to MCP (EY-06039).

451.14

NMDA RECEPTOR BLOCKADE INTERFERES WITH THE DEVELOPMENT OF RETINOTOPIC MAPS IN MAMMALS. G. Prusky[1], D.K. Simon[2], M. Constantine-Paton[1] and D.D.M. O'Leary[2], Dept of Biology, Yale Univ, New Haven, CT 06511[1] and Molecular Neurobiology Lab, The Salk Institute, La Jolla, CA 92037[2].

NMDA receptor activation is critical for maintenance of orderly retinal connections in frog tectum (Cline & Constantine-Paton, Neuron '89), establishment of order in the regenerating fish retintotectal projection (Schmidt, JNS '90), segregation of on-off afferents in ferret LGN (Hahnet al ARVO '90) and deprivation-induced plasticity in the cat geniculocortical projection (Kleinschmidt et al, Science '87). Here, we report a role for NMDA receptors in the development of topographically ordered retinal projections to the rat superior colliculus (SC). In neonatal rats, retinal axons project diffusely throughout the SC; many form arbors in topographically incorrect positions. By P12, virtually all mispositioned axons are eliminated; none arborize outside of topographically correct locations (Simon & O'Leary, Dev Bio '90). To determine if the remodeling of the initially diffuse rat retinocollicular projection depends on NMDA receptor activation, we used a technique effective in disrupting NMDA receptor function in the frog retinotectal system. A slow-release polymer (Elvax) containing the NMDA receptor antagonist AP5 or MK801, or MK801 + NMDA, or buffer alone (control), was implanted over the SC on P0. Axons arising from temporal retina (topographically matched with rostral SC) were labeled with focal DiI injections at P9 and examined at P12. By P12, in control rats, the axons develop a normal retinotopic projection. However, in rats treated with AP5, MK801, or MK801 + NMDA, many aberrant axons remain and even arborize at topographically incorrect sites, including far caudal SC. In these same animals, though, a focus of arbors forms at the topographically correct position in rostral SC, suggesting an NMDA-independent component to map formation or incomplete receptor blockade. Nevertheless, the abnormal persistance of diffusely projecting axons following NMDA receptor blockade shows that NMDA receptor function is crucial for the development of retinotopic maps in mammals. Support: NEI grants EY06039 (MC-P) & EY07025 (DO'L).

451.15

NORMAL DEVELOPMENT AND EFFECTS OF DEAFFERENTATION UPON THE MORPHOLOGY OF SUPERIOR COLLICULAR (SC) NEURONS PROJECTING TO THE LATERAL POSTERIOR NUCLEUS IN HAMSTER. S.V. Savage, T.D. King*, S.C. Hobler*, R.D. Mooney and R.W. Rhoades. Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43699.

Neurons in the superficial layers of the hamster's SC can be divided into distinct morphological classes. Neonatal enucleation has only slight effects upon the morphology of cells within a given class, but significantly reduces numbers of neurons (narrow and widefield vertical cells) with dorsally directed dendrites. Does this change reflect differential transneuronal degeneration or alterations in the dendritic arbors of surviving cells? In this study, we restricted our analysis to a specific and relatively homogeneous subpopulation of superficial layer neurons, those that project to the lateral posterior nucleus (LP). The combination of retrograde tracing and intracellular injection with Lucifer yellow demonstrated that over 75% of tecto-LP cells in normal hamsters are widefield vertical cells. Most tecto-LP cells in the neonatally enucleated adult hamsters were either horizontal cells (17.7%), giant stellate cells (25.3%), or had dendrites that were directed only toward the deep SC laminae (10.1%); less than 25% were widefield vertical cells. Differential enucleation-induced cell death could not account for all of these changes. Tecto-LP neurons were retrogradely labelled with Di-I in hamsters on postnatal day (P-) 0 through P-10. As early as P-0, most retrogradely labelled neurons could be identified as either widefield (44.6%) or narrow field (18.9%) vertical cells. These results support the conclusion that neonatal eye removal results in a reorganization of dendritic arbors that have already undergone considerable development at the time of the lesion. EY 04170, EY 08015

451.16

EFFECTS OF FETAL AND NEONATAL DEAFFERENTATION UPON ADENOSINE DEAMINASE IMMUNOREACTIVE NEURONS IN THE RAT SUPERIOR COLLICULUS. R.D. Lane*, C.A. Bennett-Clarke, N.L. Chiaia, S.V. Savage, D. Allan*, J.A. Brown, R.D. Mooney and R.W. Rhoades. Depts of Anatomy and Neurosurgery, Medical College of Ohio, Toledo, OH 43699.

It has been well-documented that neonatal enucleation causes major changes in the number and function of cells located in the superficial layers of the superior colliculus (Findlay et al., Dev. Brain Res. 28: 1, '86, Rhoades, J. Physiol., (London) 301:383, '80; Tokunaga et al., Dev. Brain Res. 23:131, '85). How this deafferentation effects specific populations of neurons in these layers has not been well defined. Adenosine deaminase immunoreactivity (ADA-IR) can be used as a marker for a population of neurons located primarily in the stratum opticum (SO). A subpopulation of these ADA-IR neurons project to the lateral posterior nucleus (LPN) of the thalamus (Miguel-Hidalgo et al., Brain Res. 476:189, '89). We undertook this study to examine the effects of fetal and neonatal deafferentation on the ADA-IR population of SO cells. Unilateral and bilateral enucleations were performed on rats at embryonic day 17 or at birth. All animals received injections of fluorescein labeled beads into the LPN. In normal animals, there was an average of 2189 ± 562 S.D. ADA-IR SO neurons per colliculus of which 498 ± 228 ADA-IR cells projected to the LPN. Surprisingly, neither the fetal enucleations or the neonatal enucleations had an effect upon the total number of ADA-IR SO neurons per deafferented colliculus (1927 ± 600 and 2086 ± 70, respectively) or the number that projected to LPN (577 ± 326 and 576 ± 28, respectively). These results indicate that a specific population of neurons in the superior colliculus are refractory to neonatal or fetal deafferentation. Support: EY04170, EY08015, HL36573.

451.17

SYNAPSES ARE LOST DURING THE NORMAL DEVELOPMENT OF THE SEROTONINERGIC INNERVATION OF THE SUPERIOR COLLICULUS (SC) IN HAMSTER. E. Arce*, C.A. Bennett-Clarke, R.D. Mooney and R.W. Rhoades. Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43699.

We have employed immunocytochemistry in conjunction with both light and electron microscopy to describe the normal development of the serotoninergic innervation of the hamster's SC. Serotonin-immunoreactive fibers in adult hamsters are present in all SC laminae and are most dense in the lower part of the stratum griseum superficiale (SGS) and stratum opticum (SO). Serotonin immunoreactive fibers were visible in the SC by embryonic day 14 (two days prior to birth). By the day of birth, some immunoreactive fibers were present just below the pial surface and others were generally oriented either parallel or orthogonal to the SC laminae. Over the next two weeks, the serotoninergic innervation of the SC increased in density and assumed the laminar distribution observed in adult animals. Electron microscopic examination of 113 5-HT-positive varicosities in single thin sections taken from the SGS and SO of adult hamsters revealed only 5 conventional synaptic contacts while similar analysis of 250 varicosities from hamsters killed on postnatal day 3 revealed 103 conventional synaptic contacts, most of which were axodendritic. Serial section analysis of additional 5-HT-positive swellings from adult hamsters provided further support for the conclusion that very few serotoninergic axons make synapses in the SC of these animals. These results indicate a substantial synaptic reorganization of the serotoninergic input to the SC in developing hamster. The role that these transient synapses might play in collicular function or development is currently unknown. EY 04170, EY 08015

451.18

CORTICAL FEEDBACK TO LGN MAY PLAY A MAJOR ROLE IN OCULAR DOMINANCE COLUMN DEVELOPMENT K.P. Unnikrishnan and Harmon S. Nine*, GM Research Labs, Warren, MI 48090 and University of Michigan, Ann Arbor, MI 48109

A model is presented to investigate the role of feedback in the development of ocular dominance columns in the primary visual cortex. In our model, summed responses of groups of cortical neurons are fed back to the LGN neurons to modify their activities and consequently their connection strengths to the cortex. The synaptic modification rule uses temporal correlations between changes in local LGN activities and the cortical feedback signals and can easily be carried out by neuronal circuitry containing NMDA-type receptors.[1]

It has been observed that during development, the subplate neurons, situated just below the cortex, project axons to the thalamus and dendrites to the deep cortical layers.[2] This provides a mechanism for sending summed cortical responses to the geniculate neurons. Recent evidence suggesting that the death of the subplate neurons[2] and inactivation of the NMDA receptors[3] coincide with the termination of ocular dominance column development also supports our model.

LGN laminae representing each eye and the cortex are modelled using 32x32 arrays. Locally correlated spontaneous retinal activity drives the LGN neurons. Summed cortical responses computed over cell aggregates are fed back to the LGN. Intracortical interaction is implemented using mexican hat type functions. Simulations of the model show robust formation of ocular dominance columns. Experimentally verifiable predictions from the model will also be presented.

1. Harth, Pandya, & Unnikrishnan, Concepts in Neurosci., *1*, 53(1990). 2. Shatz *et al.*, Cold Spr. Harb. Symp. Quant. Biol. *55*, (1991). 3. Fox, Daw, Sato, Czepita, Nature, *350*, 342(1991).

451.19

NEURONAL HYPERTROPHY IN THE NOVEL BILATERAL CORTICOTECTAL PATHWAY FOLLOWING NEONATAL CEREBRAL HEMISPHERECTOMY IN THE CAT. PD Adelson*, DA Hovda, JR Villablanca, K Tatsukawa, and YG Comair. Div. of Neurosurgery and Mental Retardation Research Cntr., UCLA Sch. of Medicine, Los Angeles, CA 90024.

To further define the anatomical reorganization associated with the cortical-tectal pathway following neonatal cerebral hemispherectomy, the left (ipsilateral to the lesion) superior colliculus (SC) of 3 intact adult cats (IN), 3 neonatal-hemispherectomized (NH), 5-15 days old, and 3 adult-hemispherectomized (AH) cats were injected (1 mm deep) with WGA-HRP (.3-.5 µl). The brains were processed for combined TMB/DAB histochemistry and the primary visual cortex (VC; areas 17 & 18) was examined (light microscopy, 1,500X). For each group, labelled neurons, which were restricted to layers IV and V, were counted and their soma was measured. For IN cats, the left (ipsilateral) VC had 1,268.7 cells (mean per cat, MPC), with a mean soma size (MSS) of 366.7 μm^2 (SD=±185.5), with the greatest percentage of cells within the 300-700 μm^2 range. There were no cells detected in the right VC of IN animals. For NH, the remaining contralateral VC, with a MPC=474.6, had a MSS=436.1 μm^2 (SD=±186.0), with a peak distribution of 400-800 μm^2. For AH, the contralateral VC had a MPC=93.6, and a MSS=486.6 μm^2, (SD=±202.5) with a peak distribution of 500-800 μm^2. In conclusion, following hemispherectomy, the cortical-tectal pathway becomes bilateral in both NH and AH, with this being more pronounced in NH; in addition, the cells of origin of this reorganized pathway become larger, perhaps in response to greater axonal arborization. USPHS R01 NS-25780, P01 HD-05958.

451.20

TEMPORAL-FREQUENCY SENSITIVITY OF LATERAL GENICULATE NEURONS IN AGING RHESUS MONKEYS. Charlene B. Y. Kim*, Rodney J. Moore, Peter D. Spear, Nina Tumosa, and Jin-Tang Xue*. Department of Psychology and Center for Neuroscience, Univ. of Wisconsin, Madison, WI and School of Optometry, Univ. of Missouri, St. Louis, MO.

Human psychophysical studies have demonstrated decrements in temporal-frequency sensitivity as a function of aging, even after pre-retinal ocular media differences have been controlled for experimentally. These studies have suggested either retinal and/or post-retinal (central visual pathways) sources for the differences in performance. In an effort to understand the nature and origin of the modifications of the visual pathways during aging, we recorded extracellularly from single neurons in the parvocellular (PCL) and magnocellular (MCL) layers of the lateral geniculate nucleus (LGN) of young (5-16 yr) and old (25-28 yr) rhesus (Macaca mulatta) monkeys. Each neuron was stimulated with gratings drifted at 10 different temporal frequencies (1-30 Hz) at its optimal spatial frequency and a sub-saturation contrast level.

Optimal temporal frequency, bandwidth, and high and low temporal-frequency cutoff values were determined for each cell. Neuronal receptive fields were matched for eccentricity (0-15 deg). Our preliminary results indicate that aging has no significant effect on temporal-frequency sensitivity of PCL or MCL neurons in the monkey LGN. This suggests that age-related neural deficits in temporal visual function may reside in more central visual structures, such as striate cortex. (Supported by EY01916, EY02545, and F32 AG05526.)

451.21

DECREASED AB5-IMMUNOREACTIVITY OF NEURONS IN LGN OF MONOCULARLY DEPRIVED CATS. S.B. Tieman and K.R. Fry. Neurobiology Research Ctr, SUNY, Albany NY 12222 and Ctr for Biotechnology, Baylor College of Medicine, The Woodlands TX 77381.

The monoclonal antibody AB5, which is specific for ganglion cells in the retina of cat and rabbit, also stains a limited subset of neurons in central nervous system. In lateral geniculate nucleus (LGN), it stains some of the larger neurons. We here report, that in monocularly deprived cats, AB5 immunoreactivity is decreased in the deprived laminae. Three long-term monocularly deprived cats were deeply anesthetized with i.v. Nembutal and perfused through the heart with buffered saline followed by 4% carbodiimide, 4% paraformaldehyde and 4% sucrose in 0.1 M phosphate buffer. Thalamus was frozen and sectioned coronally at 30 μm into 0.1 M phosphate with 0.02% azide. Sections were then immunoreacted for the presence of AB5 using standard ABC protocols. Although the staining of the retinal terminals did not appear to be affected by the monocular deprivation, staining of cell bodies was reduced in the deprived layers. These findings are reminiscent of those obtained with the monoclonal antibody CAT-301 (Sur et al. J. Neurosci., 8:874, 1988), and suggest that the AB5 staining is associated with cells that are particularly vulnerable to abnormal early experience, perhaps Y-cells. (Supported by NSF grant BNS 8811039 to SBT and PHS grant EY 06469 to KRF.)

451.22

NGF PREVENTS THE SHRINKAGE OF NEURONS IN LATERAL GENICULATE NUCLEUS (LGN) OF MONOCULARLY DEPRIVED RATS. L. Domenici , A. Cellerino * and L. Maffei *. Istituto di Neurofisiologia (CNR) and Scuola Normale Superiore, Pisa, 56100, Italy.

In cats and monkeys monocular deprivation (MD) causes the shrinkage of neuronal perykaria in the deprived laminae of LGN, in correspondence with the binocular visual field. Here we report that the NGF supply prevents this shrinkage in the rat. To identify the ipsilateral LGN laminae which project to the binocular portion of the rat visual cortex, a HRP-solution (10 ul, 30% HRP plus 2% DMSO in saline) was injected into the deprived eye and after a 24-36 hours survival time the brain was fixed, processed by standard HRP histochemistry and Nissl method. One month of MD causes a shrinkage in the LGN deprived lamina with respect to the undeprived lamina (mean difference in soma size = 22 %). By contrast, the soma size distributions for the deprived and undeprived LGN laminae extensively overlap in MD rats treated with NGF.

We conclude that NGF treatment rescues from atrophy the neurons in the LGN deprived laminae projecting to the binocular portion of the visual cortex.

451.23

DEVELOPMENT OF MEMBRANE AND SYNAPTIC PROPERTIES IN NEURONS OF THE FERRET LGN. C.A. White and M. Sur. Dept. of Brain and Cognitive Sciences, M.I.T., Cambridge, MA 02139

During development, orderly projections from the retina to the lateral geniculate nucleus (LGN) are established through remodeling of a more diffuse set of immature projections. Neuronal activity is thought to play an important role in the remodeling process (Shatz and Stryker, '88; Hahm et al., '91). We have begun to examine ferret LGN neurons in slices to determine the degree of maturation of membrane and synaptic properties during a period that retinogeniculate afferents undergo considerable change. In the ferret, segregation of retinogeniculate afferents into eye-specific layers is essentially complete by postnatal day 14 (P14) and into On and Off sublayers by P21 (Linden et al., '81; Hahm et al., '91). In vitro intracellular recordings were carried out in animals from P1 to P33. Resting membrane potentials (range 51-67 mV), input resistances (26-118 Mohms) and time constants (5-11 msec) of the LGN cells showed no clear changes with age. At all ages, depolarizing current injection could elicit action potentials. The first low-threshold spikes, common in the adult LGN, were observed at P9 and were encountered routinely thereafter. Also at P9, the first postsynaptic potentials evoked by stimulation of the optic tract were recorded. In one neuron at P9, optic tract stimulation evoked an ipsp with a reversal potential of -56mV. At later ages epsps were more commonly observed. Our data indicate that at least some cells within the LGN are electrically excitable and make functional contacts with retinal ganglion cell axons during the period of segregation of retinogeniculate afferents. Supported by EY 06297 (C.A.W.) and EY 07023 (M.S.).

451.24

NMDA ANTAGONIST INFUSION DURING ON/OFF SUBLAMINAR SEGREGATION ALTERS DENDRITIC MORPHOLOGY OF CELLS IN FERRET LGN. M. Rocha*, A. Ramoa, J. Hahm and M. Sur. Department of Brain & Cognitive Sciences, M.I.T., Cambridge, MA 02139; and Biophysical Institute, Federal University of Rio de Janeiro, 21941, Brazil.

The NMDA subtype of glutamate receptor may contribute to the establishment of specific connections in the developing central nervous system. Recently, chronic infusion of the NMDA antagonist, D-APV, has been shown to affect sublaminar segregation in the ferret LGN by altering the morphology of retinogeniculate afferents (Hahm et al., 1991). Here, we examine whether NMDA receptor blockade also affects the morphology of the postsynaptic target cells in the LGN.

The specific NMDA antagonist D-APV (0.8 mM) was chronically infused via an osmotic minipump into the thalamus of ferrets for one week from P14 to P21 (n=5) when retinogeniculate afferents normally segregate into On and Off sublaminae. Rhodamine-labeled latex microspheres were injected into visual cortex (area 17) to backfill and identify LGN relay cells. Immediately after treatment, these cells were intracellularly injected with Lucifer Yellow in living 300 μm horizontal slices.

D-APV treatment led to an increase in dendritic complexity of LGN cells, shown by an increased number of branch points (from 23±11 in normal animals at P21 to 60±28 in treated animals, p=.0001). Dendritic arbor area also increased from 26826±16626 μm^2 in the normal group (26 cells, 3 animals) to 48831±43867 μm^2 in the treated animals (19 cells, 5 animals, p=.01). These effects were particularly marked in the A laminae. Soma sizes were unaffected by NMDA receptor blockade.

These results indicate that the dendritic development of LGN cells is markedly affected by NMDA receptor blockade, and suggest that synaptic transmission through NMDA receptors may play a significant role in the concurrent maturation of presynaptic afferents and postsynaptic target cells.

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451.25

AN NMDA ANTAGONIST DOES NOT DISRUPT NORMAL EYE-SPECIFIC LAMINAR SEGREGATION OF FERRET RETINOGENICULATE AXONS. D.K. Smetters, J. Hahm and M. Sur. Dept. of Brain & Cognitive Sciences, M.I.T., Cambridge, MA 02139.

In the ferret LGN, retinogeniculate arbors undergo two major periods of segregation; the afferents first segregate into eye-specific laminae between birth and postnatal day 7, and then into ON and OFF sublaminae between P14 and P21 (Linden et al., '81; Hahm et al., '91). Neuronal activity, and NMDA receptors in particular, play a crucial role in this second segregation, which is blocked by infusion of NMDA receptor antagonists (Hahm et al., '91).

We examined the role of NMDA receptors in the formation of eye-specific laminae by chronically applying a specific antagonist, CPP, during the initial period of segregation. Ferret kits (n=9) were systemically infused with CPP (0.05mM-5mM) from P1 to P8 via a subcutaneous osmotic minipump (0.5 ul/hr). Control animals received infusion of saline (0.9%). Dosage was chosen to be in the range effective for preventing ON/OFF sublaminar segregation, allowing for the difference in weight in these much younger animals.

Intraocular injections of WGA-HRP (20%/2%) at P7 revealed robust segregation of afferents into eye-specific laminae, even at the highest dose tested (n=2). Labeling of single axons *in vitro* in treated animals (n=2) showed no significant differences from controls in arbor area, width, or height. We saw no obvious changes in LGN size or cortical thickness in treated animals, but immaturity in the sulcal pattern and behavioral changes indicated that the drug concentrations used were effective.

These results suggest that eye-specific and ON/OFF segregation differ in their degree of dependence on NMDA receptors, and may occur through different mechanisms.

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452.1

THE EFFECT OF CYCLOSPORINE-A ON EYE REMOVAL-INDUCED REJECTION OF MESENCEPHALIC RETINAL XENOGRAFTS T.Subramanian, I.F.Pollack and R.D.Lund. Depts of Neurobiol., Anat. & Cell Sc.and Neurosurg., Univ.of Pittsburgh, Pittsburgh, PA 15261.

Previous studies have shown that eye removal leads to the rejection of previously well integrated mouse retinal xenografts in the rat mesencephalon that are located close to degenerating optic axonal terminals (Lund et al.,Transpl. 46:216, 1988). In this response, lymphocytic infiltration is preceded by class I and class II major histocompatibility (MHC) antigen expression in the vicinity of the degenerating optic fibers in the superior colliculus and subsequently in and around the graft. In this study we examined the effect of the immunosuppressant Cyclosporine-A (CyA), which is known to prevent T cell activation, on this rejection response.

Fifty one postnatal day 1 (P1) Sprague-Dawley rats received embryonic day 13 CD-1 mouse retinal transplants into their mesencephalon. On P21, both the host eyes were removed and all animals began receiving systemic CyA at a dose of 10 mg/kg/day. On P36 the animals were divided into four groups. Group I animals (N=4) were sacrificed immediately and group II (N=10), were sacrificed on P51. CyA was discontinued on P51 in group III (N=17), while group IV (N=20) continued to get CyA until P81, at which point animals in both groups were sacrificed. The brain was cut in 30μm coronal sections and adjacent sections stained with cresyl violet and antibodies against MHC class I (OX18), class II (OX6) antigens, microglia (OX42), astrocytes (GFAP) and lymphocytes (anti-lym).

Although group I animals had MHC class I and II antigen expressing cells within the superior colliculus, the grafts showed no signs of rejection. All the group II animals (N=8), and 13 out of the 15 group IV animals, that had successfully engrafted had healthy appearing transplants with no evidence of rejection. In contrast all eleven group III animals with detectable grafts, showed obvious signs of rejection. These results indicate that while CyA protects mesencephalic retinal xenografts from eye removal- induced rejection, it does not eliminate the MHC antigen expression induced by this lesion. Moreover, immunoprotection is lost when CyA is withdrawn suggesting that it prevents T lymphocytes from activating the rejection response.

452.2

INTRARETINAL XENOGRAFTS OF MITOTICALLY ARRESTED HUMAN RETINOBLASTOMA CELLS. E. S. Lazar, M. del Cerro, M. F. Notter, G. Seigel, and C. del Cerro. Department of Neurobiology and Anatomy, University of Rochester Medical School, Rochester, New York, 14642, USA.

Retinoblastoma is the most common ophthalmic malignancy in childhood. Cell lines obtained from these tumors have been the topic of numerous studies at both the cellular and molecular levels. We report on the use of one of these cell lines (Y 79) for intraretinal xenografts into adult mammalian eyes. We wished to determine the survival, integration, and differentiation characteristics of these cells after they had been returned to the intraocular milieu from which the progenitor cells originated. Furthermore, we hoped to test the hypothesis that these cells could be used for cell replacement therapy in the adult mammalian retina in lieu of fetal retinal cells. 3H-thymidine labeled Y 79 cells were exposed to retinoic acid/butyrate to induce differentiation, and then were suspended in human plasma at a concentration of 4,000 cells/ μl. Using the method of Lazar and del Cerro (1989) for multisite retinal grafting, the cells were transplanted into the subretinal space of Fisher 344 rats. The host rats received daily injections of Cyclosporine A, and were sacrificed 30-60 days following transplantation. Histological examination showed that transplants survived as multiple clusters of rosette-forming cells. These cells differentiated into clearly recognizable photoreceptors. There were no signs of continued cell division or any indications of a host reaction against the grafted cells. The results indicate that appropriately treated Y 79 cells may serve as a substitute for fetal cells in retinal transplantation. (Supported by EY 05262, the Rochester Eye Bank, and private donations).

452.3

SURVIVAL OF INTRAOCULAR NEURAL RETINA GRAFTS IS INFLUENCED BY MICROENVIRONMENTAL FACTORS AS WELL AS IMMUNOGENETIC DISPARITY L.Q. Jiang and J.W. Streilein, Dept. of Microbiology & Immunology, Univ. of Miami, Sch. of Med., Miami, FL 33101

In order to achieve our ultimate goal of restoring vision to blinded eyes by use of transplantation of functioning retinal tissue, we have examined the effects of alloantigenicity on the long term survival of intraocular retinal grafts. Neural retina from neonatal BALB/c mouse eyes was implanted into the anterior chamber (AC) of syngeneic or allogeneic (C57BL/6) adult mice. The grafts were evaluated clinically and histologically at 5, 12, 17, 35, 70 and 105 days after transplantation. In this privileged site, both syngeneic and allogeneic grafts successfully implanted, reaching their maximum size at 17 days at which time all grafts displayed recognizable retinal structures, including typical rosettes (differentiated photoreceptor cells). Syngeneic grafts maintained a healthy appearance beyond 35 days, and then began to shrink, reaching a size approximately 30% of maximum at 105 days. Despite the reduction in size, these grafts still retained numerous rosettes (40% of maximum). Allogeneic grafts shrank more rapidly, being 75% of maximum at 35 days, and only 20% at 105 days. When examined histologically, few rosettes were observed in allografts at 35 days (16% of maximum), and none were found subsequently. Neither the slow deterioration of syngeneic grafts nor the much more rapid deterioration of allogeneic grafts was accompanied by clinically or histologically detectable inflammation. These results indicate that privilege can be extended briefly to allogeneic developing neural retina grafts placed in the AC. However, these implants eventually succumb to an atypical immune rejection that is nonetheless complete. In addition, the microenvironment of the AC appears to be only partially hospitable to retinal implants, presumably because the eventual success of these grafts requires appropriate growth and differentiation factors and opportunities for relevant neural connections. Thus, successful engraftment of developing retinal tissue into the eye can be expected to depend upon (a) immunogenetic disparity between graft donor and host, and (b) implantation into a site with an appropriate microenvironment. Supported by NEI Grants EY08865 and EY05678 and a grant from the National Retinitis Pigmentosa Foundation.

452.4

INTRARETINAL GRAFTS OF Y 79 CELLS INTO RATS. AN IN VIVO MODEL OF A HUMAN RETINOBLASTOMA. D. Hodari Brooks*, M. F. Notter, G. Seigel, E. S. Lazar, C. del Cerro, and M. del Cerro. Department of Neurobiology and Anatomy, University of Rochester Medical School, Rochester, New York, 14642, USA.

Retinoblastoma is the most common eye malignancy in childhood. This study reports on the use of a human retinoblastoma cell line (Y 79) for intraretinal xenografting into adult mammalian eyes. We hoped to develop an in vivo model to study the differentiation and invasive characteristics of these cells after they have been returned to the intraocular milieu from which the progenitor cells originated. Using the method of Lazar and del Cerro (1989) for multisite retinal grafting, 3H-thymidine labeled Y 79 cells were suspended in human plasma and injected under direct visualization into the subretinal space of Fisher 344 rats. The host received daily injections of Cyclosporine A, and were sacrificed 30 to 60 days following transplantation. Biomicroscopical observations showed vitreal invasion by masses of floculent material. Histologically these formations were found to be vitreal outgrowths of grafted cells lodged in subretinal and intraretinal locations. Multiple clusters of highly anaplastic cells were seen in the transplanted eyes. There were signs of continued and intense cell division within the grafts but there was no indication of cell-mediated host reaction against the transplant. These results reveal that following intraretinal xenografting, human Y 79 retinoblastoma cells survive, actively grow, and express a high degree of malignancy within the host eye. This is an attractive model for the in vivo study of human retinoblastomas and of retinal cytogenesis. (Supported by EY 05262, the Rochester Eye Bank, and private donations)

452.5

HOST PUPILLOCONSTRICTION IS LOST WITH INDUCED REJECTION OF RETINAL TRANSPLANTS R. Banerjee, J.D. Radel and R.D. Lund, Dept. Neurobiology, Anatomy & Cell Science, Univ. Pittsburgh Sch. Medicine, Pittsburgh, PA 15261.

Embryonic retinae transplanted to the the dorsal midbrain surface of newborn rats can, at maturity, mediate host pupilloconstriction in response to transplant illumination. Selective immunological destruction of retinal transplants can be achieved by peripheral sensitization of the host immune system. The functional consequences of immunological lesioning of cross-strain transplants are reported here.

Newborn Long-Evans (L-E) rats were used as hosts, and embryonic (E14) Sprague-Dawley (S-D) rats supplied retinae for transplantation. Immune rejection of transplants was initiated when skin from adult S-D rats was grafted to the flank of mature L-E hosts. Transplant-mediated pupilloconstriction was measured over a range of stimulus intensities 5 to 25 days after skin grafting. Hosts were killed and the brains processed for histology at selected intervals to determine the extent and pattern of lymphocytic infiltration of the transplant.

Transplant-mediated pupilloconstriction was relatively unchanged during the first week after skin grafting, and was lost by the end of the second week. Perivascular cuffing was initially seen in transplants 8 days after skin grafting, while lymphocytic infiltration was first observed along the external surface of transplants 10 days after skin grafting and throughout the transplant by 15 days. This approach permits a quantitative assessment of the loss of transplant-mediated pupillary responses and how it relates to progressive destruction of the transplant.

Supported by NIH grant EY 05283.

452.6

ORGANIZATION OF EMBRYONIC RABBIT RETINAL TRANSPLANTS OF AGGREGATED DONOR TISSUE. [1]M. Seiler, [1]R. Aramant, [2]A. Bergström*, [2]B. Ehinger, [1]A.R. Adolph and [3]K.R. Fry. [1]Eye Research Institute, Boston, MA 02114; [2]Dept. of Ophthalmology, Univ. of Lund, Sweden; [3]Baylor Coll. of Med., Houston, TX 77030.

Immunohistochemistry was used to characterize the development of ganglion, horizontal and rod bipolar cells in retinal transplants. Cell aggregates of pigmented (Dutch-Belted) rabbit E 16 (=embryonic day 16) retinas were transplanted with and without RPE to lesioned retinas of adult rabbit hosts. Host animals were sacrificed 8 weeks after surgery and perfused with 4% paraformaldehyde. Frozen sections were stained with the monoclonal antibody **AB5** and antibodies against **PKC** (Protein Kinase C; Amersham), **MAP 1A** (microtubule-associated protein 1A; Amersham).

The **AB5**-antibody stained retinal ganglion cells and their processes in the host retina. Sometimes, small AB5-immunoreactive cells were seen in the transplants which did not resemble retinal ganglion cells. The **MAP 1A**-antibody labelled horizontal cells and ganglion cell bodies and dendrites in the host retina. In the transplants, MAP 1A-stained horizontal cells were frequently seen in the outer plexiform layers. Cells with the morphology and location of ganglion cells were rarely observed, but some inner plexiform areas showed MAP 1A-positive fibers. **PKC**-immunoreactivity in the host retina was most prominent on bipolar terminals at the border between inner plexiform and ganglion cell layer. Bipolar cell processes, somata, and terminals in the outer plexiform layer were more faintly stained. In the transplants, PKC-immunoreactive cells were frequently observed. The distribution of stained terminals in the inner plexiform areas was irregular; terminals in outer plexiform areas were more often seen. Occasionally, graft bipolar cells appeared to terminate on host cells.

In conclusion, retinal transplants derived from cell aggregates contain horizontal and bipolar cells with disturbed organization, but very few, if any, ganglion cells. *Supported by NIH-grant R01-EY8519 to R. A.*

452.7

KINETICS OF REFLEX INHIBITION IN NORMAL AND LIGHT-DAMAGED RATS. G.P. Bowen*, J.R. Ison, and M. del Cerro, Departments of Psychology, and Neurobiology, and the Center for Visual Science, University of Rochester, Rochester, NY 14627

In normal rats, as in humans, a light flash inhibits an acoustic startle reflex elicited briefly afterwards, with the degree of inhibition determined by the intensity of the light as well as by its lead time. We will demonstrate that the initial effect of light damage to the retina in the albino rat slows the rate at which peak inhibition develops (the standard 30 ft-c. flash has its peak at about 70 ms, which shifts by about 100 ms with 48 hour exposure to 300 ft-c). Decrements in the strength of inhibition are seen only with longer exposure times. The same temporal shift precedes the loss of inhibition in RCS rats, in which photoreceptor damage occurs because of a degenerative disease. We show further that the isolated temporal shift in peak inhibition seen with early retinal damage cannot be simulated in normal rats by varying the stimulus intensity, the stimulus duration, or the degree of light-adaptation. This data reveals that these early disease states cannot be modeled solely by decrements in sensitivity or integration time, or dark-adapting mechanisms. We can conclude from this study that early retinal damage directly retards the speed of sensory processing. (Supported by NEI-05262, EY-01319, EY-08243, and generous private donations.)

452.8

RETINAL TRANSPLANTS PRODUCE VISUAL IMPROVEMENT IN LIGHT-BLINDED RATS. M. del Cerro, G. P. Bowen, J. R. Ison, D. A. Gr, E.S. Lazar, and C. del Cerro. Depts. of Psychology, Neurobiology, and Ophthalmology, University of Rochester, Rochester, New York.

Light flashes inhibit subsequent acoustic startle responses. This inhibition is abolished by retinal damage. We sought to determine if retinal grafts would restore it. Fischer 344 rats were exposed to continuous fluorescent light at 300 ft-c or 1 ft-c for 4 weeks. Following this, fetal rat retinal cells were grafted intraretinally into one eye of each of the rats exposed to 300 ft-c. Reflex inhibition was measured before, during, and after continuous light exposure, and again following transplantation. Light flashes were presented from 20 to 500 ms before a sound burst. Prior to continuous light exposure, reflex inhibition was maximal at 40 to 70 ms. Inhibition was progressively delayed during the first two days of 300 ft-c exposure, and then disappeared. During the post-exposure period, inhibition was replaced by an anomalous peak of excitation. Grafted animals showed a reduction of this anomalous facilitation, and a partial recovery of inhibition. Comparison between the "peak post-exposure performance" of grafted rats (N=9) with the "peak post-exposure performance" of non-grafted rats (N=9) showed statistically significant differences: $p < .05$ overall; $p < .05$ for the reappearance of inhibition; $p < .02$ for the loss of anomalous excitation. Neither variations in flash intensity nor dark adaptation mimic the effects of light damage on inhibition or the anomalous appearance of reflex facilitation. The control rats subsequently received retinal grafts and similar results were observed. We conclude that retinal grafts in one eye partially repair the defects caused by the blinding action of continuous light. (Supported by EY 05262, EY-01319, the Rochester Eye Bank, and private donations).

452.9

RETINAL PIGMENT EPITHELIUM (RPE) TRANSPLANTATION DELAYS AGE RELATED RETINAL CELL DEATH IN THE FISCHER RAT. Keiko Yamaguchi*, Suzanne V. Stovall**, Vinod. P. Gaur**, James E. Turner**. Department of Ophthalmology, Tohoku University School of Medicine, Sendai Japan*. Department of Neurobiology and Anatomy**, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC. USA.

Age related retinal cell death in macular degenerations may in part be due to an aging RPE which loses its capacity to support photoreceptor cells. We wished to test the hypothesis of delaying photoreceptor cell death in the aging, normal retina by introducing young RPE into older eyes of an appropriate model. We have used the Fischer-344 rat where age related death of photo-receptor cell in the peripheral retina has been demonstrated. Peripheral retinas of 3-month-old rats were transplanted with pigmented and/or nonpigmented 6-8 day-old RPE. Morphometric analyses at 3, 6, and 9 months after transplantation indicated a significant delay in the age related cell death in the outer and inner nuclear layers and the two plexiform layers. Upto 9 months after transplantation, the grafted eyes showed significant neuronal rescue in the various layers. RPE transplantation appears to have benefical effects on the aging eye.

452.10

OPTIC INPUTS TO THE OLIVARY PRETECTAL NUCLEUS FROM HOST AND TRANSPLANTED RETINAE: SEGREGATION, CONVERGENCE AND IMPLICATIONS FOR BEHAVIOR
J.D. Radel, D. Kustra* and R.D. Lund, Dept. Neurobiology, Anatomy & Cell Science, Univ. Pittsburgh Sch. Medicine, Pittsburgh, PA 15261.

Embryonic rodent retinae transplanted over the midbrain of newborn host rats establish connections with the host's olivary pretectal nucleus (OPN), an obligatory nucleus in the neural circuit subserving the pupillary light reflex. Pupilloconstriction can be elicited in the host by direct illumination of the transplant, and transplant-mediated responses are enhanced when host optic input is eliminated. No such change is observed in the pupillary reflex of control rats in response to illumination of the contralateral eye after intracranial sectioning of the ipsilateral optic nerve.

Anatomical studies after labelling the retinal projections of the transplant and host eye (in experimental rats) or both eyes (in normal rats) with different markers shows that the transplant and host inputs converge in the host OPN, while inputs from the eyes of normal rats are segregated to different regions of the nucleus. The convergence of transplant and host inputs may be reflected in the interaction of transplant and host inputs to determine host pupil diameter, and provides an anatomical basis for the rapid enhancement of transplant-mediated pupilloconstriction when host optic input is eliminated. Segregation of the left and right retinal inputs in normal rats, on the other hand, may serve to limit the functional interaction of inputs from the two eyes.

Supported by NIH grant EY 05283.

452.11

TECTAL GRAFTS PROMOTE RECOVERY FROM BEHAVIORAL DEFICITS AFTER INFERIOR COLLICULUS LESIONS. M.C. Zrull, J.R. Coleman and C. Bennett*. Dept. of Psychology, Univ. of South Carolina, Columbia, SC 29208.

Fetal tectal tissue grafted to inferior colliculus (IC) lesion sites has certain neuronal and cytoarchitectonic features similar to adult rat IC. Cell populations of tectal grafts exhibit sound-evoked activity and could play a functional role in the damaged auditory pathway. Neurons of the IC provide a substrate across which the spatial loci of sound sources may be encoded from convergent binaural afferent information. We examined deficits in spatial audition after bilateral lesions of adult rat IC and recovery from deficits following tectal grafts into the lesion sites.

In a lick suppression paradigm, hooded rats ($N=13$) were trained to stop licking when noise bursts (30 to 45 dB SPL) were presented at random loci in the horizontal plane. Responses were quantified as a detection ratio (DR) which reduced correct detection by the proportion of incorrect guesses (hits-hits*[false alarms]). Rats perform the spatial detection task well across intensity levels, DR=0.70±.02 (*M±SEM*). Under anesthesia (ketamine:xylazine, 50:10 mg/kg, ip), 5 rats received IC lesions (LO; 1.2 mA DC), 5 rats received grafts of E18 tectal tissue 1 week after lesions (LG), and 3 rats were sham operated. At 21 days post-surgery LO rats exhibited behavioral deficits, DR=0.49±.04, and LG rats had less behavioral loss, DR=0.62±.02. Both groups improved (LO, 0.54±.03; LG, 0.67±.04) by test sessions 35 days post-operative. Comparison of grafted and sham rats (DR=0.78±.02) to LO animals accounted for 65% of the recovery effect, $F'(1,2.5)=20.5$, $p<.025$. Analysis of brain sections shows similar lesion sites in LO and LG rats, and cell loss in the dorsal nuclei of lateral lemniscus (DNLL), a source of IC afferents, in LO animals. Sparing of DNLL neurons is observed in brains of grafted rats. In hooded rats, tectal grafts ameliorate, but do not completely reverse, deficits in spatial audition.

452.12

DIFFERENTIAL SURVIVAL OF SENSORY ELEMENTS IN INTRACRANIAL OTIC TRANSPLANTS. S.E. Hughes and M.S. Silverman. Sensory Neuroscience Lab, Central Institute for the Deaf, St. Louis, MO 63110.

We have previously shown that isolated embryonic rat inner ear can be transplanted to the anterior chamber of the eye of an adult. Initially, E15 inner ears were transplanted in isolation. While a considerable degree of organotypic development occurred in the transplants, the sensory structures present were limited to those found in the vestibular division: cristae and macular surfaces (Silverman and Hughes, 1987, ARO Abstr. 10: 219). When the E15 inner ear is cotransplanted with a portion of E15 brainstem, a considerably different outcome is obtained. With cotransplanted brain present in the anterior chamber, the cochlear duct develops recognizable sensory elements, including spiral ganglion cells and an organ of Corti (Hughes and Silverman, 1989, ARO Abstr. 12: 296).

In order to investigate the possibility that more mature brain might exert a similar supporting role on cochlear development, we transplanted E15 inner ears to either the cortex or the brainstem of a newborn rat. After survival times ranging from 3 to 5 weeks, we found that more than half the transplants survived and developed sensory structures. Transplants made to the cortex only showed evidence of the cristae and macular surfaces of the vestibular division. However, those transplants placed within the brainstem demonstrated organotypic development of the cochlear duct, with an organ of Corti apparent on the basilar membrane.

Other workers have shown that long-term survival of intracranial transplants of embryonic retinae depends on the presence of target cues to guide innervation (Sefton et al., 1987, Dev. Brain Res. 33: 145). While we as yet have no evidence of innervation of the brain by the transplanted inner ear, the improved cochlear development seen in the intraocular cotransplants and brainstem transplants suggests that the proximity of appropriate target cues also may be an important factor for the development of cochlear sensory elements.

453.1

EFFECTS OF TRANSPLANTS OF FETAL HIPPOCAMPAL TISSUE INTO HIPPOCAMPAL LESION CAVITIES ON ACQUISITION OF CUE OR PLACE TASKS BY RATS. R. L. Cannon, R. H. Baisden and M. L. Woodruff. Dept. of Anatomy, J. H. Quillen College of Medicine, East Tennessee State University, Johnson City, TN 37614.

Kimble et al. (Brain Res. 363: 356-363, 1986) found that transplants of fetal hippocampus reduced hippocampal lesion-induced deficits in the Rabinovitch-Rosvold mazes. Woodruff et al. (Prog. Brain Res., 82:367376, 1990) found that rats with hippocampal transplants were more impaired than hippocampal lesioned rats in acquisition of the Morris water maze. These contradictory results may be due to difference in use of internal vs. external cues. The present study tested the hypothesis that hippocampal transplants would effect performance in a radial arm maze with internal discriminative cues differently than performance in the same maze without the cues (i.e. a "spatial" maze). The results indicated that rats with transplants exhibited more working memory errors in both mazes than rats with just hippocampal lesions. These results are compatible with those of Woodruff et al. (Supported by NIH Grant ES 04070-05 to MLW.)

453.2

NORADRENALINE RELEASE FROM GRAFTS OF SUPERIOR CERVICAL GANGLIA OR FETAL LOCUS COERULEUS TRANSPLANTED TO THE HIPPOCAMPUS AS STUDIED BY IN VIVO MICRODIALYSIS M.A. Cenci*, O.G. Nilsson*, P. Kalén* and A. Björklund, Dept. of Medical Cell Research, University of Lund, Sweden.

Extracellular noradrenaline (NA) was monitored in the hippocampus (HPC) after a lesion of the normal NA innervation followed by grafting of either fetal locus coeruleus (LC) or superior cervical ganglia (SCG) tissue. Rats received a bilateral intraventricular injection of 6-hydroxydopamine. Two weeks later, the fornix-fimbria was unilaterally removed by aspiration. Solid grafts of either autologous SCG or fetal LC (E14) were transplanted in the resulting cavity. One year later, a microdialysis probe was implanted in the HPC. Samples were collected in 30 min fractions and analysed for NA by a radioenzymatic assay. At the end of the experiment, the brains were processed for dopamine-ß-hydroxylase (DBH) immunohistochemistry. Basal extracellular levels of NA were higher than normal in LC-grafted rats (47 vs 23 fmol/30 µl), and much lower than normal in SGC-grafted rats (4 fmol/30 µl). The addition of 100 mM KCl to the perfusion fluid for 30 min enhanced NA levels by 7-8-fold in normal, LC- and SCG-grafted animals, and the addition of 5 µM desipramine (DMI), a NA uptake blocker, induced a 4-5-fold increase in NA levels. The subsequent infusion of 1 µM tetrodotoxin (TTX) significantly reduced NA overflow in all three groups. In lesioned controls, infusion of KCl, DMI, and TTX did not modify NA levels, which were close to, or below the limit of detection of the assay (1 fmol) throughout the experiment. Morphological analysis revealed that both LC- and SCG-grafts had developed an extensive DBH-immunoreactive reinnervation in the whole HPC. Thus, although both LC- and SCG-grafts produced rich fiber outgrowth, spontaneous release of NA appeared to occur at a higher rate from central NA neurons. However, pharmacological manipulations modulated NA release from both types of grafts in a similar fashion.

453.3

SURVIVAL AND CONNECTIVITY OF NEURAL GRAFTS IN NORMAL AND DAMAGED HIPPOCAMPAL TISSUE VISUALIZED WITH VOLTAGE-SENSITIVE PROBES. D.M. Senseman[1], J. Fitzgerald*[2], B. Cobb[3], L.J. Ferguson*[4], J.P. Amaya*[1], J. Gonzalez*[1], and G.A. Mickley[3]. [1]Center for Information Visualization, Univ. of Texas at San Antonio, San Antonio, TX 78285; [2]Dept. of Biological Sciences, Rutgers Univ., Newark, NJ 07102; [3]USAF School of Aerospace Medicine, Brooks AFB, TX 78235; [4]AFFRI, Bethesda, MD 20814

The ability of neural grafts to attenuate behavioral effects of radiation-induced hypoplysia of dentata granule cells depends, in part, on the source and final location of the donor neural tissue (Mickley *et al.*, Brain Res. 509: 280, 1990). In this study the long-term survival (>2 yrs) and functional connectivity of fetal neural grafts were directly visualized using optical recording techniques (Senseman *et al.*, NATO ASI, H 39: 330-347, 1990). Both homolgous grafts (hippocampal neurons) and non-homologous grafts (cerebral cortical cells) were investigated. Evoked neural activity was recorded optically from "thick" hippocampal slices (400 um) stained with the voltage-sensitive dye RH155 following either focal and/or field stimulation. Focal stimulation of both homolgous and non-homolgous grafts produced relatively large optical signals that propagated radially from the site of stimulation. In the three non-homologous grafts studied, we failed to find evidence of functional connections between grafted cells and surrounding host neurons. However the spatial/temporal spread of activity from the point of stimulation suggests that ectopically grafted cortical neurons were capable of supporting regenerative electrical activity and may have developed a neural plexus within the graft. By comparision, in the two homologous grafts studied, focal stimulation of the graft evoked propagated responses that transversed the graft boundary and invaded surrounding host tissue. These results support the view that homologous grafts are more likely to form functional connections with surrounding host neurons than non-homolgous grafts. *Support by: AFSOR-89-0118, ARP 2227, BNS-0794 & AFRRI B4163*

453.4

INTRAHIPPOCAMPAL GRAFTS FROM FETAL STRIATUM AMELIORATE SPATIAL MEMORY DEFICITS IN RATS WITH FIMBRIA-FORNIX LESIONS. Y.J. Li[1], J.R. Simon[2], W.C. Low[1], [1]Dept. of Neurosurgery, Univ. of Minnesota Medical School, Minneapolis, MN 55455, and [2]Dept. of Psychiatry, Indiana Univ. School of Medicine, Indianapolis, IN 46202.

Previous studies have shown that intrahippocampal grafts of cholinergic septal neurons are capable of ameliorating spatial memory deficits in rats with bilateral fornix lesions. In the present study, we have examined the behavioral and biochemical effects of transplanting cholinergic neurons derived from the striatum of fetal rats. Embryonic striatal neurons were transplanted into the hippocampal formation of adult rats with fimbria-fornix lesions. Spatial reference memory and spatial navigation performance were evaluated using a Morris water maze, and spatial working memory performance was evaluated using a T-maze. Animals with striatal grafts exhibited significant improvements in performance on all three spatial tasks in comparison to animals with lesions alone. Atropine administration dramatically disrupted spatial navigation performance in animals with grafts and sham controls. Regression analysis indicated that improvements in spatial memory function were significantly correlated with increases in high affinity choline uptake. We thus conclude that intrahippocampal grafts of striatal tissue produce similar effects as grafts derived from the medial septal nucleus.

453.5

FETAL HIPPOCAMPAL TRANSPLANT PROMOTES RECOVERY OF WIN-SHIFT BEHAVIOR AFTER HIPPOCAMPAL LESIONS. J.D. Chudik* and A.G. Gittis. Psychology Department. Westminster College, New Wilmington, PA 16172.

The extent to which fetal hippocampal tissue, extracted from embryos (17-19 gestation days) could ameliorate the alternation deficit seen in rats after hippocampal lesions was investigated. Prior to a hippocampal lesion, subjects were trained on a win-shift alternation task to a criterion of 18/20 correct responses over two days. In the first surgical procedure, the dorsal hippocampus and overlying cortex was removed via aspiration and win-shift behavior was reassessed. Only animals which demonstrated a marked win-shift deficit (less than 50% accuracy over two sessions) were continued in the study. A second surgical procedure was performed. Half received fetal transplants, the others underwent a sham surgery. Win-shift behavior was reassessed at 2 weeks and 3 months after the second surgery. Experimenters who conducted the behavioral testing were unaware of an animal's group assignment. Hippocampal animals, throughout testing, were severely impaired in win-shift performance demonstrating characteristic response perseveration, alternating less than 10% of the time. Transplant animals tended to alternate slightly more at 2 weeks post-surgery (38%) and improved even more at 3 months (59%). Histological analysis of cresyl violet and Kluver-Barrera stained sections revealed that dorsal hippocampal tissue was absent between 1.8 and 4.8 mm posterior to bregma in both the transplant and non-transplant subjects. The brains of fetal tissue recipients had anomalous cellular aggregations adhering to and incorporated within thalamic tissue in the evacuated hippocampal cavity. Thus it appears that transplanted fetal hippocampal tissue can reduce the response perseverative tendencies in hippocampal subjects in a win-shift task.

453.6

DENTATE GRANULE CELL TRANSPLANTS RESCUE MOLECULAR LAYER INPUTS. J. Wells and B.P. Vietje. Department of Anatomy and Neurobiology, University of Vermont, Burlington, VT 05405.

When dentate granule cells were axotomized by fluid injections into the infragranular cleavage plane, the molecular layer (ML) shrank, due to the loss of both the inputs to the ML and their targets, the granule cell dendrites. If the fluid which created the axotomy also contained immature neurons, the same injection which made the lesion could replace the adult granule cells with potential target neurons. When immature granule cells replaced the adult granule cells, the ML was not as thin as the lesioned ML without transplants and cytochrome oxidase staining was restored in the ML. AChE-positive fibers also increased and were redistributed without distinct laminae. When immature septal cells replaced the adult granule cells, the lesioned ML continued to shrink and there was no cytochrome oxidase staining in the lesioned ML. Granule cell transplants rescued the lesioned ML and could have restored the normal circuit. Septal cell transplants were not adequate targets for ML inputs but grew into the intact molecular layer and perhaps established new circuits. Supported by PHS NS23266

453.7

TERMINAL FIELD SPECIFIC MONOCLONAL ANTIBODIES THAT DEMONSTRATE REINNERVATION OF ADULT RAT DENTATE GYRUS BY EMBRYONIC ENTORHINAL GRAFTS. P.L. Woodhams,* H. Kawano,[1] G. Raisman. (SPON: Brain Research Association). Norman & Sadie Lee Research Centre, Nat. Inst. for Medical Research, London NW7 1AA, U.K. [1]Dept. Anatomy, University of Tokushima, Japan.

We have derived monoclonal antibodies which give complementary staining patterns in the dentate molecular layer: OM-1 to OM-4 selectively recognise the outer two-thirds (the entorhinal terminal field), and IM-1 the inner one-third (the commissural-associational zone). OM-1 recognises a 93 kDa glycoprotein antigen, OM-2 to OM-4 a second glycoprotein of 36 kDa, and IM-1 multiple antigens of 120-260 kDa.

After unilateral entorhinal lesions, OM immunostaining was abolished in the ipsilateral entorhinal afferent zone of the dentate gyrus. At the same time the IM-imunoreactive inner zone acquired OM-1 staining and expanded by 10 μm in width, while the distal zone shrank by 80 μm. When grafts of embryonic entorhinal cortex were placed in the entorhinally deafferented distal zone of adult hosts, 14 out of 26 cases showed restoration of OM-1 immunoreactivity which was restricted to a band in the middle third of the molecular layer. However, staining for the antigen recognised by antibodies OM-2 to OM-4 was restored throughout the entire outer two-thirds of the dentate molecular layer.

Our results suggest that the membrane-associated glycoprotein antigens detected by the OM monoclonals may be part of an intrinsic signalling system used in normal development and in reinnervation of the adult by embryonic entorhinal transplants to direct and restrict the distribution of entorhinal axons to the appropriate part of the dentate molecular layer, the distal two-thirds. This hypothesis is currently being tested in vitro, using roller tube cultures of hippocampal slices.

453.8

DENDRITIC GROWTH FROM FETAL HIPPOCAMPAL CELLS TRANSPLANTED AFTER TRANSIENT FOREBRAIN ISCHEMIA IN RATS AS DEMONSTRATED BY MICROTUBULE-ASSOCIATED PROTEIN 2 (MAP2) IMMUNOHISTOCHEMISTRY. Morimichi Koshinaga*, Yoichi Katayama, Shuhei Miyazaki*, Takashi Tsubokawa* . *Department of Neurological Surgery , Nihon University School of Medicine, Tokyo 173, Japan*

Cell suspensions prepared from 17-18 gestational hippocampi were transplanted into the area just above the hippocampus of male Wistar rats (300-350g) following transient forebrain ischemia, and the dendritic growth patterns of the transplanted cells were examined by microtubule-associated protein 2 (MAP2) immunohistochemistry. The ischemic insult was carried out by hemorrhagic hypotension and bilateral occlusion of the carotid arteries at 7 days before transplantation. Four weeks after transplantation, the animals were perfused transcardially and fixed in 2% paraformaldehyde, and the brains were removed and embedded in paraffin. Consecutive paraffin sections of 5μm in thickness were stained by the ABC method, employing the anti-serrum rabbit anti-pig MAP2 polyclonal antibody (Penninsula Laboratories Europe) and anti-calbindin D monoclonal antibody (Sigma). It was found that far more transplanted cells survived and extensive dendritic growth occurred when complete death of CA1 pyramidal cells had been induced by the ischemic insult. The dendrites demonstrated two growth patterns: the cells located in the dorsal part gave off dendrites towords the dorsal direction, and the dendrites of the cells located in the ventral part oriented themselves towards the ventral direction, much like the parallel arrangements of apical dendrites of normal CA1 pyramidal cells. These findings suggest that dendritic growth from transplanted hippocampal cells is dependent on the absence of host hippocampal cells and is guided by the presence of remaining free terminals of afferent fibers.

453.9

REARRANGEMENT OF THE SEROTONERGIC AND DOPAMINERGIC FIBERS IN THE HIPPOCAMPAL FORMATION OF THE RAT. INTRAVENTRICULAR FETAL RAPHE TRANSPLANT AND INTRAOCULAR DOUBLE-GRAFT OF FETAL RAPHE AND HIPPOCAMPUS. S. Ueda' and M. Kawata. Dept. of Anatomy, Kyoto Pref. Univ. of Med., Kyoto 602, Japan.

Pieces of fetal midbrain raphe tissue were transplanted into the third ventricle near the dentate gyrus of adult host rats that had previously been denervated by treatment with 5,6-dihydroxytryptamine. In another experiment, the fetal midbrain raphe tissue was co-grafted with the fetal hippocampus including the dentate gyrus in the anterior chamber of the eye of adult rats. One and three months after transplantation, the extent of axonal outgrowth of raphe tissue into the hippocampus was studied using serotonin and tyrosine hydroxylase (TH) immunohistochemistry. Serotonin fibers were densely distributed throughout the raphe graft tissue, while TH fibers were restricted to an area near the somata of TH positive neurons. A large number of serotonin fibers were distributed in the hippocampus, especially in the molecular layer of dentate gyrus, while only a few TH fibers were observed in the hippocampus. These observations suggest that the serotonergic and dopaminergic neurons located in transplants of midbrain raphe have different reinnervation patterns in the hippocampal tissue.

AGING PROCESSES IV

454.1

AGE DIFFERENCES IN RABBITS IN THE DELAY CLASSICAL CONDITIONING PARADIGM USING 400 AND 750 MSEC CS-US INTERVALS. D.K. Sasse, J.M. Coffin, & D.S. Woodruff-Pak. Dept. of Psych., Temple Univ., Phila., PA 19122.

To explore aspects of eyeblink conditioning which are most impaired by processes of aging, we compared the performance of young (Y) and older (O; minimum of 24 months old) rabbits in the 400 and 750 msec CS-US interval delay paradigms. For all rabbits a 1 KHz, 85 dB tone was the CS, and a 3 psi, 100 msec corneal airpuff was the US. Twenty-four Y and 18 O rabbits were run for 90 trials/day until they attained a learning criterion of 8/9 CRs and then were overtrained a minimum of one day. Both O and Y rabbits required significantly more trials to reach criterion with a CS-US interval of 750 msec ($F=14.88$, $p<.01$). In the 400 msec paradigm O rabbits required 300 trials to criterion, while they required 713 trials to criterion in the 750 msec paradigm. Y rabbits required 171 and 343 trials to criterion with a 400 and 750 msec paradigms, respectively. These age differences were highly significant ($F=20.52$, $p<.01$). The age by paradigm interaction was significant at the .1 level ($F=3.47$), suggesting that O rabbits are more impaired at the longer 750 msec CS-US interval. Longer CS-US intervals impair acquisition in rabbits, and processes of aging exacerbate the difficulty at longer intervals. *Supported by a Research Incentive Fund Grant from Temple University.*

454.2

MULTIVARIATE ANALYSIS OF BEHAVIORAL AGING HIGHLIGHTS SOME UNEXPECTED FEATURES OF COMPLEX SYSTEMS ORGANIZATION. A. Giuliani *, O. Ghirardi *, A. Caprioli *, S. Di Serio *, G. Taglialatela, M.T. Ramacci , L. Angelucci [1]. Sigma Tau, Institute for Research on Senescence, Pomezia, Rome; [1]Institute of Pharmacology II, La Sapienza University, Rome, Italy

Male Fischer 344 rats aged 5 and 24 months were subjected to 10 behavioral tests for a total of 22 variables, including spontaneous activities, memory, and learning measures.

The behavioral performances of the two age groups were significantly different in most tests, indicating a clear factorial structure of the whole data set. Principal components analysis has evidenced a first factor describing some 40% of the information concerning the 22 variables with a 100% discriminating efficiency between the two groups. This first component may be taken as a comprehensive measure of the animals' "behavioral age". On the contrary, the same analysis performed separately on the two groups revealed a relationship pattern between variables, which was different for the two ages, thereby indicating different meanings for the same parameters and their different behavioral utilization in the two classes. With the aid of the spin glasses physical model, these results lead to the definition of the difference between causal linking and correlations driven by an external parameter.

454.3

TREATMENT OF d-AMPHETAMINE REDUCE AGE-RELATED MEMORY DEFICITS IN RATS. G.L. Dunbar, G.A. Smith, L.S. Janis, B.J. Snyder, and L.A. Mead. Dept. of Psychology, Central Michigan University, Mt. Pleasant, MI. 48858.

Since previous studies have indicated that some age-related memory deficits may be a result of reduced levels of catecholamines, we have attempted to counteract these behavioral deficits by treating rats 6, 12, or 24 months of age with either 0.5, 1.0, or 2.0 mg/kg d-amphetamine or saline (control). All rats were tested for 8 days on a Morris water maze task. Daily IP injections were given 20 minutes prior to testing. Rats were perfused 24 hours following the final testing day and their brains were processed using cytochrome-oxidase (CYO) histochemistry. Both the 12 and 24 month-old control rats took more time and swam further searching for the platform than did the 6 month old controls. These deficits were reduced by treatments of 1 mg/kg d-amphetamine in the 24 month old rats, while the 2 mg/kg dose caused further impairments in these aged rats. Age-related deficits in CYO activity were observed in the dorsalmedial thalamus, nucleus basalis, and caudate nucleus of the 24 month old rats and in the dorsalmedial thalamus of the 12 month old rats, irrespective of treatment. However, these histochemical deficits did not correlate significantly with either of the behavioral measures used, suggesting that amphetamine is affecting behavior in other ways.

454.4

PRINCIPAL COMPONENT ANALYSIS HELPS TO CLARIFY THE STRUCTURE OF A DATA SET: A CASE STUDY ON FOUR BEHAVIORAL PARAMETERS. S. Algeri*, A. Giuliani*, N. Pitsikas[1]*, Sigma Tau Institute for Research on Senescence, Pomezia, Rome; [1]Institute Pharmacol. Res., M. Negri, Milano, Italy.

Sprague Dawley rats (N. = 86) of different ages, fed standard or hypocaloric diet, were subject to a radial water maze test in order to assess the effect of age and diet on their performances. Four performance indeces were singled out from the test: 1) Spatial reference memory 2) Spatial working memory, 3) Non spatial reference memory 4) Non spatial working memory. These parameters, measure over 10 subsequent sessions, were globally analyzed with the aid of the principal component analysis (PCA), which highlighted a first factor explaining 65% of total variability. This factor was highly correlated with time course as measured by the successive sessions (R=0.9). Such a relation individuated the factor as "learning": all the parameters had a significant factor loading on this component.

When PCA was performed separately at each session, thus eliminating the "learning effect", the 4 kinds of memory resulted to be completely uncorrelated, thence pointing out to the role of "order parameter" exerted by learning with respect to the measured parameters.

Aging was demonstrated to decouple learning and working memory performances and this decoupling was antagonized by an hypocaloric diet.

454.5

HIPPOCAMPAL MOSSY FIBER ZINC AND SPATIAL MEMORY IN AGED RATS. D. Guidolin*, P. Polato*, A. Zanotti*, E. Mocchegiani*[1], N. Fabris*[1] and M.G. Nunzi. Fidia Research Laboratories, 35031 Abano Terme (PD) and [1]Center of Immunology, Dept. of Gerontologic Research, INRCA, 60121 Ancona, Italy.

Zinc is known to play a key role in synaptic function of the mossy fiber system, a pathway deeply involved in the modulation of processes related to spatial learning and memory. We evaluated the amount of histochemically detectable zinc in the mossy fibers from young adult (5 mo) and aged (25 mo) rats tested for spatial performance. Computer-assisted microdensitometry and x-ray microanalysis showed a significant difference (40%) on the neo-Timm's staining between memory-impaired and non-impaired aged rats, thereby suggesting a correlation between age-associated memory deficits and localized changes in hippocampal zinc levels.

Furthermore chronic oral administration of phosphatidylserine has been reported to significantly improve spatial memory in aged rats with a severe place-navigation deficit (Zanotti et al., 1989, Psychopharmacol., 99:316). Since phosphatidylserine restores plasma zinc levels in aged rats, we are currently evaluating the effects of this phospholipid on hippocampal zinc levels in aged memory-impaired rats.

454.6

A NEW TEST DESIGNED TO ASSESS MOTOR IMPAIRMENT IN AGED RATS. F. García-Hernández and R. Drucker-Colín. Instituto de Fisiología Celular, UNAM. Apdo. Postal 70-600, México. D. F.

Neurodegenerative disorders which involve motor impairment is characteristic of old age. Although there are a few tests which attempt to assess motor incapacities, many have utilized scales which have either a great deal of subjective evaluations or are subject to learning-performance complexities. This study describes a method able to measure motor impairment of aging rats which is subject to dopaminergic influences and has negligible practice effects. The test is designed so that rats have to traverse 2 meter beams of 15° inclination whose widths 3,6,12,18 and 24 mm are changed on each test session using a table of random numbers. The time ceiling allowed for traversing the 2 m beams was established at 120 sec. 3 month old rats (N=20) and aged rats (N=20) with a mean age of 26.5 ± 3.8 months ranging from 23 to 34 months were utilized in this study. All young rats traversed the beams, independently of beam width, while virtually none of the old rats traversed the 3 and 6 mm beams. However, as the beam width increased more and more aged rats ascended the beam. Nevertheless, there were always a few old rats who were unable to cross even on widest beam. When young rats were fitted with a lead belt which increased their body weight by approximately 40%, they still traversed all beam widths. On the other hand, Haloperidol (.1 and .2 mg/kg) severly impaired the performance of young rats. Conversely administration of amphetamine (1.0 mg/kg) or L-dopa (50 mg/kg) to old rats substantially improved their performance. The results suggest that beam taxis appears to be an effective and efficient test for screening motor dysfunction in aged rats and can be amenable to studies on changes of motor capabilities as a result of drugs and/or brain damage.

This work was partially supported by FIIRESIN.

454.7

AGE DEPENDENT ALTERATION OF NERVE TERMINAL ACTIVITY AT THE NEUROMUSCULAR JUNCTION (NMJ) OF RAT; A MICROELECTRODE STUDY. M.D. Sokoll, B. Bhattacharyya,* and K. Tsen*. Department of Anesthesiology, University of Iowa College of Medicine, Iowa City, Iowa 52242

Rat NMJ has been chosen by several workers to study synaptic aging. Using the two microelectrode voltage-clamp technique, we studied age induced alterations of ACh release in phrenic nerve-diaphragm preparation of young (3 month old) and old (30 month old) Sprague-Dawley rats. Results of this study indicate that spontaneous release of ACH measured by recording miniature end-plate current (MEPC frequency) was significantly higher in old rats. Quantum content calculated by the direct method (recording MEPC and end-plate current (EPC evoked at 0.4 Hz from same cell)) indicate a larger quantum content in old muscle compared to the young. The EPC quantum content was also studied by recording MEPC's immediately before the in trains of EPC's elicited at 40 Hz. In these studies, quantum content of first EPC as well as the EPC in the plateau phase in a train is greater in old muscle than in young. A significant increase in neurotransmitter mobilization is also observed in old muscle. These results suggest altered capabilities for sustaining synaptic transmission by increasing nerve terminal activity in a continuously active muscle of senescent rats.

This study was supported by American Federation for Aging Research and Department of Anesthesia Trust Fund.

454.8

ACETYL-L-CARNITINE RELEAVES AGE- AND TRAUMA-INDUCED NEUROPATHIES IN RATS. C. De Angelis*, C. Scarfò*, M. Falcinelli*, A. Bellucci*, L. Pacifici*, E. Reda*, M.T. Ramacci, L. Angelucci[1] Sigma Tau Institute for Research on Senescence, Pomezia, Rome; [1] Institute of Pharmacology II, La Sapienza University, Rome, Italy

The neuronal trophic effects of Acetyl-L-Carnitine (ALCAR) prompted investigation on the substance favorably affecting peripheral neuropathies, both age-related (reduced neuromuscular conduction velocity (NMCV)); and trauma-induced (distal axonal degeneration, denervation of the soleus and extensor digitorum longus (EDL), and changed morphology of regenerated motor end-plate). NMCV was evaluated in the sciatic nerve soleus muscle preparation in anesthetized 3 - and 24 - month - old Fischer 344 rats, following acute (50-100 mg/kg i.v.), or 6 - month long-term treatment (150 mg/kg/day in drinking water) with ALCAR, and resulted to be increased. Anesthetized 3-month-old Sprague Dawley rats were subjected to sciatic nerve crush and morphological and morphometric evaluations were carried out on sections of muscle (combined silver-cholinesterase stain) and on tibial nerve sections (toluidine blue and antineurofilaments antibodies). Treatment with ALCAR (15-60 days, 150 mg/kg/day in drinking water) increased number, area and diameter of regenerating axons; decreased the number of degenerating elements; and maintained the ratio of axon/ myelinated fiber diameter within the range of the optimal values for conduction velocity. The number of branching points of nerve terminals x end-plate length rose significantly, and morphology of the end-plate was similar to that of unlesioned animals. Besides possessing a neurotrophic effect, ALCAR favors the activity of sprouting factors released from the denervated muscle, thereby enhancing synaptical transmission.

454.9

FURTHER ANALYSIS OF SENSORIMOTOR DYSFUNCTIONS IN AGED RATS: ADHESIVE PAPER TEST. T. Schuurman* and M. de Jonge. Troponwerke, Inst. for Neurobiology, Dept. of Geronto-pharmacology, Berliner Str. 156, 5000 Köln 80, FRG

Until now little research has been directed to the study of age-related sensorimotor dysfunctions compared with the number of studies on age-dependent cognitive deficits. Our previous studies showed an impairment of motor coordination of old rats subjected to balance rod and pole climbing tests. An analysis of the footprints of aged rats showed a disturbance of the coordination of the movements of the hindlegs during walking. In two experiments we assessed differences between young and old rats in stimulus-directed movements of the forepaws. In the first experiment, small pieces of adhesive paper were placed on various parts of the rat's snout or forepaws. The time needed to remove the sticky paper was measured. 25-month-old rats needed 2-4 times longer than 3-month-old rats to remove the stimulus. In the second experiment young and old rats were repeatedly subjected to the adhesive paper test. Latencies to remove the sticky paper decreased significantly in 3-month-old rats. In contrast, 25 month-old rats showed no improvement of their sensorimotor skills.

454.10

QUANTITATIVE ASSESSMENT OF CROSSED PHRENIC NERVE ACTIVITY AT VARIOUS TIME INTERVALS AFTER SPINAL CORD HEMISECTION IN OLD RATS. H. G. Goshgarian and X.-J. Yu*. Department of Anatomy, Wayne State Univ. Sch. of Med., Detroit, MI 48201.

A recent quantitative electrophysiological study from our lab has shown that there is a significant augmentation of crossed phrenic reflex nerve activity that can be induced as early as 2 hours after spinal hemisection in 3-4 month old young adult rats (Exp. Neurol 111:224-250, 1991). The present study utilized the same techniques on older rats (9-10 months old) to determine if the time course for the augmentation of the reflex between the two age groups was similar. Mean integrated areas under phrenic nerve compound action potentials during the crossed phrenic reflex at 4 hours (26.60 ± 8.62 mm^2) and 12 hours (35.56 ± 14.91 mm^2) were not significantly greater than the mean area under the initial recordings taken at 30 minutes post hemisection (29.29 ± 9.67 mm^2). The first significant ($P<0.01$) increase of mean integrated area occurred 24 hours after hemisection (86.29 ± 26.12 mm^2). Activity measured at 7 days (86.66 ± 23.54 mm^2) and 30 days (99.48 ± 27.65 mm^2) was not significantly different from the enhanced activity measured at 24 hours. From these data, we conclude that crossed phrenic nerve activity increases significantly in older rats after spinal cord injury, but the augmentation of the reflex occurs more slowly than it does in young adult rats.

454.11

THE LONG-TERM EFFECTS OF MUSCLE USAGE ON AXONAL SPROUTING FOLLOWING PARTIAL DENERVATION IN RAT. B.R.Pachter and A.Eberstein*. Dept. of Rehab. Med., N.Y.U. Med. Ctr., New York, NY 10016.

The present study examined the effects of long-term muscle usage on axonal sprouting (intranodal, preterminal, and intraterminal) as well as terminal branch number and endplate area following 1,3,6,9, and 12 months of partial denervation induced by radicular nerve L4 transection in rat. We found that in the partially denervated (PD) plantaris muscles, there was a progressive increase in all types of axonal sprouting up to 9 months post-surgery as compared to sham-operated controls. The number of terminal branches per endplate in the PD muscles remained constant and less than controls from 1 to 6 months post-surgery; at 9 months post-surgery, it approximately doubled from previous values and was about 1.5 times greater than in controls. The endplate area in PD muscles at 3 to 12 months post-surgery was greater than in controls. At 12 months, axonal sprouting and number of terminal branches were decreased when compared to sham controls; denervated endplates and endplates exhibiting degenerative changes were also seen in PD muscles. It would appear that there is an earlier onset of aging-like changes in the PD muscles possibly induced by enhanced stress on the remaining motoneurons which are hyperfunctioning due to a greatly enlarged peripheral field of muscle fibers. Supported by NIH grant NS25624.

454.12

THE EFFECTS OF DIFFERENT LIGHT CYCLES ON LIFESPAN IN CARDIOMYOPATHIC HAMSTERS. S.D. Drastal*, B.H. Natelson, W.N. Tapp, and J.E. Ottenweller*. Dept of Neurosci., N.J. Medical School and VA Medical Center, East Orange, NJ 07019.

Our earlier work showed that hamsters with heart disease lived significantly longer in an environment devoid of time cues than in light/dark (LD) 12:12. To evaluate these results, male cardiomyopathic hamsters (CMHs) were housed two/cage in four lighting conditions: LD 12:12, LD 6:18, LD 6:30, and LL (constant light). In addition, CMHs were housed in LD 12:12, five/cage, but in larger cages. The LD 12:12 and LL groups were chosen to replicate our original finding while the other groups were chosen to evaluate the effects of a short photoperiod (LD 6:18) and an entrained non-24 hour LD schedule (LD 6:30) on lifespan. Survival curves were generated and tested for statistical significance compared to the LD 12:12 two/cage group. Compared to that control group, LL extended life over the entire survival curve, LD 6:18 did so over the first half of the survival curve, and LD 12:12 five/cage group did so over the second half of the curve. These data confirm our earlier work on the life-extending effects of constant light and suggest that other environmental factors related to either cage size or animal density also are important. Supported by V.A. Medical Research.

454.13

SIBERIAN HAMSTERS AGE AT DIFFERENT RATES IN LONG AND SHORT PHOTOPERIODS. C.M. Finley, and I. Zucker, Dept. of Psychology, Univ. of California, Berkeley, CA 94720. In small mammals dry eye lens weight is a reliable marker of chronological age. Lens weights continue to increase throughout the life span even when body weight remains stable. In Siberian hamsters maintained in either long (LD 16:8) or short (LD 8:16) photoperiods after weaning on day 18, eye lenses increase in weight at comparable rates until day 35. Lens weights sampled at 15 weeks of age, however, were significantly greater in short than long-day animals (19.1 ± 0.2 vs 17.3 ± 0.2 mg, $p< 0.001$), though body, testes and uterine weights were significantly greater in long-day than short-day hamsters. Because older animals had heavier lenses than younger ones, these results indicate that Siberian hamsters age at a more rapid rate in short than long photoperiods. This may reflect seasonal differences in aging of free living hamsters.

454.14

NOCICEPTION INCREASES AND THE EFFECTIVENESS OF ANALGESICS DECREASE IN AGING. E.E. Quinton.Lab of Psychobio.,Univ. Louisville, Louisville,ky.40292.

The present study was undertaken to further characterize the age-nociception interaction in the mouse. Two age groups of C57BL/6j mice were used; old (27-30 mo) and adult (8-12 mo). In exp. 1 mice from both groups were inj with saline, morphine (15 mg/k), or CCK (400 µg/k) and tested on a hot plate (55° C) 8 times from 10 min to 120 min after inj. The old-sal group was more responsive through 30 min after inj but did not differ from the adult-sal group thereafter. CCK was analgesic in both groups through 45 min, but the old and adult groups did not differ. Mor. was more analgesic in the adults through 45 min, but less analgesic thereafter. In exp. 2 the formalin test for chronic pain was used and paw-lick was monitored for 60 min after inj of the formalin. Mor. (2 mg/k), CCK (400 µg/k), or sal was inj 5 min before the formalin. The old-sal group had a higher rate of paw-lick from 25min to 60min after formalin than the adult-sal group. Mor. was less effective in the old. CCK produced a short term analgesia followed by hyperalgesia in the adult, and had a stronger effect than mor. in the old group over the duration of the test interval. In exp. 3 & 4 stress-induced analgesia and tolerance to mor. was less in the old mice.

454.15

VENTROMEDIAL HYPOTHALAMIC IMPAIRMENTS DURING AGING? C.V. Mobbs. Rockefeller Univ., New York, NY 10021.

Rats with VMH impairments evince an increased adiposity setpoint which is defended by increased conversion of calories into fat and increased eating, especially during che light phase. To examine if the age-related increase in adiposity is similarly defended, 6-, 12-, and 18-month-old male Sprague-Dawley rats (n=12/age) were fasted for 72 hours. For 1 week after fasting, rats were restricted to consuming only as much food as before the fast. From 1 to 2 weeks after fasting, rats were allowed to eat *ad lib*. Rats weighed more with increasing age; older rats lost the same weight during fasting as younger rats. Rats of all ages gained weight even when restricted to pre-fast consumption, ate more during the *ad lib* period, and by two weeks most rats had regained initial body weights. At all times, the ratio of food eaten during the light to food eaten in the dark (L:D) was greater in older than younger rats.

Food eaten (L:D X 100):	6 Mo.	12 Mo.	18 Mo.
Before fast	10±2	18±2	34±10
After fast (restricted)	0.1±0.1	4±2	13±4
After fast (*ad lib*)	14±2	26±3	29±5

Thus older rats will defend the age-related increase in adiposity in a manner similar to VMH-lesioned rats. We will next examine if the age-related changes in body weight set-point correlates with changes in VMH gene expression. Supported by the Glenn Foundation.

456.16

ENHANCED ACQUISITION OF A SPATIAL MEMORY TASK BY AGED RATS RECEIVING CHRONIC NOREPINEPHRINE (NE) INFUSION OUTLASTS NE TREATMENT. T.J. Collier and P.D. Danielson*. Department of Neurobiology and Anatomy, University of Rochester School of Medicine, Rochester, N.Y. 14642.

Studies of aged rodents (Leslie et al., Brain Res. 359:292, 1985; Collier et al., N.Y. Acad Sci. 495:396, 1987) and non-human primates (Arnsten and Goldman-Rakic, Science 230:1273, 1985) suggest that age-related declines in the locus coeruleus NE system contribute to age-related declines in learning and memory function. As part of our ongoing studies on aging, the NE system and memory function in rats, we examined the effects of chronic intraventricular NE infusion on acquisition and retention of a spatial task in the Morris swim maze. Male F344 rats, 22 months of age, were screened for baseline performance of the spatial task, then implanted with an osmotic mini-pump that delivered 5ug NE/hour into the lateral ventricle over 14 days. During the last eight days of NE infusion, rats were tested for acquistion and retention of a new spatial location in the swim maze. Seven days after termination of NE infusion, rats were tested for learning and memory of a third spatial location in the maze. Acquisition of the spatial task was improved during NE infusion in aged rats that were initially classified as "impaired" at the spatial task (n=4), as well as those aged animals that were relatively "unimpaired" (n=4). One week after termination of NE infusion, improved acquisition performance was maintained, and indeed, was slightly improved over performance during NE infusion. In contrast, 24 hour retention of the spatial location, as measured by distribution of swim-search on a no-platform probe trial, was not improved during or after NE treatment. These findings are consistent with the view that chronic NE replacement in aged rats enhances acquisition of a spatial location in the swim maze, and that NE replacement can influence the system to yield behavioral improvement that outlasts the presence of exogenous NE. In contrast, NE replacement appears to have a minimal influence on retrieval of spatial memory. Supported by R29 AG08133.

NEUROGLIA AND MYELIN IV

455.1

TUMOR NECROSIS FACTOR-α AND INTERLEUKIN-1β INCREASE THE PHOSPHORYLATION OF TWO 30,000 M_R (HEAT SHOCK) PROTEINS IN ASTROCYTES. P.L. Mobley* and D.L. Combs. Dept. Pharmacology, Univ. Texas Hlth. Sci. Cntr., San Antonio, TX 78284.

Cytokines affect various aspects of astrocyte function but little is known about the action of these agents on protein phosphorylation. Cultures of rat astrocytes were labeled with ^{32}P-orthophosphate for 2 hrs and then treated with 1 ng/ml interleukin-1β (IL-1β), 100 U/ml interferon-γ (IF-γ), or 100 ng/ml tumor necrosis factor-α (TNF-α) for 10 min. Treatment with IL-1β and TNF-α increased ^{32}P-incorporation into two 30,000 M_R proteins with pI values of 5.7 and 5.5; however, no effect was observed with IF-γ. Based on the apparent molecular weight, pI values, and phosphorylation response to other agents, these 30,000 M_R proteins are likely the low molecular weight heat shock proteins. Treatment with protein kinase C (PK-C) activators also increased ^{32}P-incorporation into these proteins, however, the effects of IL-1β and TNF-α are observed in PK-C-depleted astrocytes suggesting that their action is not mediated by PK-C. The 30,000 M_R proteins can also be phosphorylated in response to agents which mimic or increase cyclic AMP along with a number of other proteins including glial fibrillary acidic protein (GFAP) and vimentin. However, GFAP and vimentin are not phosphorylated in response to TNF-α and IL-1β suggesting that their action is not mediated by cyclic AMP-dependent protein kinase (PK-A). These studies suggest that protein phosphorylation, mediated by a signal transduction pathway independent of PK-C and PK-A, may play an important role in the action of TNF-α and IL-1β in astrocytes.

455.2

IMMUNOLOCALIZATION OF CALPAIN II IN RAT PERIPHERAL NERVE. M.Mata and D.J.Fink. Dept. Neurology, University of Michigan, and GRECC VAMC, Ann Arbor, MI 48105

Calpain II is a thiol protease which is activated by millimolar Ca^{++}. The protein is a dimer composed of an 80K catalytic subunit and a 30K regulatory subunit. We have used a rabbit polyclonal antibody raised against rat platelet Calpain II (a gift of J. Elce) which recognizes only the 80K subunit in Western blot of homogenate of rat peripheral nerve.

Light microscopic immunocytochemistry of 1 micron cryo-semithin sections of adult rat sciatic nerve using biotin streptavidin peroxidase detection revealed prominent staining of the Schwann cell cytoplasm which extended to the plasmalemma of the Schwann cell. No immunoreactivity was seen in the myelin, or in the axon using this method.

Electron microscopic immunocytochemistry of cryo-ultrathin sections using secondary antibodies bound to colloidal gold confirmed diffuse staining of Schwann cell cytoplasm. There was prominent staining of the plasma membrane and along the external surface of the Schwann cell.

These results suggest that calpain II in nerve may be exposed to the millimolar Ca^{++} concentration required for activation in the extracellular space, and may therefore play a role either at the Schwann cell membrane or in modulation of the extracellular matrix.

455.3

DISTRIBUTION OF mCALPAIN IN RAT BRAIN AND SPINAL CORD. Z.Li*, J.Xu, N.L.Banik and E.L.Hogan. Dept. of Neurology, Med.Univ.of SC, Charleston, SC 29425.

The calcium-activated proteinase calpains are involved in the pathological process in spinal cord injury. By using immunocytochemical ABC technique, we observed the morphological distribution of mcalpain in CNS. mCalpain immunoreactivity (IR) is widely distributed in adult rat CNS.

In spinal cord, mcalpain-containing structures include neurons, fibers, glia. The IR neurons with weak staining appear in laminae III, IV, V, VII, IX and X but the cell membrane and nuclear membrane of motoneurons are distinct. Sparse numbers of IR fibers appear in gray and white matter and dorsal root. Glia in particular fibrous astrocytes with the strongest staining were seen in both gray and white matter and were most dense at the surface of the spinal cord just beneath the pia mater.

In the brain, a few weak-staining IR neurons and sparse fibers appear in hypoglossal and vestibular nuclei, nuclei of trigeminal spinal tract and solitary tract, reticular formation, substantia nigra, hypothalamus, purkinje cells, dentate nucleus and layer V,VI of neocortex. Glia, ependyma and endothelium stained most prominently. Supported by NS-11066 and MS Soc.Grant-2130.

455.4

SEQUENCE ANALYSIS OF J1-160/180 cDNA. B.Fuss* and M.Schachner. Dept. Neurobiology, Swiss Federal Institute of Technology, Honggerberg, 8093 Zurich, Switzerland.

The J1 glycoproteins are glia-derived components of the extracellular matrix which are involved in cell recognition processes. The higher molecular weight J1 forms (200-240 kD) are designated J1/tenascin because they are structurally related to chicken tenascin. The lower molecular weight J1 forms (160 and 180 kD) are expressed by oligodendrocytes and in CNS myelin. Functional studies demonstrate their adhesive substrate properties for astrocytes and their repulsive substrate properties for neurons (Morganti et al.,Exp Neurol 109:98,1990; Pesheva et al.,J Cell Biol 109:1765,1989). Recent findings implicate the neuronal adhesion molecule F3 in the initial recognition process (Pesheva et al., submitted for publication). To characterize structure-function relationships we have isolated a J1-160/180 cDNA clone from a rat oligodendrocyte library (Fuss et al., J Neurosci Res,in press). The cDNA sequence coding for at least one of the lower molecular weight forms shows highest homology to tenascin from mouse fibroblasts. Sequence analysis reveals 4.5 EGF-like domains, 9 fibronectin type III repeats and a C-terminal region homologous to fibrinogen. Members of the J1 family thus appear to be composed of structurally closely related, yet functionally distinct glycoproteins.

455.5

DISTRIBUTION OF ASTROCYTES ALONG THE FREQUENCY AXIS OF THE GERBIL LSO. A. Hafidi*, D. H. Sanes, and W. Cammer. Dept. Otolaryngol and Physiol & Biophys, NYU Med Ctr, NYC, NY 10016

We are interested in the extrinsic signals that may lead to specific neuronal shape along the frequency axis of a central auditory nucleus, the lateral superior olive (LSO; Sanes et al., J Comp Neurol 294: 443, 1990). Since glial cells have been implicated in many aspects of neural development, the purpose of the present study was to determine the distribution of astrocytes along the tonotopic axis of the LSO. Antibodies directed against glial fibrillary acidic protein (GFAP) and glutamine synthetase (GS) were employed on sections of paraffin embedded tissue from adult gerbils (*Meriones unguiculatus*), and visualized with an avidin-biotin-peroxidase amplification system (Vector). In addition, the density of astrocytes was determined from toluidine blue stained semi-thin sections through the LSO.

GS immunoreactivity was restricted to the cytoplasm of small non-neuronal cells. The number of GS-positive cell bodies appeared greater in the low frequency region of the LSO. GFAP immunoreactivity was more prominent in astrocytic processes than their cell bodies, and some of these processes terminated on the blood vessel endothelium within the LSO. The GFAP immunoreactivity was much greater in the low frequency region of the LSO: Preliminary counts of GFAP-positive cells showed a threefold difference between the low frequency and high frequency regions. Preliminary counts of all astrocytes in toluidine blue stained material also showed a heterogeneous distribution. The present results suggest that glial cell distribution along the tonotopic axis of the LSO is correlated with a known difference in dendritic form.

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455.6

AXONAL ALTERATION OF ACTION POTENTIAL CODING IN DEMYELINATED FIBERS. Peter Shrager, Dept. of Physiology, Univ. of Rochester Medical Center, Rochester, NY 14642-8642.

Demyelination may result in propagation delays, failure to transmit impulses at high frequencies, and total conduction block. However, there may also be mechanisms conferring hyperexcitability. Myelinated axons have, in addition to the usual refractory period, a supernormal phase, lasting typically from 10-100 msec after the termination of an impulse. During this time, the threshold for a subsequent action potential is reduced. While this phenomenon may not be apparent in firing patterns in normal fibers, it may play a significant role at sites of reduced safety factor. It has, for example, been shown that threshold changes are accentuated at demyelinated regions (Bostock and Grafe, J. Physiol. 365, 239, 1985). We have used optical techniques to follow conduction along single, identified axons that have been focally demyelinated by an intraneural injection of lysolecithin. Short bursts (1-8 impulses) are repeated 64 times at 10 Hz for signal averaging. By increasing the temperature the safety factor is gradually decreased until bursts consisting of just one stimulus fail to propagate. If the burst is now changed so that it contains 3 stimuli with an intraburst interval of 10 msec an unusual pattern may develop in which one or more of the initial spikes are missing. If the burst was elicited with, for example, 8 stimuli, then the final one or more impulses may also be deleted in demyelinated axons, so that only the middle few action potentials are transmitted. This pattern has been seen in demyelinated internodes, and also in presumptive new nodes of Ranvier forming during early stages of remyelination. We present evidence for the site of integration responsible for these events, and also for possible mechanisms, including the role of paranodal and internodal ionic channels.

Supported by the NIH and the National Multiple Sclerosis Society.

455.7

RELEASE OF CALCIUM FROM INTRACELLULAR STORES OF ISOLATED RETINAL GLIAL (MULLER) CELLS. S.A. Keirstead and R.F. Miller. Dept. of Physiology and Neuroscience Graduate Program, University of Minnesota, Minneapolis, MN 55455.

Previous studies in our laboratory have shown that dissociated Muller cells from the tiger salamander respond to an elevation in extracellular potassium ($[K^+]_o$) with an increase in intracellular calcium ($[Ca^{++}]_i$), even in the absence of Ca^{++} in the extracellular medium. This suggests that the K^+-evoked depolarization causes a release of Ca^{++} from intracellular stores. In the present experiments, we have used Fura-2 imaging techniques to study the calcium release mechanism in single dissociated Muller cells from the tiger salamander retina.

Muller cells responded to bath application of caffeine (10 mM) in Ca-free Ringer (2 mM EGTA) with an increase in $[Ca^{++}]_i$. Ryanodine (10-50 µM; Ca-free Ringer) also evoked an increase in intracellular Ca concentration, and reduced or blocked the Muller cell response to caffeine (10 mM). The changes in $[Ca^{++}]_i$ evoked by ryanodine and caffeine were more prominent in the apical region and soma of the Muller cell and less evident or delayed in the specialized endfoot region. This same spatial pattern was often observed in response to an elevation in $[K^+]_o$ in the absence of $[Ca^{++}]_o$. These findings suggest the presence of a ryanodine-sensitive mechanism in Muller cells which may be involved in the K^+-evoked increase in $[Ca^{++}]_i$ that we report here. This type of mechanism could serve to mediate long term interactions between neuronally mediated changes in external K^+ and alterations in Muller cell homeostatic mechanisms. Supported by NEI grant EY03014.

455.8

EFFECTS OF VASOPRESSIN (VP) AND ATRIOPEPTIN (AP) ON THE WATER CONTENT OF CULTURED RAT ASTROGLIAL CELLS. H.F.Cserr, L.Latzkovits, C.S. Patlak, K.D. Pettigrew, and A.Rimanoczy. Section of Physiology, Brown University, Providence RI 02912.

VP and AP are synthesized and released within the brain. This study evaluates the hypothesis that VP increases brain cell water content (DePasquale et al., Am. J. Physiol. 256:F1059-F1066, 1989) and examines interactions between this peptide and AP in cultured astroglial cells. Cultures were derived from newborn rats with minor modification of the method of Rudge et al. (Dev. Brain Res. 19:161-172, 1985). Cell water was estimated in defined medium as the 3-O-methyl[^{14}C]-D-glucose space (Kimelberg & Frangakis, Brain Res. 361:125-134, 1985) in control cells and in cells treated for 30 minutes with VP (10^{-5} to 10^{-7} M) and/or AP (10^{-6} M). VP increased glial cell water content (mean±SD, µl/mg protein) by an average of 19% (P<0.001), from 4.22±0.19 (n=30) in control cells to 5.01±0.32 (n= 54). In contrast, cell water did not differ from control (P>0.5) in the presence of AP alone, 4.21±0.23 (n=18), or AP plus VP, 4.23±0.28 (n=18), indicating that AP blocks the VP-dependent increase in glial water content. Results support a role for centrally-released VP and AP in the homeostasis of brain water content. Supported by NS-11050.

455.9

GLIONEXIN, A GLIAL GLYCOPROTEIN, IS SELECTIVELY EXPRESSED IN INSECT MECHANORECEPTORS. L.H.Field*, M.R. Meyer*, and J.S.Edwards. Dept. of Zoology, NJ-15, Univ. of Washington, Seattle, WA 98195.

Glionexin (GX), a high molecular weight extracellular matrix (ECM) glycoprotein recognized by the 5B12 antibody [(Meyer et al., J. Neurosci 7:512 ('87); Dev. Biol.130:374 ('88)], is associated with discrete types of glia in the CNS of embryonic and adult cricket (*Acheta domesticus*). We now show that GX is also expressed selectively in the peripheral nervous system.

GX is found in: (a) mechanoreceptor hairs (cercal filiform and clavate sensilla) and (b) chordotonal organs (subgenual, tympanal, femoral, Johnston's) in legs and antennae. GX is excluded from fine trichoid mechano/chemoreceptors on cerci, antennae, legs, and palps, as well as from retinal photoreceptors. Therefore, GX expression in the periphery is sensory modality selective.

GX is transiently expressed on basal lamina of embryonic ectoderm and is present within the mechanoreceptor organs in developing limbs and cerci; mechanoreceptor labelling persists throughout development. Peripheral GX immunoreactivity appears to be non-neuronal, confined to the ECM investing sense organs. Highest levels of GX expression are usually associated with either ECM regions surrounding sensory neuron somata or with sheath cells which wrap sensory axons. Moreover, various types of mechanoreceptor organs show differential patterns of GX labelling. Thus, peripheral GX expression may be both receptor class- and non-neural cell type-selective. Supported by NS-07778.

455.10

SECRETION OF PEPTIDES BY CHOROID PLEXUS AND EPENDYMAL CELLS IN CULTURE. R.H. Angeletti, P. Gee* and C.H. Rhodes. Albert Einstein College of Medicine, Bronx NY 10461 & Dartmouth Medical School, Hanover NH 03756

Immunohistochemical studies with sequence-specific antibodies to the peptidylglycine alpha-amidation enzyme (PAM) have shown that this enzyme is found in glial cells: Schwann cells, astrocytes, ependyma, choroid plexus, subependymal and subpial glia [J Histochem Cytochem 38:1301 (1990)]. These data are consistent with recent reports that the mRNA for the endocrine carboxypeptidase (CPE), another neuropeptide processing enzyme, is also present in glia [Birch *et al.*, Mol Brain Res 7:53 (1990); MacCumber *et al.*, J Neurosci 10:2850 (1990). In order to study their secretion, we have set up primary cultures of rat ependyma and choroid plexus, as well as cultures of a choroid plexus cell line. Pulse-chase experiments with ^{35}S-methionine, ^{3}H-glutamic acid, ^{3}H-leucine, and other labeled amino acids show that a discrete set of polypeptides is secreted, ranging in molecular weight from >200,000 daltons to less than 10,000 daltons. These peptides are being isolated, so that their identity can be determined. Neuropeptide processing enzymes are also being measured by activity, cDNA and immunoaffinity probes. Further studies have demonstrated the presence of PAM immunoreactivity in human ependymomas. (supported by NIH grant NS-22697)

455.11

Peptidylglycine amidating monooxygenase (PAM), an enzyme involved in neuropeptide biosynthesis, is present in normal glia and glial tumors. C. H. Rhodes, C. Honsinger, R. H. Angeletti, and F. A. McMorris, Dartmouth Medical School, Hanover, NH; Albert Einstein College of Medicine, Bronx, NY; Wistar Institute, Philadelphia, PA

The enzyme peptidylglycine amidating mono-oxygenase (PAM) is required for the biosynthesis of about half of the known neuroendocrine peptides. PAM-like immunoreactivity has been demonstrated not only in neurons, but also in Schwann cells, normal ependyma and some glia (J Histochem. Cytochem., 38:1301-1311 (1990)). The presence of PAM in primary glial cultures was demonstrated by experiments which showed the production of PAM enzymatic activity, PAM immunoreactivity, and PAM message by those cultures. These results suggest that some glia may be producing an amidated peptide of as yet unknown function. The modulation of this system during neoplastic transformation is examined by studies of PAM expression in human glioma tissue.

455.12

ORGANIZATION AND STRUCTURE OF ASTROCYTES IN ORGANOTYPIC CULTURES OF RAT HIPPOCAMPUS. Lucas D. Pozzo Miller* and Dennis M.D. Landis. Depts. of Neurology and Neurosciences, School of Medicine, Case Western Reserve University, Cleveland, OH 44106-4901.

In organotypic cultures, the initial cellular relationships at the time of plating are essentially those of the tissue from which slices were made. We have prepared organotypic cultures of hippocampal slices obtained from 6-7 days old rats and maintained for 14-21 days in vitro. Astrocytes in such slices phagocytose large amounts of cellular debris, and appear to form a glial limitans. Among the neuronal elements, however, the patterns of astrocytic investments resemble those present in developing hippocampus.

In cultures prepared by aldehyde fixation and freeze fracture techniques, astrocytic processes in the superficial 15-20μm of the slice are characterized by very large gap junctions, and numerous "assemblies". These arrays of orthogonally-packed intramembrane particles are normally found in astrocytic processes investing blood vessels or forming the glial limitans in situ. Astrocytes in secondary cultures also express assemblies, but with lower densities than those observed in tissue or in organotypic cultures. Cytoplasmic organization in astrocytes appears similar in cultures prepared by aldehyde fixation and in cultures prepared by rapid freezing and freeze substitution fixation.

We find that selective neuronal death occurs in particular regions and at particular times in the cultured slices. These cultures provide an opportunity to examine the response of astrocytes to both the initial damage of making the slice, and to subsequent neuronal death, in the relative or complete absence of blood-borne macrophages.

455.13

PHENOTYPIC AND FREEZE-FRACTURE ANALYSIS OF TRANSFORMED ASTROCYTES. J.R. Fredieu*, J.W. Jacobergert, S. Collins, and D.M.D. Landis. Depts. of Neurology and Neuroscience, †Dept. of Genetics, Case Western Reserve University, Cleveland, OH 44106.

SV40 T-antigen transformed astrocytic cell lines offer several potential advantages for study with biochemical and molecular biological techniques. However, the transformed cell lines may differ from primary astrocyte cultures in significant aspects of differentiation. We compare two cell lines derived from aged murine astrocyte cultures with primary rat astrocyte cultures derived from neonatal rats.

Primary rat astrocytes express GFAP, vimentin, and Mab 8C10 immunoreactivity as assessed by immunocytochemical methods. Mouse astrocyte cell lines (Mastr) #6 and #11, however, express less GFAP, and did not express vimentin, galactocerebroside-C (a marker of oligodendroglia), and Mab 8C10 immunoreactivity.

Freeze fracture electron microscopic methods reveal characteristic, orthogonally-packed arrays of intramembrane particles (assemblies) associated with the cytoplasmic half of fractured astrocyte membranes; the density of these assemblies is increased in confluent primary astrocyte cultures by treatment with dexamethasone (dex). Mastr #6 expressed assemblies without dex, but no assemblies were found when dex was added to the culture media. Mastr #11, on the other hand, did not express assemblies in the presence or absence of dex.

Thus, clonal astrocytic cells lines may resemble astrocytes as visualized with phase contrast optics, but have significant differences in the expression of several epitopes and in membrane structure regulation. Assessing the utility of such cell lines for isolation of cell-type specific cDNAs requires careful characterization of specific cell lines and culture conditions.

455.14

IN SITU AND IN VITRO EXPRESSION OF TENASCIN BY SUPRAOPTIC NUCLEUS ASTROCYTES. A. K. Salm and P. S. Klinkhachorn. Department of Anatomy, West Virginia University, Morgantown, WV 26506.

The dendritic arbors of hypothalamic supraoptic nucleus (SON) neurons of adult rats undergo activity-related remodelling to form bundles. Tenascin is an extracellular matrix glycoprotein that is expressed by astrocytes, primarily during development. Appearing in restricted neural regions, it is believed to play a role in histological patterning by serving as a "boundary molecule" to outgrowing neurites. The morphological plasticity of the adult SON suggested the hypothesis that astrocytes in the adult SON express this morphogenetic molecule. Twelve adult rats were anesthetized, perfused with fixatives and histologically processed. Double-label immunocytochemistry with primary antibodies against tenascin and glial fibrillary acidic protein (GFAP) was used to assess 5-10 μm thick sections through the SON. We observed a consistent, generally overlapping, staining for tenascin and GFAP of those SON astrocytes with cell bodies in the subjacent glial limitans and processes extending into the nucleus. Staining was usually confined to fibers of the glial limitans and dendritic zone, but in four animals was seen to encircle neuronal cell bodies. Primary cultures prepared from the SON produced both flat and stellate GFAP+ astroglia. When immunostained for tenascin without permeabilization, a subpopulation of these cells clearly expressed a surface patchwork of the molecule. With permeabilization however, nearly all GFAP+ cells displayed tenascin+ perinuclear "granules", the distribution of which closely paralleled that of the glial filaments. These results indicate that some astrocytes whose processes distribute in the adult SON synthesize and express tenascin. The in vitro results suggest that synthesis and surface expression of this glycoprotein may be differentially regulated. Whether and how the presence of tenascin in the SON is related to structural remodelling, and the possibility of tenascin expression by other SON cell types, remains to be determined.

455.15

AGE AND SPECIES DETERMINANTS OF PROLIFERATION OF ASTROCYTES TO RECOMBINANT GAMMA-INTERFERON. T. Tejada-Bergés, F.P. Yong and V.W. Yong, Montreal Neurological Institute, Montreal, Quebec, Canada H3A 2B4

Determining the signals involved in regulating the proliferation of astrocytes is important to an understanding of normal brain development as well as pathological processes such as reactive gliosis. Our laboratory has previously reported that recombinant human gamma interferon (rhIFN; 10-1000 u/ml) is mitogenic (7-fold) for adult human astrocytes in vitro as assessed by GFAP/BrdU double immunofluorescence. Recombinant murine gamma interferon (rmIFN) inhibits the proliferation of cultured neonatal mouse astrocytes as measured by ^{3}H-Thymidine incorporation (50% reduction at 100 u/ml). The results from the immunofluorescence method of BrdU incorporation correspond well with measurements of ^{3}H-Thymidine. In order to determine whether the differential response was age- or species-dependent, human fetal astrocytes were examined for their response to rhIFN. RhIFN (10-1000 u/ml) induced a 2-fold increase in the proliferation of human fetal astrocytes in vitro as evaluated by both BrdU and ^{3}H-Thymidine incorporation. We are currently testing the mitogenic capacity of rm IFN on cultured adult murine astrocytes. The current data suggest that the differential effects of gamma-IFN on human and murine astrocytes reflect species-specific differences.

455.16

EXPRESSION OF A CARBOHYDRATE BINDING PROTEIN ON A SUBSET OF ASTROCYTES DURING DEVELOPMENT. M.J. Carson*, Y. Aizenman, F.-T Liu*, and R. J. Milner. Research. Institute of Scripps Clinic, La Jolla, CA 92037.

Increased attention has been focused on carbohydrate binding proteins (CBP's) for their potential contribution to the development of both neuronal and nonneuronal tissue as well as their potential role in cellular proliferation. One such CBP was defined in rat as an IgE binding protein (EBP). EBP is also known as RL-29 and is homologous to the mouse protein, CBP-35, which is identical to the Mac-2 antigen. Because EBP has been detected in rat brain, we examined EBP expression in rat brains and cell culture using an antibody against EBP. Interestingly, the antibody labeled all cells in two cell lines which express glial markers: C6 glioma cells and 36C.21 cells. In contrast, in primary astrocyte cultures, the antibody labeled only a subset of astrocytes (GFAP+ cells) and a few unidentified (GFAP-) cells. Similarly, in brain sections from 4 different ages (P1, P8, P15, and adult) two cellular populations were labeled with the EBP antibody: a subset of astrocytes (GFAP+ cells) and a set of unidentified cells (GFAP-). In sections from 1-day old rat pups, the labeled cells were located primarily around the ventricles, and few of these cells were also GFAP+. In sections from 8-day old rats, labeling was concentrated primarily in the cerebellum and around the ventricles and many of the EBP labeled cells were also GFAP +. This same pattern of labeling was visible, with greater intensity in sections from 15-day old rats. In sections from adult rats, labeling with the antibody against EBP was no longer concentrated in the cerebellum but was weak throughout the brain and slightly concentrated around the ventricles. Thus, the expression of EBP appears to be developmentally regulated on a subset of astrocytes in the rat brain. This work was supported by a fellowship from the National Multiple Sclerosis Society (MJC) and grants NS 22347 and MH47680.

455.17

INFLUENCE OF GRAFTED PURKINJE CELLS ON BERGMANN GLIAL ENZYME EXPRESSION. M. Fisher and P. Trimmer. Dept's. of Anatomy & Cell Biology and Neuroscience, University of Virginia, Charlottesville, VA 22908

Following the postnatal loss of Purkinje cells (Pcs), Bergmann glia (Bg) in the Lurcher (Lc) mouse cerebellum show a striking metabolic response that includes decreased expression of the enzyme glycerol-3-phosphate dehydrogenase (GPDH). Analysis of Lc chimeric mice showed that Lc Bg were competent to maintain normal GPDH levels as long as they were situated within the domain of at least one surviving wild-type Pc (Fisher, M. 1990, J. Neurogenet. 6:183.). Grafts of wild-type fetal Pcs into Lc mutant cerebella have been used to determine whether already down-regulated Bg can be reinduced to express a high level of GPDH. Grafts were made into hosts 3-8 weeks old and analyzed 6-8 weeks after surgery. Only grafts into very young hosts result in significant observable glial GPDH immunoreactivity. The host age restriction and the distribution pattern of GPDH-positive Bg suggest that grafted Pcs can sustain GPDH expression in Bg that have not completely shut down, but they can not reinduce expression in those cells that have already shut down. EM analysis shows a significant reduction in the extent to which Bg ensheath dendrites of grafted Pcs relative to that seen in normal cerebellum. The results are consistent with the idea that Bg require continuous extensive interaction with Pcs to maintain normal adult levels of GPDH. Supported by NS25350 from NINDS.

455.18

ENZYME EXPRESSION IN BERGMANN GLIA OF ADULT MICE REMAINS SENSITIVE TO PURKINJE CELL PRESENCE. G.Ruthel and M.Fisher. Department of Anatomy and Cell Biology, University of Virginia, Charlottesville, VA 22908.

Bergmann glia (Bg) in certain mutant mice respond to the early loss of Purkinje cells (Pcs) with metabolic changes that include a reduction in glycerol-3-phosphate dehydrogenase (GPDH) levels. Studies with mutants suggested that normal glial enzyme expression requires the presence of Pcs at least through the first postnatal month (Fisher, J. Neurogenet., 6:183, 1990). To determine whether glial GPDH expression requires the presence of Pcs in adults, intracerebellar injection of kainic acid (1 µL, 0.5 mg/mL) was used to produce areas of selective Pc loss in mice at 7, 10, and 13 weeks of age. By 3 to 4 weeks post injection, there was a noticeable decrease in GPDH immunoreactivity in Bg in areas devoid of Pcs. By 8 weeks post injection, GPDH immunoreactivity was almost entirely absent from glial cell bodies and reduced in the glial processes. The presence and normal appearance of radial Bergmann fibers in the affected areas was confirmed in sections stained with antibody to glial fibrillary acidic protein. In some cases, isolated Bg of high GPDH immunoreactivity were observed within an affected area. Visualization of Pcs in adjacent sections with antibody to calbindin showed such Bg to be within the dendritic field of a similarly isolated Purkinje cell. These results suggest that normal glial GPDH expression in adults depends on the continued presence of Purkinje cells.

455.19

QUANTIFICATION OF THE CELLULAR GROWTH BALANCE OF GLIOMA CELLS USING MULTIPARAMETER FLOW CYTOMETRY. Herbert H. Engelhard, Division of Neurological Surgery, Department of Surgery, Northwestern University, Chicago, IL 60611

Proliferating cells in the process of balanced cellular growth double their protein content before entering mitosis. Alterations in cellular growth balance produce changes in the ratio of total cellular protein (TCP):DNA, and the expression of specific proliferation-associated proteins. In this study, a new three-color, dual-laser flow cytometric technique was used to measure cellular DNA, TCP and c-myc protein content using cell staining with the fluorescent dyes DAPI, SR101 and FITC. Modulations in the cellular growth balance of A-172, U118MG and U138MG (human glioma) cells were produced using growth-inhibitory conditions including serum deprivation, stationary phase growth and treatment with DMSO and sodium butyrate (SB). Flow cytometric data were confirmed using immunoblot analysis and the Lowry method.

Growth-inhibited cells exhibited changes in DNA, TCP and c-myc protein content which could be quantified using the new technique. SB-treated and stationary phase A-172 cells exhibited the most pronounced derangement in cellular growth balance. In these cells, the ratio of protein content in G2/M:G0/G1 cells increased from 1.45 to 1.70 (±0.05), while c-myc protein:TCP dropped from 1.00 to 0.31 (±0.02). The new dual-laser flow cytometric method should be useful for understanding other complex cell processes.

NEUROGLIA AND MYELIN V

456.1

CHARACTERIZATION OF PERIPHERAL-TYPE GFAP. D.L. Feinstein, G. Weinmaster*, R.J. Milner and D.J. Reis. Div. of Neurobiol., Dept. of Neurol. & Neurosci., Cornell Univ. Med. Coll., New York, NY 10021; Research Institute of Scripps Clinic & The Salk Institute, San Diego, CA 92037.

Immunological studies have shown that an antigenically-distinct form of glial fibrillary acidic protein (GFAP) is expressed in various peripheral tissues. In these studies, we have investigated the structure and expression of PNS-type GFA mRNA and protein. Tryptic mapping of GFAP isolated from primary cultures of rat Schwann cells revealed differences between the CNS and PNS GFA proteins. Screening of a cultured rat Schwann cell library with rat brain GFAP cDNA resulted in the isolation of clone rGFA15, whose sequence differed from the rat brain mRNA (GFAP-α) by the presence of an extended 5'UTR. A polymerase chain reaction assay was developed to allow detection of the longer GFAP mRNA (GFAP-β) in tissue and cell samples. GFAP-β mRNA is expressed in various Schwann cell lines which express exclusively the PNS-GFA protein. GFAP-β mRNA is also expressed at low levels in cultured rat astrocytes, and its expression could be influenced by prior treatment of the cells with dbcAMP. In the CNS, GFAP-β mRNA was detected in RNA isolated from postnatal day 5 whole brain, and at lower levels in samples from postnatal day 25. These results suggest that in the CNS, GFAP-β mRNA may represent a developmentally regulated form of GFAP.

456.2

REGIONAL VARIATION OF ASTROCYTIC GAP JUNCTIONS D.K. Batter, D.C. Spray, E.L. Hertzberg and J.A. Kessler, Albert Einstein College of Medicine, Bronx, NY 10461.

The predominant protein comprising astrocyte gap junctions is connexin 43. Here we show that astrocytes derived from two brain regions express different levels of connexin 43 and of the resulting functional gap junctions. In this study, confluent cultures containing >90% astrocytes were prepared from neonatal rat striatum or hypothalmus. Cell homogenates were subjected to western blot analysis probing with specific antisera that recognize both phosphorylated and unphosphorylated forms of connexin 43. Densitometric analysis of the resulting signals revealed that hypothalamic astrocytes contained 5-fold more connexin 43 per mg protein than striatal astrocytes. Cell cultures from both regions were processed for connexin 43 immunocytochemistry. Consistent with the western blot results, hypothalamic astrocytes displayed a significantly higher level of connexin 43 immunofluorescence than those from the striatum. Interestingly, in cultures from both brain areas the immunostaining was much stronger for some cells than for others, suggesting the existence of astrocyte heterogeneity even within a particular brain region. To determine if these differences were reflected in functional gap junctions, astrocytes from both brain regions were injected with Lucifer yellow and the dye spread was monitored. These experiments showed that while coupling of striatal astrocytes was generally restricted to cells adjacent to the injected one, dye spread following injection of hypothalamic astrocytes often extended to fourth order cells and occasionally beyond. Taken together, our data indicate region-specific expression of connexin 43 levels in astrocytes resulting in a quantitative difference in coupling of these cells in different brain areas. If these data reflect the situation *in vivo*, differential coupling of astrocytes may have important implications for regional brain function.

456.3

TEMPERATURE EFFECTS ON GROWTH OF GRADIENT PURIFIED MURINE CNS CELLS IN VITRO: RAPIDLY PROLIFERATING GFAP EXPRESSING ASTROCYTES AT 34°C DIFFER IN DENSITY FROM SLOWLY PROLIFERATING CELLS WITH LOWER GFAP CONTENT

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Neurology Svc, Wm S Middleton Mem VA Hosp and Univer of Wisconsin-Madison Sch of Med, Neurol & Med Microbiol Dept, Madison, WI 53705

Temperature sensitive retroviruses provide a means to study of the pathogenesis of the CNS neurodegenerative diseases produced by murine neurotropic retroviruses [Neurol 41(Suppl 1):143, 1991]. In order to study the specific vulnerability of CNS cells to these retroviruses in vitro, purified CNS cells were obtained from 5-10 day old FVB mice. Cerebral cortex [CTX] subcortex [SCTX] and cerebellum [CBL] were dissected, enzyme dissociated and separated by Dextran step gradient sedimentation into microvessels and other CNS tissue elements [Ann Neurol 28:291,1990]. The Dextran supernatant cells were separated by sedimentation through 30% Percoll into three bands at the following densities: [1] 1.018, [2] 1.033 and [3] 1.098 gm/cm^3. Because rapidly proliferating cells may permit more virus replication, proliferation rates of murine CNS cells from CTX, SCTX and CBL were measured at 34, 37 and 39°C. Cells from band 1 grew poorly at all temperatures. Cells in band 2 from all three regions grew significantly [$p<0.03$] better at 34°C [4.7 $\pm$ 0.6 (standard deviation) x 10^5 cells/cm^2 at 15 days in vitro (DIV)] compared to 39°C [2.6 $\pm$ 0.9]. Cells in band 3 from all CNS regions did not grow differently at 34°C [2.0 $\pm$ 1.4] from 39°C [2.8 $\pm$ 1.1]. CNS cells from different regions were characterized in vitro by immunocytochemistry and immunoblotting with cytoskeletal antibodies, fluorescent activated cell sorting with lectins and acetylated low density lipoprotein binding. Rapidly proliferating astrocytes in band 2 had a higher GFAP content than cells from band 3.

456.4

DISTRIBUTION AND MORPHOLOGY OF HIPPOCAMPAL MICROGLIA FOLLOWING CHRONIC EXPOSURE TO ETHANOL.

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Microglial cells are potential immunoeffector cells which respond to neuronal injury and death by morphologic and phenotypic transformations. Chronic ethanol treatment (CET) has been shown to produce a loss of neurons in the rodent hippocampal formation. This study examined the effects of CET on microglial distribution and morphology in the rodent hippocampus. Animals in the ethanol (E) and control (S) groups were fed liquid diets containing either ethanol or sucrose for 28 weeks followed by an 8 week period of abstinence. Microglia were visualized in vibratome sections from the dorsal, central and ventral hippocampus using lectin histochemistry with the B_4-isolectin from *Griffonia simplicifolia*.

Resting microglia from S-group animals possessed extensive process staining and were scattered throughout all subfields and laminae, being most prominent in the dentate hilus and entorhinal cortex. Microglia were differentially distributed in various subfields and regions within the dorsal-ventral extent of the hippocampus. On the other hand, microglia from the hippocampi of E-group animals appeared reduced in number with a greater reduction in the dorsal as compared to ventral hippocampus, particularly in area CA1. In addition, animals possessed fewer and more truncated processes than observed in S-group animals. These results suggest that abnormal microglial cell function may be related to the reduction in reactive synaptogenesis following chronic exposure to ethanol.

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456.5

ATTENUATION OF NEURITE OUTGROWTH PROMOTION AND NCAM AND HNK-1 EXPRESSION DURING IN VITRO MATURATION OF CNS ASTROCYTES. P. S. Frisa* and J. Jacobberger. Case Western Reserve University, Dept. of Genetics, Cleveland, OH 44106.

The attenuation of neuronal regeneration capacity during CNS maturation has been correlated with a decrease in the neurite outgrowth promoting properties of astrocytes and, in vitro, the ability of neonatal astrocytes to promote neurite outgrowth is dependent on the length of time in culture (Smith, G. M. et al., 1990. Dev. Biol. 138:377). Neurite outgrowth on astrocytes has been linked to the expression of cell surface molecules such as NCAM, laminin and N-cadherin. Changes in the neurite outgrowth-promoting ability of astrocytes can be expected to occur in step with changes in levels of these cell surface molecules. We have compared neurite outgrowth promotion and expression of NCAM and HNK-1 in astrocytes as they mature during culture in vitro.

A time course study of neurite outgrowth from embryonic chick retinal ganglion cells on neonatal and in vitro-aged astrocytes showed that a 12h outgrowth period was optimal for detecting differences between them. 12h neurite outgrowth on mouse cortical astrocytes was maximal at 2 days of culture and progressively declined, generally beginning to level off on astrocytes cultured for 6 days in vitro. NCAM and HNK-1 expression of the astrocytes was measured by flow cytometry. HNK-1 expression was maximal at 2 days of culture while NCAM reached peak levels at 4 days. Both HNK-1 and NCAM declined to low levels by 14 days.

These time dependent changes in vitro in neurite outgrowth promotion and expression of NCAM and HNK-1 by astrocytes correlate with the critical period for regeneration during development in vivo (Smith, G. M. et al., 1986. J. Comp. Neurol. 251:23).

456.6

CALCIUM WAVES PROPAGATE VIA GAP JUNCTIONS IN GLIOMA CELLS TRANSFECTED WITH CONNEXIN43 A. Charles, C. Naus+, D. Zhu+, E. Dirksen* and M. Sanderson*. Depts. of Neurology and Anatomy and Cell Biology, UCLA School of Medicine, Los Angeles CA 90024 and + Dept. of Anatomy, Univ. of Western Ontario, London Ontario, Canada N6A 5C1

C6 glioma cells in culture express low levels of connexin43 and have correspondingly weak gap junctional communication as evidenced by dye-coupling. These cells have been transfected with the cDNA encoding connexin43, resulting in clones which exhibit different levels of connexin43 expression and dye coupling, and decreased proliferation. We have previously reported that mechanical stimulation of a single cell in mixed glial or purified astrocyte culture induces a wave of increased $[Ca^{2+}]_i$ that is communicated from the stimulated cell to surrounding cells. In C6 glioma cells, mechanical stimulation induced a Ca^{2+} wave that was either not communicated or spread only to 1 or 2 adjacent cells. By contrast, C6 cells transfected with connexin43 cDNA showed extensive intercellular propagation of Ca^{2+} waves, with the distance and rate of intercellular propagation in different clones correlating directly with the level of connexin expression. A clone showing moderate expression of connexin showed propagation of Ca^{2+} waves to 5-10 adjacent cells, whereas a clone showing greater expression of connexin showed propagation of Ca^{2+} waves to 10-40 adjacent cells. These results provide direct evidence that Ca^{2+} waves are propagated intercellularly via gap junctions. Since transfection with connexin43 has been shown to reduce proliferation of C6 cells, intercellular Ca^{2+} signalling may participate in the regulation of cell proliferation.

456.7

PROTEIN PHOSPHORYLATION IN HYPOOSMOTIC-TREATED ASTROCYTES. JT Neary, AS Bender, J Blicharska* and MD Norenberg. Lab. Neuropathology, VA Med. Ctr. & Dept. Pathology, Univ. Miami Sch. of Med., Miami, FL 33101.

Calcium appears to play an important role in hypoosmotically-induced swelling and regulatory volume decrease (RVD). Previous studies from our laboratory using inhibitors of calcium-dependent protein kinases (Bender et al, 1990) suggested that the volume-related effects of calcium may be mediated by protein phosphorylation systems. To test this possibility, we studied ^{32}P incorporation in intact astrocytes during hypoosmotically-induced swelling and RVD. After a 1 min exposure to a hypoosmotic (105 mOsm) salt solution (maximum time for swelling), there was a reduction in ^{32}P incorporation in 24 and 21 kDa proteins (ca. 90% and 50%, respectively). After a 5 min exposure to the hypoosmotic solution (when volume regulation has begun), ^{32}P incorporation in these two proteins returned to nearly normal levels. The dephosphorylation of these proteins appears to be regulated by calcium because (a) treatment with ionomycin, a calcium ionophore, for 1 min leads to similar decreases in ^{32}P incorporation in the 24 and 21 kDa proteins and (b) application of extracellular ATP (100 uM, 1 min), which also increases intracellular calcium in astrocytes, causes a marked dephosphorylation of these proteins. We also found that dephosphorylation of 24 and 21 kDa proteins did not occur in a swelling model which does not undergo volume regulation (exposure to 80 mM external potassium). Taken together, our studies indicate that calcium-dependent protein phosphorylation/dephosphorylation systems play a role in hypoosmotically-induced swelling and volume regulation.

456.8

ASTROGLIOSIS IN SUBCORTICAL WHITE MATTER OF AMYOTROPHIC LATERAL SCLEROSIS (ALS).

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ALS Research Foundation, Pacific Presbyterian Medical Center, San Francisco, CA 94115

Histopathological features of ALS are commonly described as the isolated degeneration of upper and lower motor neurons and a pronounced demyelination in the primary motor tracts. We have discovered astrogliosis in ALS uniquely within the subcortical white matter. In all cases of ALS examined: 1) the GFAP-immunoreactive astrocytes have the morphological parameters of an on-going, "reactive" process and 2) the astrogliosis is widespread, as it is observed in multiple cortical areas, the midfrontal, inferior parietal, temporal, cingulate, and occipital cortices, as well as the motor cortex. To address the issue of neuronal degeneration and astrogliosis directly, we developed Marchi staining on cryosections and compared Marchi degeneration products in parallel with GFAP immunoreactivity. The amount of astrogliosis did not correlate with Marchi products. ALS was compared to eight different neurological diseases; the gliosis was similar to that in other diseases with gliosis ($p < .001$). Cytologically ALS astrogliosis resembles that present in cases of cerebral infarction, both focal and multiple microinfarction. Preliminary studies of Guam ALS have reiterated the findings of astrogliosis in the subcortical white matter. These data indicate a pathophysiological process in ALS beyond the motor system and implicate the subcortical white matter and its astrocytes as a special region of involvement.

456.9

FETAL GLIAL CONDITIONED MEDIUM STIMULATES THE PROLIFERATION AND DIFFERENTIATION IN VITRO OF TYPE I AND TYPE II ASTROCYTES ISOLATED FROM ADULT SPINAL CORD. L. Webster,* A. Tessler, P. Levitt. Department of Anatomy and Neurobiology, Medical College of Pennsylvania, Philadelphia, PA 19129

We are currently investigating the processes involved in the growth and differentiation of astrocytes derived from adult spinal cord in an effort to determine ways of modifying the environment to enhance axon growth after injury. Our previous study demonstrated that adult astrocytes do not survive well when grown alone in vitro, but survive well when they are grown in co-culture with E18 astrocytes and allowed to share medium but prohibited from direct contact. In order to determine the effects of fetal glial cells on adult glial cells, we examined the proliferation capabilities of adult cells grown in co-culture using bromodeoxyuridine (BrdU) as an indicator of proliferation. Preliminary data show that adult cells, grown in co-culture with E18 astrocytes, are capable of dividing and differentiating to produce GFAP+ cells with distinct type I and type II morphologies. Cells of connective tissue origin and PDGF do not have a similar capability to stimulate adult glial growth. It is likely that the E18 astrocytes are secreting a factor(s) that is capable of promoting the division of both quiescent type I astrocytes and O-2A progenitor cells in vitro. Other factors known to stimulate glial growth are currently being tested for proliferation-stimulating activity. Supported by VA Medical Research Service, USAMRDC grant 5193002, and NIH grant NS24707.

456.10

RESPONSES OF ASTROCYTES, MICROGLIA, AND MACROPHAGES TO PSEUDORABIES VIRUS (PRV) TRANSPORTED THROUGH CENTRAL VAGAL CIRCUITS. L. Rinaman[1], L.W. Enquist*[2], and J.P. Card[2]. *Department of Anatomy & Neurobiology, Medical College of Pennsylvania, Philadelphia, PA 19129[1] and Viral Diseases Group, duPont-Merck Pharmaceutical Co., Wilmington, DE 19898[2].*

The responses of astrocytes, microglia, and other phagocytic cells of monocyte origin were examined in the rat CNS following the peripheral uptake and subsequent central transneuronal passage of PRV (a swine α-herpes virus). PRV was injected into the ventral stomach wall or pancreas, and rats were perfused 48-100 hrs later. PRV-positive cells were identified with Rb134 antibody, astrocytes were identified with GFAP antibody, and microglia and macrophages were identified with OX42 antibody (anti-C3bi complement receptor). ED1 antibody (anti-monocyte/macrophage cytoplasmic antigen) was also used to identify phagocytic cells of monocyte origin. Rb134 immunoreactivity was first seen in motoneurons in the dorsal motor nucleus of the vagus and was subsequently observed in neurons in synaptically-associated nuclei, consistent with transsynaptic passage of the virus. A time-course dependent proliferation of GFAP-, OX42-, and ED1-positive cells was observed in virus-infected areas. In nuclei containing severely infected neurons, some of the GFAP-, OX42-, and ED1-positive cells were also Rb134-positive. We suggest that the non-neuronal cells that proliferate following PRV infection sequester virus released from severely infected neurons, thereby reducing non-specific virus spread and contributing to the specific transneuronal transport of PRV that has been demonstrated in central neural circuits.

456.11

ELECTRICAL PROPERTIES OF CULTURED MOUSE ASTROCYTES AFTER BARIUM EXPOSURE RESEMBLE PROPERTIES OF RAT ASTROCYTES. N.S. Magoski* and W. Walz. Dept. of Physiology, Univ. of Saskatchewan, Saskatoon, S7N 0W0, Canada.

The issue of cultured mouse astrocytes having a 20-fold or higher channel-mediated ^{42}K turnover than cultured rat astrocytes (Brain Res 340:333, 1985) has yet to be addressed. Electrophysiological studies to date have used rat astrocytes almost exclusively, with many authors being unaware of this difference and ignoring its importance in interpretating results. The membrane potential and input resistance of type-1-like cortical astrocytes, cultured from newborn mice, were recorded with and without barium (1 mM) exposure. Barium decreased the membrane response to 0 and 35mM K^+. It also revealed a Na^+/K^+ATPase mediated electrogenic component, as evident by a biphasic depolarization to ouabain (1mM), which was monophasic without barium. GABA or glutamate elicited small, monophasic depolarizations in untreated astrocytes. Barium-treated astrocytyes showed further transient responses to either transmitter, similar to rat astrocyte responses as reported in the literature. Uptake blockers for either amino acid did not change the responses. This electrophysiological study confirms that mouse derived astrocytes short-circuit electrogenic components and transmitter responses. The low channel-mediated K^+ flux in cultured rat astrocytes must be considered when using this preparation as a model of astrocyte function.

456.12

WITHDRAWAL OF EXOGENOUS GANGLIOSIDE GM1 ALTERS THE PATTERN OF B-CHOLERA TOXIN BINDING TO THE ASTROCYTE MEMBRANE. Ad J. Dekker and Mike S. Manley, Dept. Neurosciences, UCSD, CA92037.

Cultures of rat cerebral astrocytes were treated with ganglioside GM1 (30 μM) for 22 h, followed by ganglioside-free medium (2 h) or GM1 (30 μM, 2 h). The distribution of GM1 in the astrocyte membrane was then evaluated by indirect immunofluorescence using the B-subunit of cholera toxin (BCT). Approximately 60% of the astrocytes showed BCT-binding, with considerable variability across cells. After treatment with GM1, 70% of the astrocytes showed BCT-binding. In cultures where exogenous GM1 was previously withdrawn, BCT bound predominantly to the outer portions of the cells. These results suggest that the conformation or distribution of GM1 in the astrocyte membrane changes upon withdrawal of exogenous GM1.

456.13

MODULATION OF EPIDERMAL GROWTH FACTOR BINDING IN ASTROCYTES BY HORMONE AND PHOSPHORYLATION INFLUENCES. K.Huff and H.Tang*. Harbor/UCLA Med Ctr & Childrens Hospital, Los Angeles, CA, 90509.

The regulation of EGF binding including transmodulation of the receptor is multifactorial in many systems but not well understood in astrocytes where the majority of the CNS binding sites reside. We have studied 125-I labelled ligand binding in cultured neonatal rat astrocytes after 48 hr stabilization in serum free media. Increase in binding was a rapid response to elevators of intracellular cAMP, inhibitors of protein Kinase A, and to insulin, which have cell membrane sites of action. Protein kinase C inhibitors partially reversed Fibroblast Growth Factor down regulation. Decrease in binding was seen as a slower response to dexamethasone, and no response in binding changes was seen to thyroid hormone, although this hormone produced other effects. Regulation of receptor ligand effects may occur through a number of receptor related pathways in addition to transmodulation by other growth factors.

456.14

In Vitro Differentiation Inhibits the Migration of Cultured Neonatal Rat Cortical Astrocytes Transplanted to Neonatal Rat Cerebrum. JD Hatton and HS U*, Div. of Neurosurgery UCSD and VAMC, La Jolla, CA

Transplanted neonatal rat astrocytes have been shown to migrate throughout much of the rat host brain. In the present study, cortical astrocytes were collected from the brains of rats 1 to 3 days postpartum and purified by culturing them in DME medium supplemented with 10% calf serum. After 14 to 21 days, astrocytes were labelled with fluorescein-tagged latex microspheres. After 16 hours, the label was removed and replaced with either fresh medium or fresh serum-free medium plus 1mm dbcAMP. After 24 hours, cells were harvested and then transplanted into the right frontal cerebrum of neonatal rats at 3 days postpartum by injection with a hand-held Hamilton syringe. Animals were sacrificed at 3, 6, 9, 15, 21 and 28 days after inoculation. Brains were fixed by immersion in aldehydes, sectioned on a cryostat and examined with fluorescence microscopy. Undifferentiated astrocytes migrated along the corpus callosum, internal capsule, glial limitans, ventricular linings and the hippocampal structure. They also appeared to migrate in a radial fashion toward the periphery from the ventricular lining. Differentiated astrocytes did not appear to migrate into the neonatal parenchyma, remaining confined to the injection site or to the ventricular spaces. Thus neonatal cortical astrocytes migrate outward in a pattern similar to that defined by the radial glia. Differentiated glia, however, do not appear to migrate to any large degree in this environment, suggesting that differentiation may represent an end-point to glial migration in the neonatal host brain.

456.15

DELAYED RECTIFIER CURRENT EXPRESSED BY CULTURED RAT MICROGLIA. A. R. Korotzer and C. W. Cotman. Department of Psychobiology, University of California, Irvine CA 92717.

Microglia are thought to be the resident macrophages of the brain. Because many functions of immune system cells, such as interleukin 1 production by macrophages, appear to require K^+ channel expression, we have recorded from cultured rat microglia using the whole-cell patch clamp technique. Two types of voltage-gated K^+ currents were expressed: a previously described inward rectifier current (J Neurosci Res 26:278) and a delayed rectifier current (Ik). Ik activates at potentials positive to -40 mV, and the rates of activation and deactivation are voltage-dependent. The tail currents for Ik reverse close to the expected E_K in both 4.5 mM and 100 mM extracellular K^+. TEA (10 mM) reduces the magnitude of Ik. The rate of inactivation of Ik during a sustained depolarizing pulse varies from cell to cell. Many cells displayed slow recovery from inactivation, as reflected by the amount of use dependence of Ik. Ik is therefore similar to delayed rectifier currents described in many preparations.

456.16

TRANSPLANTATION OF PURIFIED GLIAL CELL POPULATIONS INTO THE MYELIN DEFICIENT RAT. I.D. Duncan, C. Paino*, P.M. Wood*, D.R. Archer*, and R.Hoffman*, School of Veterinary Medicine, University of Wisconsin, Madison, WI and The Miami Project, University of Miami, Miami, FL

Previous studies on glial cell transplantations into the CNS of the myelin deficient (md) rat have shown that transplanted dissociated CNS cell suspensions from neonatal or fetal rats can myelinate many axons in the area of the injection site. Whether the myelin produced by the grafted cells was derived from oligodendrocytes that were mature at the time of transplantation or from progenitors was not critically evaluated. The present study was designed to compare the capacity of mature oligodendrocytes and progenitors to produce myelin in the md rat. Progenitor cells from normal, neonatal littermates labelled with antibody to A2B5, or mature oligodendrocytes from normal adults labeled with antibody to O1 were purified by fluorescence activated cell sorting. The final purity of sorted cells was 95% or greater. Following sorting, 75,000 - 100,000 cells were injected into the spinal cord of 2-7 day old md rats and the rats perfused 14-20 days later. A number of rats were injected with freshly dissociated, non-sorted glial cell suspensions from adult rats. Patches of myelin were seen after injection of either progenitor or adult oligodendrocytes; however, the greatest amount of myelin was consistently seen in the rats injected with unsorted cells. These results show that: 1) transplanted A2B5 positive progenitor cells, purified by cell sorting, are capable of generating myelinating oligodendrocytes, and 2) mature oligodendrocytes from adult animals are also capable of myelinating md rat axons. (Supported by NMSS grants, RG-2219-A-1 and RG-1990-A-2.)

456.17

MYELINATION IN THE MYELIN DEFICIENT RAT BY TRANSPLANTED CANINE GLIAL CELLS. D.R. Archer*, R. Hoffman*, V. Miletic, and I.D. Duncan, School of Veterinary Medicine, University of Wisconsin, Madison, WI 53706.

Studies of allografts of mixed glial cell suspensions injected into the CNS of the myelin deficient (md) rat have shown that transplanted glial cells are capable of creating focal areas of myelination. In this study we examined the capacity of canine glial cells to myelinate host axons following transplantation into the md rat CNS. Mixed glial cell suspensions were dissociated from the spinal cord of a seven week old dog. After 24 hours in culture approximately 100,000 cells were injected into the T13/L1 region of the md rat spinal cord. Fifteen days later the animals were euthanized by perfusion fixation and the transplant site examined by light and electron microscopy. Patches of myelination attributable to transplanted oligodendrocytes were found in the dorsal columns of the graft recipients. In order to test the effect of cryostorage on the myelinating capacity of oligodendrocytes, a similar cell preparation from a four week old dog was frozen under liquid nitrogen and then thawed prior to transplantation. Again, approximately 100,000 cells were injected into the spinal cords of md rat hosts. The tissue was removed and examined thirteen days after transplantation. Patches of myelination were also found in the dorsal columns of the md rat spinal cord.

Two conclusions may be drawn from this work: 1) xenografts of canine glial cells are capable of myelinating md rat axons and 2) cryo-preserved canine glial cells retain their capacity to myelinate following transplantation into the md rat. (supported by NIH grant NS 23124 and the Myelin Project)

456.18

ADULT OLIGODENDROCYTES FAIL TO FORM MYELIN AFTER TRANSPLANTATION INTO CYCLOSPORINE-TREATED MYELIN-DEFICIENT RATS (MDR). J. Rosenbluth, Z. Liu, D. Guo & R. Schiff. Depts. Physiology and Rehab. Med., NYU School of Med., New York, N.Y. 10016.

Oligodendrocytes (OL) from adult rats do not form myelin within two weeks after transplantation into juvenile mdr spinal cord, while fetal glial cells do (J. Neurocytol. 19:718). One possible cause is a host immune response directed against donor OL antigens not expressed by precursor cells. To test this, we cultured adult (P32-55) or fetal (E15-16) rat OL, labeled them with fast blue and transplanted them into spinal cords of P8-9 rats treated with cyclosporine (10mg/kg/day). 11-14 days later, mdr that had received adult transplants showed fast blue and macrophages within the dorsal columns, but still no significant myelin formation. In contrast, fetal donor cells, lacking mature (GC+) OL at the time of transplantation, formed large islands of myelin that exhibited normal periodicity and formed normal axo-glial junctions. The results do not support an immune-mediated mechanism for the failure of transplanted adult OL to form myelin and are consistent with the view that immature rather than mature cells are needed for initiation of myelination. Supp. by NIH & NMSS.

456.19

A DIFFERENTIAL SCREENING APPROACH TO ISOLATE NEW OLIGODENDROCYTE-SPECIFIC cDNA CLONES. N.Schaeren-Wiemers, C. Schaefer*, G. Yancopoulos[1] and M.E. Schwab. Brain Research Institute, University of Zurich, August-Forel-Strasse 1, CH-8029 Zurich/Switzerland. [1]Regeneron Pharmaceuticals, Tarrytown, N.Y.

Oligodendrocytes are a very special and important cell type, both, from a functional and from a cell biological point of view. In addition to forming myelin they also express specific proteins inhibiting neurite growth. To identify and characterize new oligodendrocyte-specific gene products a postnatal P16 rat spinal cord cDNA library was differentially screened with either probes from first strand cDNA synthesis of P16 spinal cord (plus probe) or probes from X-irradiated (at P0) P16 spinal cord in which oligodendrocytes are absent (minus probe). From both probes "house keeping genes" were subtracted by hybridization against liver mRNA. Clones coding for the known myelin proteins MBP, PLP, MAG, and CNPase were identified by S-blots and discarded. By Northern analysis two groups of interesting clones emerged: 1.) Clones whose corresponding mRNAs are CNS-specific, most highly expressed around P16, lower in the adult, and absent in E17 spinal cord qualify as possibly oligodendrocyte-specific. 3 of these clones (mRNA sizes of 1.5-3.5 kb) were partially sequenced and found to represent unknown proteins. The coding strands were identified by defined riboprobes in N-blots and corresponded to those found by in vitro transcription-translation experiments. - 2.) Clones with a similar CNS expression pattern but additional expression in sciatic nerve probably correspond to oligodendrocyte-Schwann cell clones. 12 of these clones were analysed and showed mRNA sizes of 1.7 to 4.0 kb; some of them show 2 mRNAs probably resulting from differential splicing.

456.20

CHARACTERIZATION OF ANGIOTENSIN II RECEPTOR SUBTYPES IN RAT ASTROGLIAL CULTURES DERIVED FROM DIFFERENT BRAIN REGIONS. S.P. Bottari*, N. Obermuller*, K.L. Zahs[1] and C.F. Deschepper[1], Cardiovascular Research, Ciba-Geigy, 4002 Basel, Switzerland and [1]Dept of Physiology, U.C. San Francisco, CA 94143-0444

Recent studies with new, selective angiotensin II (AII) antagonists have revealed two main AII receptor subtypes, referred to as AT_1 and AT_2. To determine which subtypes were present on astrocytes, we have tested the effect of the selective antagonists Dup 753 (AT_1) and CGP 42112A (AT_2) on the binding of [^{125}I]-Sar^{1}Ile^8-AII. Membranes prepared fom cultured astrocytes were incubated for 90 min with 0.2 nM [^{125}]-Sar^{1}Ile^8-AII, and the bound tracer was separated from the free by filtration. Non-specific binding was measured in the presence of 1μM AII. The tracer bound to high (Ki = 54 $\pm$ 12 pM) and low affinity (Ki = 11 $\pm$ 3 nM) sites. The Ki of competition for tracer binding with Dup 753 was 24 $\pm$ 7 nM, while the Ki of competition with CGP 42112A was greater than 1μM. These values are characteristic of AT_1 sites, and indicate the absence of AT_2 receptors. The B_{max} of tracer binding on the membranes astrocytes from pons or diencephalon ranged from 1 to 4 pM/mg protein. On the other hand, much lower levels of AII-binding sites were detected on the membranes of astrocytes from frontal cortex. We conclude that (1) no AT_2 receptors are detectable on astrocytes; (2) high capacity AT1 receptors are present on astrocytes from subcortical regions; (3) astrocytes derived from cortex had less AII receptors than astrocytes from subcortical regions. Supported in part by HL29714 and HL38774.

457.1

VACCINIA VIRUS-MEDIATED EXPRESSION OF PLASMA MEMBRANE TRANSPORTERS: FUNCTIONAL ANALYSIS OF THE CLONED HUMAN L-NOREPINEPHRINE TRANSPORTER.

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We have characterized a novel vaccinia-virus based transient expression method suitable for the rapid functional characterization of neurotransmitter transporters and applied this system to an analysis of the activity of the cloned human Na+/L-norepinephrine carrier. The expression system utilizes a vaccinia-virus encoded T7 RNA polymerase to synthesize, in infected cells, cytoplasmic transcripts from transfected plasmids bearing T7 RNA promoters. To characterize and optimize the expression methodology, we transfected separately the intestinal Na+/glucose and brain Na+/GABA transporters into vaccinia-T7 infected fibroblasts and demonstrated rapid (4-12hr) appearance of [^{14}C]α-methyl D-glucopyranoside and [^{3}H]GABA uptake, respectively, bearing pharmacological sensitivities previously documented in *Xenopus laevis* oocytes. Upon identification of a candidate cDNA encoding the human Na+/L-norepinephrine transporter (NET), we applied this system to document the functional properties of the expressed transporter in HeLa fibroblasts. Following transfection with NET, vaccinia-T7 infected cells expressed saturable (K_t=457 nM), sodium-dependent uptake of [^{3}H]L-norepinephrine which is antagonized by antidepressants (desipramine> nortryptyline> imipramine), D-amphetamine (K_i=56 nM) and cocaine (K_i=140 nM). GBR 12909 and paroxetine exhibited weak potency (K_i>100 nM) relative to their actions on brain dopamine and serotonin transporters, respectively. Anatogonists of adrenergic receptors, other plasma membrane transporters, and vesicular amine transport failed to block NET activity. Further studies are underway to characterize and localize the direct binding of radiolabeled antagonists to the cloned carrier. These results demonstrate that a single cDNA encodes many if not all of the readily observable properties of the high affinity, human norepinephrine reuptake system.

457.2

HUMAN MONOAMINE OXIDASE A AND B GENES EXHIBIT IDENTICAL EXON-INTRON ORGANIZATION.

J. Grimsby, K. Chen*, L-J Wang*, N.C. Lan and J.C. Shih. Dept. of Mol. Pharm. & Tox., Sch. of Pharm., Univ. of South. Calif., L.A., Ca. 90033

Monoamine oxidase (MAO) A and B oxidize a number of neuroactive amines and they are coded by separate genes (PNAS:85,4934,1988). Recently, we have isolated genes encoding human MAOA and B by screening four bacteriophage libraries with human liver MAOA and B cDNA subfragments. Twenty-one MAOA positive clones and forty-three MAOB positive clones were isolated. Southern blot analysis of phage DNA isolated from these bacteriophage clones identified 6 MAOA and 7 MAOB positive clones. By extensive mapping and sequencing the exons of MAOA and B were aligned based on the nucleotide sequences of MAOA and B cDNAs. Our results show that human MAOA and B genes span at least 60 kb, consist of 15 exons, and exhibit identical exon-intron organization. Exon 12 codes for the covalent FAD-binding site and is the most conserved exon; the amino acid sequences deduced from these exons share 93.9% identity between MAOA and B. These results suggest that MAOA and B are derived from duplication of a common ancestral gene and provide insight on the structural/functional relation of the enzyme. (Supported by NIMH grants R37 MH39085 (merit award), K05 MHH00796, R01MH37020, and Welin professorship).

457.3

THE ROLE OF SPLICE SITE-ENCODED AMINO ACIDS ON THE SUBSTRATE AND INHIBITOR SENSITIVITIES OF MAOB.

H.F. Wu, K. Chen* and J.C. Shih., Dept. of Mol. Pharm. & Tox., Sch. of Pharm., Univ. of South. Calif., L.A. Ca. 90033

We have recently isolated human MAOA and B genes and showed that they both consist of 15 exons and exhibit identical exon-intron organization. When the splice site-encoded amino acids in MAOA are compared with those in MAOB, 11 of 14 amino acids encoded at the 5'boundary and 9 of 14 encoded at the 3'boundary of the introns are identical. Among the different ones only 5 amino acids are not conserved. Thus, we made five MAOB mutants in which the splice-site coded amino acid was mutated to corresponding amino acid in MAOA. These MAOB mutants were prepared by oligonucleotide-directed mutagenesis. Each mutagenic clone was sequenced then cloned into an expression vector, pECE. High efficiency CaPO4 precipitation transient transfection was performed. The amount of MAOB mutants transfected was determined by Western blotting. The substrate specificities were determined by using ^{3}H-serotonin or ^{14}C-phenylethlyamine radioassay. The sensitivities to clorgyline or deprenyl was also determined. The role of each of these amino acids on the catalytic properties of MAOB will be discussed. (Supported by NIMH grants R37 MH39085 (Merit Award), K05 MH00796, R01 MH37020 and Welin professorship).

457.4

MOLECULAR ANALYSIS OF THE CATECHOL-O-METHYLTRANSFERASE GENE IN FISCHER AND WISTAR-FURTH STRAINS OF RAT.

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In mammals, catechol-O-methyltransferase (COMT) is a physiologically important enzyme for the metabolism of catecholamine neurotransmitters and catechol drugs. Soluble and membrane-bound forms of the enzyme exist, which differ in their substrate affinity, and apparent MW and pI. Breeding of Fischer-344 (F-344) and Wistar-Furth (W-F) rat strains has demonstrated monogenic inheritance of hepatic as well as renal COMT activities. The recent cloning of the gene for rat liver soluble COMT has enabled us to examine the molecular basis of these inherited variations. For this study, full-length DNA fragments were generated by PCR amplification of total poly+A mRNA, from both F-344 and W-4 strains, using synthetic oligonucleotide primers complementary to both ends of the coding region. Multiple clones from each strain were completely sequenced by the dideoxy method. Sequence analysis using the DNAStar program Lasergene indicates several nucleotide differences between the strains. A change exists from an A to G at nucleotide #192 which does not change the amino acid coded for by that triplet. Also, a possible deletion of a cytosine at nucleotide #33 alon with an insertion of an extra guanine at #42 is found in both strains compared to published sequence. Restriction fragment length polymorphism (RFLP) analysis has been performed on DNA from parents (F-344 and W-F), F1, F2, and backcross animals after digestion with a variety of restriction enzymes. A potential polymorphism (RFLP) is seen with the enzyme HhaI (recognition site GCGC), which agrees with the sequence variations seen around either #33 or #42 nucleotide. There also appears to be an RFLP that is identified by the PstI enzyme. Verification of this finding and complete analysis of flanking sequence is presently underway. (Supported by NIH grant NS24066 to MHG).

457.5

NITRIC OXIDE SYNTHASE: MOLECULAR CLONING OF THE MACROPHAGE GENE.

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Nitric oxide (NO) is a novel messenger molecule, acting as an endothelial-derived relaxing factor, and as a neurotransmitter released by discrete neuronal populations in the brain and periphery. Macrophages generate NO to elicit their bactericidal and tumoricidal effects via an NO synthase (NOS) which is distinct from the form found in brain. These macrophages probably include the microglial cells of the central nervous system. The goal of this work is to sequence the gene encoding the macrophage NOS.

Cerebellar NOS has been purified (P.N.A.S. 87:682, 1990) and molecularly cloned (TIPS 12:125, 1991). With this sequence data, various hybridization techniques were used to isolate the macrophage NOS. Using a probe of the cerebellar NOS gene, low stringency screening of a phage library of activated macrophage cDNA clones failed to isolate any homologous plaques. Using degenerate oligonucleotides that are homologous to various portions of the cerebellar NOS gene, the polymerase chain reaction of reverse transcribed mRNA templates from activated macrophages produced 300 bp and 500 bp fragments which are approximately 30% homologous to the cerebellar nitric oxide synthase sequence. A Northern blot of activated macrophage RNA shows that this message size is 4.5 kb.

These fragments have been used to screen a phage library of activated macrophage cDNA clones. When the entire gene is sequenced, the localization in the brain and other tissues will be studied, and the enzyme will be expressed and characterized.

457.6

CHARACTERIZATION AND CHROMOSOMAL MAPPING OF A CANDIDATE GENE FOR 3-HYDROXYANTHRANILATE OXYGENASE.

C. Ambrose*, B. Navia*, A. McClatchey*, E. Okuno*, R. Schwarcz, J. Gusella and M. MacDonald*. Neurogen. Lab, Mass. Gen. Hosp., Charlestown, MA 02129 and Maryland Psych. Ctr. Baltimore, MD 21228.

3-hydroxyanthranilate oxygenase (3-HAO) is the enzyme responsible for the synthesis of quinolinic acid (Quin) in the mammalian brain. An endogenous excitotoxin, Quin, has been postulated to be involved in several neurodegenerative disorders including Huntington's disease (HD). A candidate cDNA for 3-HAO, isolated from a rat liver library using a polyclonal antibody, has been previously reported (Neurosci. Abs., 1988). Here we report the further characterization of this clone and others obtained from cDNA and genomic libraries. Northern blot analysis has revealed that the 3-HAO mRNA is approx. 2.2 kb in rat and mouse and about 1.7 kb in baboon. 3-HAO mRNA is easily detected in kidney and liver total RNA, but not in whole brain RNA. However, 3-HAO cDNA fragments can be amplified from total brain RNA using PCR. The rat cDNA clone contains an inverted repeat at its 5' end which has the potential to form a 100 bp stem and a 155 bp loop. This 355 bp sequence has been shown to exist on 4 exons in a mouse cosmid. Sequence analysis of the genomic fragment containing the most 5' exon has identified a potential initiator methionine and TATA sequence. Cross-species hybridization on somatic cell hybrids, has localized the candidate 3-HAO gene to human chromosome 17q. A Spretus mouse cross allowed us to map the gene to mouse chromosome 11 and determine that it is tightly linked to the murine thymidine kinase gene. The location of the this gene eliminates it as a candidate gene for HD since the disease gene maps to chromosome 4p.

457.7

EXPRESSION, ORGANIZATION AND LOCALIZATION OF THE HUMAN N-CHIMAERIN GENE.

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The novel brain-specific cDNA, *n-chimaerin*, encodes a predicted product with significant similarities to both the C1b cysteine-rich motif $CX_2CX_{13}CX_2CX_2CX_7C$ of protein kinase C and to BCR, the product of the *breakpoint cluster region* gene involved in Philadelphia chromosome translocation. The N-terminal half of n-chimaerin shares almost 50% identity with corresponding sequences in the regulatory domain of PKC. The C-terminal half of n-chimaerin has 42% identity with residues 1050 to 1225 of BCR. *n-chimaerin* RNA is specifically expressed in the brain with highest abundance in the hippocampus and the cerebral cortex. The RNA expression pattern is neuronal. The human *n-chimaerin* gene was localised to a specific chromosome by analysis of somatic cell hybrid DNA and a *n-chimaerin* related locus was detected on a different specific chromosome. Genomic clones containing the *n-chimaerin* gene show that three exon boundaries in the *n-chimaerin* gene precisely corresponded to those in the human *BCR* gene. A further exon boundary corresponded to a (drosophila) *protein kinase C* splice junction, in the region encoding the cysteine-rich zinc finger motif. The opposite end of the same *n-chimaerin* exon contains a region of sequence similarity to BCR.

457.8

MOLECULAR BIOLOGY OF THE D1 AND D5 DOPAMINE RECEPTOR GENES. D.K. Grandy, Q.-Y. Zhou*, Y. Zhang*, C. Bouvier*, L. Allen*, R.A. Johnson*, O. Civelli. Vollum Institute for Advanced Biomedical Research, Oregon Health Sciences University, Portland, OR 97201.

The complex pharmacological and physiological effects of dopamine are mediated by several different G protein-coupled receptors. In an effort to clone and characterize these molecules we have combined PCR with homology screening. Using degenerate primers directed against conserved regions of the catecholamine receptors we cloned both the rat and human D_1 receptor genes (Zhou et al., 1990). A search for regulatory elements that control D_1 gene expression has resulted in the identification of consensus sequences for AP1, AP2, CRE, and SP1. In experiments designed to identify other D_1-like genes we performed Southern blotting analyses of human DNA. Under condiditions of lowered stringency the D_1 gene hybridizes to a 5kb EcoRI fragment. Contained within this fragment is an open reading frame whose deduced amino acid sequence is 49% identical to D_1. This gene encodes a new dopamine receptor, D_5, which has a 10 fold higher affinity for dopamine than the D_1 receptor. Furthermore, D_5 is able to stimulate adenylyl cyclase in response to dopamine at concentrations 30 times lower than needed to activate D_1 receptors. This gene is also interesting because in contrast to rats, there are three copies in the human genome and at least one is a pseudogene. Therefore, at present only humans have been shown to contain a D_5 pseudogene. This is the first reported case of a pseudogene in the G protein-coupled receptor family which raises some interesting evolutionary questions.

457.9

A VERSATILE CLONING AND EXPRESSION SYSTEM TO ANALYZE FUNCTIONAL PROPERTIES OF HUMAN D2 RECEPTOR ALLELES. G.M. Lawless[1,2], C.H.J. Ruppert[1], T. Ritchie[2], E.P. Noble[2,3], and A.J. Tobin[1,3,4]. [1]Department ofBiology, [2]Alcohol Research Center, [3]Brain Research Institute, and [4]Molecular Biology Institute, University of California, Los Angeles, CA, 90024.

Dopamine receptors are widely studied because of their role in many neuropsychiatric disorders. A RFLP in the D2 receptor gene has been found to be associated with patients suffering from severe alcoholism (JAMA, 263:2055-2060). To analyze the functional properties of D2 receptors of different alleles we have developed a PCR-based method to clone and express specific cDNAs. Total RNA is purified from limited amounts of postmortem brain samples, and D2 receptor mRNA is transcribed into cDNA utilizing human D2 receptor specific primers. Two sets of primers allow us to clone the complete open reading frame of receptor sequence after amplification in a thermal cycler. Expression of the cloned sequence in a cell culture system will enable us to analyze and compare the biochemical and pharmacological properties of D2 receptor derived from the two human alleles A1 and A2 for its possible involvement in the appearance of severe alcoholism. (Supported in part by The Seaver Foundation.)

457.10

GENE STRUCTURES OF $GABA_A$ RECEPTOR β SUBUNITS.

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Subtypes of $GABA_A$ receptor subunits (eg. β1, β2, β3), are highly homologous but exhibit distinct temporal and regional patterns of expression in brain. As an initial step to study how expression of these genes is regulated, genomic DNA encoding β subunit subtypes has been cloned and characterized. Over 5kb of human genomic DNA, upstream of the β1 initiation ATG was cloned and mapped. Analysis of reverse-transcribed RNA by PCR, indicates that the exon containing the start of coding sequence extends only 70-140bp upstream of the initiation ATG. However, primer extension studies, and PCR analysis of human brain cDNA libraries, has uncovered cDNAs that contain exonic sequences 200-350bp upstream of the initiation ATG. These upstream sequences are not contiguous with the cloned genomic DNA and indicate the existence of one or more upstream introns.

The gene encoding the β3 subunit was found to be deleted from patients with Angelman syndrome and Prader-Willi syndrome, and its absence may play a role in the pathogenesis of one or both of these conditions (Am. J. Hum. Genet., in press). Genomic DNA, extending over 10kb upstream of the β3 initiation ATG was cloned from rat and human libraries. These genomic fragments, containing the first three coding exons, exhibit conservation of intron/exon junctions with the β1 gene. Gene fragments have been linked to a CAT reporter gene and used to transfect several cell lines, in order to define important regulatory features of the genes.

457.11

CLONING AND EXPRESSION OF THE PHOSPHATE ACTIVATED GLUTAMINASE GENE FROM RAT BRAIN. R.G. King*, H. Haleem-Smith*, H.J. Purohit*, V.P. Kedar*, and J.F. Mill. Lab. of Molecular Biology, NINDS, NIH, Bethesda, MD 20892.

L-glutamate (Glu) is the principal excitatory neurotransmitter in the vertebrate CNS. One of the primary enzymes involved in the metabolism of Glu is phosphate activated glutaminase (PAG).

Using a previously cloned partial cDNA to PAG (PGA1, Banner et al., 1988) as a probe, we isolated a PAG cDNA clone 4 Kb in length (OGA4K). This clone contains all of the sequence previously described in PGA1 as well as an additional 57 bases of coding sequence on the 5′ end and 2.7 Kb of 3′ untranslated region which includes the polyadenylation signal.

Antisense riboprobes generated from this clone were used in S1 nuclease protection assays to study the expression of PAG mRNA in rat cardiac and skeletal muscle, lung, kidney, intestine, liver and brain. PAG expression was also invetigated in several cell lines as well as primary astrocytes and granule cells.

A genomic clone for the rat PAG gene was isolated through homology to the OGA4K cDNA clone. Partial characterization of this clone has demonstrated that it extends to the 3′ terminus of the gene, and in two exons extends into the coding region of the gene. The two exons terminate in consensus sequences for splice sites.

457.12

GENES FOR RAT GLUTAMIC ACID DECARBOXYLASES.

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The brains of rats and humans contain at least two forms of glutamate decarboxylase (GAD_{65} and GAD_{67}), which differ in sequence and size, and which derive from different genes. The human GAD_{67} gene contains two promoters whose usage differs between adult and embryonic brain. We have isolated rat genomic DNAs for GAD_{65} and GAD_{67}. The restriction maps of these cloned DNAs suggest that the rat GAD_{65} gene spans a length of approximately 34 kb and the rat GAD_{67} approximately 32 kb. The two rat GADs derive from different genes: GAD_{65} cDNA hybridizes to genomic restriction fragments distinct from those that hybridize to GAD_{67} cDNA, and it does not hybridize to GAD_{67} genomic clones. The sequence of the putative promoter region of the rat GAD_{65} gene resembles, in several respects, that of the corresponding region of the human GAD_{67} gene. Functional analyses are now under way to determine the molecular basis of the coexpression and the independent regulation of the two GADs. (Supported by NIH grants NS 20356 and NS 22256)

457.13

HUMAN GLUTAMATE DECARBOXYLASES: cDNA CLONING, SEQUENCING, BACTERIAL EXPRESSION AND LOCALIZATION ON TWO CHROMOSOMES. D.F. Bu[1], M.G. Erlander[1], B.C. Hitz[1,*], N.J.K. Tillakaratne[1], C. Wagner[2,*], G.A. Evans[2,*] and A.J. Tobin[1,3,4]. [1]Department of Biology, [3]Brain Research Institute, and [4]Molecular Biology Institute, University of California, Los Angeles, CA 90024; [2]Molecular Genetics Laboratory, The Salk Institute, San Diego, CA 92186.

Using previously described feline and rat cDNAs, we isolated human GAD_{67} and GAD_{65} cDNAs that contain complete open reading frames. GAD_{65} cDNA encodes a polypeptide of 585 amino acids, while GAD_{67} cDNA a polypeptide of 596 amino acid residues. Northern blots reveal a single RNA species for each GAD using extracts of both fetal and adult human brain, with GAD_{65} mRNA 5.7 kb long, and GAD_{67} 3.7 kb long. Sequence analysis shows high conservation of each coding sequence between rats and humans, about 90% at the nucleotide level and 96% at the amino acid level. To obtain human GAD_{67} and GAD_{65} protein from recombinant DNA, we cloned the coding region of human GAD_{67} and GAD_{65} cDNA into bacterial expression vectors and expressed enzymatically active protein in E. coli. Using human GAD_{67} and GAD_{65} genomic clones, we determined the chromosomal localization of the two GAD genes by in situ hybridization. (Supported by NIH grants NS20356 & NS 22256)

457.14

GENE AND mRNA STRUCTURE OF HUMAN B-50 (GAP-43)
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The phosphoprotein B-50 (GAP-43) has been implicated in neuronal development, axonal regeneration and signal transduction. We previously identified the protein encoding part of the human gene, contained in 3 exons (Schrama et al. 1990, *Soc. Neurosci. Abstr.* 16:154.11).

We now report on the 3' and 5' regions of the human B-50 gene. Two polyadenylation signals are present in exon 3; they start respectively 506 and 580 bp downstream from the stop codon. Analysis of the 5' region of the gene revealed two purine-rich areas in the 358 bp upstream of the startcodon; sequences analogous to known transcriptional regulatory elements are not present however. Primer extension on adult human poly(A)$^+$ RNA using a primer in exon 2 showed multiple transcription starts. The extension products found thus far suggest two major transcription starts located approximately 130 bp and 340 bp upstream from the translation start. In addition, PCR analysis of the cDNA showed an uninterrupted extension product up to 200 bp upstream from the translation start. Both the length and the sequence of the untranslated regions of the human mRNA, as shown by primer extension, PCR and sequence analysis show a high degree of homology with the rat B-50 messenger. The combined results regarding the 3' and 5' regions suggest the existence of mRNA of at least 1.6 kb, not including the poly(A)-tail. Interestingly, this is the length reported by others (Kosik et al. 1988, *Neuron* 1:127) for the polyadenylated mRNA.

457.15

Identification of a mouse secretogranin III.

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Chromogranins/Secretogranins are family of acidic secretory vesicle proteins that are found in a variety of endocrine cells and neurons. Recently, the elucidation of the primary structure of a novel rat secretogranin, termed secretogranin III (SgIII), has been accomplished (Ottiger et al., J. Neurosci. 10, 3135 (1990). We have now extended this analysis to the mouse. (i) The generation of null mutants, where the gene for SgIII has been completely abolished should allow us to gain insights into the function of the proteins in normal physiology (Kingsley et al., EMBO J. 9, 395 1990). (ii) We report here the identification and determination of the primary structure of a mouse secretogranin III cDNA. Northern blot analysis confirmed the neuro-endocrine specific expression of SgIII. The deduced protein encodes for a 57-KDa protein and sequence specific antibodies detect a target of the same size in SDS-extracts of brain, but not liver or kidney. These data demonstrate that the SgIII genes are highly conserved between species.

457.16

CLONING OF THE THYMOSIN BETA-10 GENE: CHARACTERIZATION OF ITS STRUCTURE AND NUCLEOTIDE SEQUENCE.

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Elevated levels of thymosin B10 gene expression have been shown to be associated with the early phases of embryonic/fetal human brain development. How thymosin B10 transcription is regulated is currently not understood.

In order to elucidate the regulation of thymosin B10 transcription we have isolated the human thymosin B10 gene, and determined its exon/intron structure. The gene is composed of three exons and two introns. Exon two encodes amino acids 1-32 and contains the first nucleotide for amino acid 33, exon three encodes the remainder of the protein and the entire 3' untranslated portion of the gene. Currently we are sequencing the 5' untranslated region in an attempt to define the start of exon one as well as the promoter region. Preliminary primer extension studies map the start of transcription approximately 80 nucleotides upstream of the translational start site. The presumptive promoter region contains a putative CAT box consensus sequence also a Spl binding sequence. Future studies are aimed at determining promoter activity in vitro using CAT as the reporter gene.

Supported by NIH/NCI grant CA 49422-01 to A.K. Hall.

SYNAPTIC STRUCTURE AND FUNCTION I

458.1

PROTEIN METHYLATION IN SYNAPTOSOMES. L.S. Wright* and F.L. Siegel, Depts. of Pediatrics and Physiological Chemistry, Univ. of Wisconsin, Madison, WI 53706.

Synaptosomes from five regions of adult rat brain (cortex, cerebellum, hippocampus, brain stem and striatum) and three regions of 2 day old rat brain (cerebellum, brain stem and remaining brain) were isolated and used in studies of protein methylation. Synaptosomes were incubated with [methyl-^{3}H]-S-adenosyl-L-methionine at pH 6.5 and at pH 8, and methylated proteins visualized by SDS-PAGE and fluorography. For adult brain, methylated profiles in synaptosomes were compared with those in P_1, P_2, P_3 and cytosolic fractions. At pH 8, a protein of 68kDa was methylated synaptosomes from all brain regions to a greater extent than in other subcellular fractions, and in cerebellar synaptosomes a protein of 56kDa was a major methyl acceptor protein. Neither of these proteins was methylated at pH 6.5, indicating that this is not aspartate carboxylmethylation. In synaptosomes from the two day old rat, methylation of 68kDa and 48kDa (all brain regions), but not 56kDa was found. A methyl acceptor protein of 35kDa was found in all fractions and a family of 22kDa-26kDa methylated proteins was present in all membrane fractions. These results indicate an age-dependent, region specific methylation of synaptosomal proteins. (Supported by NIH grants NS24969, GM38497 and HD03352.)

458.2

AUTOPHOSPHORYLATION OF CEREBELLAR AND FOREBRAIN FORMS OF CAM-KINASE-Gr. Q.B. McDonald* and N. Sanyoun Wellcome Research Laboratories, 3030 Cornwallis Road, RTP, NC 27709.

A neuronal Ca^{2+}/calmodulin-dependent protein kinase, CaM Kinase-Gr, occurs as M_r 65,000 and 67,000 polypeptides in the rat cerebellum and as a M_r 65,000 polypeptide in the rat forebrain. Both enzyme forms were purified and were found to undergo stoichiometric autophosphorylation on a serine residue. Autophosphorylation caused a characteristic decrease in the electrophoretic mobility of both polypeptides. This altered migration on denaturing gels could be reversed by the action of an endogenous brain Mg^{2+}-dependent phosphoprotein phosphatase, or by the addition of an acid phosphatase. The shifts in gel mobility were confirmed by silver staining, autoradiography and immunoblotting with a CaM Kinase-Gr antiserum. Autophosphorylation of the cerebellar and forebrain enzymes acquired an autonomous, Ca^{2+}/calmodulin-independent activity,and displayed greatly increased sensitivity to subsequent exposure to Ca^{2+}/calmodulin.

458.3

Ca^{2+}/CALMODULIN-DEPENDENT PROTEIN KINASE II IS ASSOCIATED WITH PURIFIED SMALL SYNAPTIC VESICLES. J. L. Rubenstein*, A. J. Czernik and P. Greengard. Laboratory of Molecular and Cellular Neuroscience, The Rockefeller University, New York, NY 10021.

Ca^{2+}/calmodulin-dependent protein kinase II (CaM kinase II) appears to play an important role in the regulation of neurotransmitter release. CaM kinase II-dependent phosphorylation of synapsin I, a major synaptic vesicle-associated substrate, is thought to regulate the availability of vesicles for fusion at the presynaptic active zone. We now provide evidence that CaM kinase II is associated with a highly purified preparation of small synaptic vesicles from rat cerebral cortex and characterize this interaction.

When purified vesicles were incubated in the presence of 1mM $CaCl_2$, 10mM $MgCl_2$ and 50 μM [γ-^{32}P]ATP, little phosphorylation of endogenous vesicle proteins was evident. Addition of calmodulin (10 μg/ml) markedly stimulated the phosphorylation of a number of vesicle proteins, which were resolved by SDS/PAGE. Synapsin I, specifically phosphorylated at sites 2 and 3, was a prominent ^{32}P-labeled protein, as were proteins migrating at 48 kD and 58-60 kD, respectively, corresponding to the autophosphorylated subunits of CaM kinase II. Addition of the CaM kinase II inhibitory peptide [281-302]Ala286 reduced all calmodulin-dependent phosphorylation in a dose-dependent manner. The peptide inhibited the phosphorylation of synapsin I with an IC_{50}=10 μM.

Immunoblots of synaptic vesicle proteins with affinity-purified antibodies against CaM kinase II demonstrated that both the α and β/β' subunits are associated with purified synaptic vesicles. Quantitative immunoblotting indicated that CaM kinase II was a major vesicle protein, representing 4.4% of total vesicle protein by weight (α subunit=2.8%; β/β' subunit=1.6%). Moreover, the molar ratio of CaM kinase II:synapsin I per vesicle approached 1. Extraction trials of vesicle proteins using low and high salt, EDTA, EGTA and Triton X-100 indicated that the kinase was tightly associated with the vesicle membrane. These results raise the possibility that CaM kinase II and synapsin I form a functional complex on the synaptic vesicle membrane (see Benfenati *et al.*, this meeting).

458.4

GROUPS OF STRUCTURAL COMPONENTS CO-LOCALIZED IN BRAIN SUBCELLULAR FRACTIONS ARE DIFFERENTIALLY AFFECTED BY A CALCIUM ACTIVATED PROTEASE. B.L. Bakus*, B.A. Bahr, E.T. Esteban*, A.C. Godshall*, J. Black & G. Lynch. Center for the Neurobiology of Learning and Memory, University of California, Irvine, California 92717

Integrins act as transmembrane linkages between components of the extracellular matrix and the intracellular cytoskeleton and play an important role in forming and maintaining contacts between cells. The present study used immunochemical methods to test the hypothesis that integrin-like proteins and their ligands found in synaptic membranes differ in important ways from those found in extra-synaptic regions. Antibodies against three integrins labeled proteins with much larger molecular weights in crude synaptosomes than in synaptosomal membrane fractions (SPMs). The high M_r (175 to 225 kDa) antigens which correspond to conventional integrin subunits co-migrated with synaptosomes and low density myelin in equilibrium buoyant density sucrose gradients, whereas the antigens of low M_r (27 to 65 kDa) that are concentrated 20 to 40-fold in SPMs migrated to higher density fractions including those containing synaptosomes and/or mitochondria. A similar pattern was evident for high and low M_r antigens recognized by antibodies against the integrin ligands fibronectin and laminin. Note, proteins of 43 and 40 kDa labeled respectively by anti-laminin and anti-fibrinogen were shown not to be the same, but were both concentrated 10 to 15-fold in SPMs compared to homogenates for all brain regions tested. A striking difference between the groups of antigens is that those in crude synaptosomes increased in concentration during early postnatal development while those in SPMs did not. Moreover, the synaptosomal polypeptides containing integrin epitopes were reduced in concentration by a typical amount of 81 ± 8% ($x \pm$ s.d., n = 8) following incubation with a calcium-activated protease (calpain), while those antigens in SPMs were unaffected by the enzyme. These results suggest that adhesion in synaptic zones differs from that in extra-synaptic regions, both in terms of the species of proteins involved and in the factors which regulate them. (Supported by NIA grant #AG00538 and the Pew Foundation.)

458.5

TWO LOCI WITH EXTENSIVE POLYMORPHISM ENCODE SNAP-25 IN GOLDFISH. C. Risinger and D. Larhammar. Dept of Medical Genetics, Box 589, Uppsala University, S-751 23 Uppsala, Sweden.

SNAP-25 (synaptosome-associated protein) is a 25-kDa protein which is expressed exclusively by neurons. It is localized to presynaptic nerve terminals and is associated with synaptosomal membranes. Its expression starts at the time of synaptogenesis.

We have previously shown that SNAP-25 is an extremely well-conserved protein which implies important functions. The chicken and mouse SNAP-25 proteins are identical throughout the 206 amino acids (S. Catsicas et al., PNAS 88, 785-789, 1991), and SNAP of goldfish, ray *(Torpedo marmorata)*, river lamprey, and *Drosophila melanogaster* show extensive similarity to the mouse protein (Abstract 154.14, Neuroscience Meeting 1990).

Surprisingly, all six clones that we have isolated from a goldfish retina cDNA library are distinct at the nucleotide level. They can be sorted into two groups according to degree of sequence identity. Within each group, the clones are virtually identical at the amino acid level. Between the two groups there is only 90% amino acid sequence identity. Furthermore, some clones contain mutations which preclude synthesis of a functional SNAP protein. Our results suggest that there are two loci for SNAP in goldfish in agreement with chromosome analyses which have shown that goldfish are tetraploid. The gene duplication has presumably allowed the redundant gene copy to accumulate deleterious mutations.

Our findings of SNAP gene duplication and polymorphism suggest that also other cloning results in goldfish should be regarded with caution until multiple clones have been analysed.

458.6

GENE ORGANIZATION OF SYNAPTIC PROTEIN SNAP-25 IN *DROSOPHILA*. I. Lundell*, C. Risinger, A. G. Blomqvist, C. Bark#, and D. Larhammar. Department of Medical Genetics, #) Department of Developmental Biology, Uppsala University, S-751 23 Uppsala, Sweden.

SNAP-25 is a 25-kDa protein which is expressed exclusively by neurons. It is associated with the inner side of the cell membrane in presynaptic nerve terminals. The expression of SNAP-25 starts concomitantly with synaptogenesis, indicating a role in this process or in the functions of the mature synapse.

We have previously shown that the chicken and mouse SNAP-25 proteins are identical throughout the 206 amino acids (S. Catsicas et al., PNAS 88, 785-789, 1991) and that SNAP of goldfish and *Torpedo* show approximately 90% similarity with the mouse sequence (see abstract by C. R. and D. L.).

Using low-stringency hybridizations, we have isolated SNAP-25 cDNA clones from a *Drosophila melanogaster* library. *Drosophila* SNAP-25 displays 60% sequence identity to the mouse protein, and a segment of 60 amino acids shows 85% sequence identity. We have also isolated several genomic SNAP-25 clones from a phage library. Determination of the exon organization will reveal whether the *Drosophila* SNAP-25 gene structure is as complex as that of the chicken SNAP-25 gene which consists of eight exons spanning more than 65 kbp.

These studies show that SNAP-25 is one of the most highly conserved synaptic proteins known which suggests a crucial role for SNAP-25 in synapse function.

458.7

TUBULIN IS IN A LARGE, HYDROPHOBIC COMPLEX ON SYNAPTIC VESICLES FROM RAT BRAIN. H. Qi* and E. Floor, Dept of Physiology & Cell Biology, University of Kansas, Lawrence, KS 66045.

Tubulin is present on a subpopulation (~30%) of synaptic vesicles purified from rat brain. We are analyzing the mode of association of tubulin with synaptic vesicles to gain insight into its possible function in these organelles. Both α- and β-tubulins are present, apparently externally, since all tubulin is removed from vesicle suspensions by mild protease digestion or by immunoprecipitation of vesicles with tubulin antibodies. Tubulin is not likely bound by association with MAPs, since it is not removed in 1M NaCl, although it does dissociate from synaptic vesicles in 50mM NaOH and is therefore not an integral membrane protein. Extraction of synaptic vesicles with Triton X-114 suggests that vesicular tubulin is hydrophobic; it partitions into the detergent-rich phase in contrast to whole brain tubulin, which acts like typical soluble proteins and is recovered in the detergent-poor (aqueous) phase. Vesicular tubulin may be hydrophobic because it is post-translationally modified, e.g. by acylation, or because it binds to a hydrophobic protein. To look for the latter, synaptic vesicles were treated with the covalent cross-linking reagent, DTSSP (Pierce). A discrete, large complex (~5,000K) was observed by chromatography on Sephacryl S-500HR in 0.1% SDS. Analysis by a two-dimensional gel electrophoresis method in which the crosslinker is cleaved by thiol reagent did not reveal any other proteins besides tubulin in this complex. These results indicate that a distinctive form of tubulin associates with synaptic vesicle membranes.

Supported by NIH Grant NS 24890.

458.8

IDENTIFICATION OF AN ALTERNATIVELY SPLICED MEMBER OF THE SYNAPTOPHYSIN GENE FAMILY. J. L. Bixby. Dept. of Molec. & Cell. Pharmacology, Univ. of Miami, Miami, FL 33136.

Synaptic vesicle-associated membrane proteins are important in the regulation of transmitter release, and as markers for nerve terminal differentiation. One such protein, synaptophysin, has been shown (in mammals) to be part of a gene family with at least one other member, synaptoporin. In a search for related proteins, we screened a chick brain cDNA library at low stringency with a rat synaptophysin probe. Two classes of clones were isolated, with sizes of 2.3 and 2.5 kB, which differ in their use of alternative polyadenylation signals. DNA sequencing revealed that each class contained cDNAs that also differ in the N-terminal protein coding region. One such cDNA (psyn2A) has a deduced aa sequence 83% identical with rat synaptoporin. The other (psyn1B) is identical to psyn2A starting at aa 29, and continuing through the coding region and 1.6 kB of 3' untranslated DNA. The N-terminal 28 aa deduced from psyn1B are 60% identical with rat synaptophysin, and the C-terminal aa sequence shared by psyn1B and psyn2A is equally similar to synaptoporin and synaptophysin. Southern blotting with chick genomic DNA suggested that psyn1B and psyn2A are encoded by a single gene. Northern blots suggested that the corresponding mRNAs are moderately abundant in a variety of CNS tissues. Experiments are underway to determine whether the two forms are differentially expressed in different brain regions and at different developmental stages. The results suggest that the synaptophysin gene family is more complex than had been appreciated. Supported by grants from the NIH and the PMAF.

458.9

ISOLATION AND PRELIMINARY CHARACTERIZATION OF SYNAPTIC VESICLE-PLASMA MEMBRANE COMPLEXES ("ACTIVE ZONES"). C. Walch-Solimena* and R. Jahn. Max-Planck-Institute for Psychiatry, Dept. of Neurochemistry, Am Klopferspitz 18A, D-8033 Planegg-Martinsried, Germany.

At present, little is known about the molecular constituents of the contact sites between synaptic vesicles and the plasma membrane ("active zones") in nerve terminals. Here, we report the isolation and preliminary characterization of such complexes from rat brain. The isolation involves purification and lysis of nerve terminals, followed by separation of membrane-bound from free synaptic vesicles and finally immunoadsorption of membrane-bound material to a bead matrix coated with monoclonal antibodies against synaptic vesicle proteins. Electron microscopic analysis revealed that the adsorbed material contains synaptic vesicles attached to membrane fragments, resembling complexes of pre- and postsynaptic membranes. Using immunoblotting, a coenrichment of synaptophysin and synaptotagmin, two membrane proteins of synaptic vesicles, with the immunoisolated material was found. Separation of the protein constituents by SDS-polyacrylamide electrophoresis revealed the presence of membrane proteins different from those found in purified synaptic vesicles. A comprehensive analysis of the protein content is currently in progress.

458.10

THE ISOLATION AND PURIFICATION OF A PROTEIN ASSOCIATED WITH THE RIBBON SYNAPTIC STRUCTURE. Grant W. Balkema, S. Burt, M. Hatton, A. Duffy, and K. Bachman, Department of Biology, Boston College, MA 02167.

A monoclonal antibody, B16 (sub-type IgM), has been produced that recognizes a protein determinant associated with synapses throughout the brain and retina. Within the retina the antigen is concentrated at the ribbon synaptic structure in the photoreceptor terminal. We have found this antigen in every species that we have examined (fish, frog, lizard, mouse, rat, rabbit, cat, cow, monkey).

We are presently purifying the antigen with two aims in mind: 1) the purified protein will used as antigen to produce a panel of IgG monoclonal antibodies to the orginal protein. 2) the purified protein can be partially sequenced with the intent of producing primers for PCR. We have purified the protein using differential centrifugation, IEF column chromatagraphy, and SDS-PAGE. The protein is fairly soluble, requiring 70% salt to precipitate. Thus, with differential centrifugation we have been able to purify the B16 protein by 30-40 fold. This semi-purified fraction was then applied to an Iso-Electric-Focusing column (LKB) and focused for 48 hours. The fraction containing the B16 protein eluted at roughly pH 7.3 +/- 0.2 and resulted in a 3,600 fold purification. The 7.3 pH fraction was then applied to a 5-15% gradient SDS-PAGE and Western blotted. The B16 88kDa band was cut out of the blot and the protein eluted via 1%SDS-1%Triton. The purity is 100% as determined by initial amino acid analysis. From 100 grams of brain we have recovered 50 µg of purified protein. (Supported by the NSF BNS-8919829 and the Boston College Research Fund.)

458.11

DETECTION OF DYSTROPHIN IN A POSTSYNAPTIC DENSITY (PSD) FRACTION ISOLATED FROM ADULT RAT BRAIN. Kim, T.W.[1,2*], Wu, K.[2], Byers, T.J.[3], Sherwood, A.C.[2*], Xu, J.L.[2*] and Black, I.B.[2]. [1]Graduate program in Physiol. and Neurobiol., Rutgers-The State Univ. of NJ and UMDNJ/Robert Wood Johnson Medical School,[2]Dept. of Neurosci. and Cell Biol., UMDNJ/Robert Wood Johnson Medical School, Piscataway, NJ and [3]Children's Hospital and Howard Hughes Medical Institute, Harvard Medical School, Boston, MA.

Moderate non-progressive cognitive impairment is a consistent feature of Duchenne muscular dystrophy (DMD); underlying mechanisms remain unknown. Only recently, dystrophin was found to be present in brain. Preliminary immunocytochemical analysis has shown that the dystrophin is localized in the PSD. In the present study, we directly demonstrate the presence of dystrophin in the PSD isolated from cerebral cortex (CTX), cerebellum (CBL) and olfactory bulb (OB) of adult rat brains. The amount of dystrophin present in the structure was determined by western blot analysis using the antibody, anti 6-10. In all PSD fractions examined, the antibody detected multiple proteins with apparent molecular weight higher than 110 kDa under denaturing and reducing conditions. The relative proportion of proteins differed in CTX, OB and CBL, suggesting differential regional expression. Moreover, in CTX, there was 10-fold greater dystrophin in PSD than in synaptic membrane or total homogenate, confirming previous immunocytochemical localization. We are presently characterizing dystrophin in PSD from developing and DMD brains. Our results indicate that dystrophin is a component of the PSD, potentially suggesting abnormalities of dystrophin function at the synapse that underly cognitive deficits. (Supported by grants from NINDS, NICHD and McKnight Foundation)

458.12

NEUROCHEMICAL CHARACTERISTICS OF A POSTSYNAPTIC DENSITY (PSD) FRACTION ISOLATED FROM OLFACTORY BULB (OB) OF ADULT RATS. Huang, Y.[1*], Wu, K.[2], Stanley, M.[1], Xu, J.L.[2*], Cherry, J.A.[1], Wu, K.M.[3], Kim, T.W.[1*] and Black, I.B.[2] [1]Div. of Neurosci., [3]Div. of Neuropathal., NYSPI, New York, N.Y. and [2]Dept. of Neurosci. and Cell Biol., UMDNJ/RWJMS, Piscataway, N.J.

Abundant evidence suggests that the PSD, a proteinaceous disc-shaped structure attached to the postsynaptic membrane, is central to the function of the synapse. We have previously shown that environmental input regulates a specific novel major PSD protein (mPSDp). Moreover, we found that the molecular characteristics of the PSD differ in the cerebral cortex (CTX), cerebellum (CBL) and hippocampus (HI). To further examine regional differences in PSD characteristics throughout the brain, we chose to study the OB, a forebrain structure richly innervated by both peripheral and central afferents. Highly purified PSD was obtained from synaptosomal fractions after treatment with 0.5% Triton X-100. The yield was 21.7 mg PSD per 7.5 gm of OB, at least a 10-fold greater yield per unit tissue than CTX, HI or CBL. Morphologically, the OB-PSD exhibited a typically distinct disc-like structure. SDS-gel profile revealed that the proteins in the OB-PSD were qualitatively similar to but quantitatively different from the PSD in the aforementioned brain regions. Further, the mPSDp found in CTX and HI represented only a minor portion of the total OB-PSD protein, similar to that found for the CBL. The OB-PSD exhibited Ca^{2+}/calmodulin- and cAMP-dependent protein kinase activities. There were 5 proteins that bound calmodulin in a Ca^{2+}-dependent manner. Presently, we are examining the binding characteristics of the neurotransmitters GABA and dopamine in the OB-PSD. This is the first demonstration of a PSD fraction isolated from the OB. The high yield of the PSD, the presence of protein kinases and neurotransmitter receptors, as well as the characteristic calmodulin binding suggest that the OB-PSD may constitute a useful model to study the regulation of synaptic molecular architecture by environmental factors.

458.13

A PROTEOGLYCAN-LIKE HEPARIN BINDING PROTEIN OF HIGH MOLECULAR WEIGHT FOUND CONCENTRATED IN SYNAPTOSOMAL MEMBRANES. D. Capaldi*, B.A. Bahr, H. Lim*, K. Norenberg[1] & G. Lynch. Center for the Neurobiology of Learning and Memory, Univ. of Calif., Irvine, CA 92717 and [1]Dept. of Chem., Univ. of Calif., Santa Barbara, CA 93106

Structural molecules such as proteoglycans have been suggested to be involved in nerve terminal adhesion. It is conceivable that due to their adhesive nature proteoglycans contribute to the stable synaptic morphology which survives most subcellular fractionations. In an attempt to identify candidate proteoglycans positioned appropriately in synapses, synaptic plasma membranes (SPMs) were isolated and probed with antibodies against structural elements and [^{3}H]heparin using immunoblotting and gel overlay techniques. Anti-keratin strongly recognized a high M_r (400-600 kDa) species of heterogeneous electrophoretic mobility which is characteristic of a proteoglycan whose protein core is modified by the addition of many glycosaminoglycan side chains. This species was 20 to 50-fold concentrated in SPMs compared to homogenized tissue for all brain regions tested, and was sensitive to trifluoromethanesulfonic acid treatment. An antigen recognized by anti-keratan sulfate was shown to be very similar in locale and molecular weight to the species labeled by anti-keratin. Keratanase appeared to reduce the antigenicity of this species. The two antibodies, however, failed to recognize a similar species in other subcellular fractions such as myelin, mitochondria, or synaptic vesicles, nor in tissues throughout the body. SPMs but not brain homogenate exhibited a proteoglycan-like species in polyacrylamide gels stained with alcian blue or overlaid with [^{3}H]heparin which strongly resembled that labeled by anti-keratin. Hippocampal SPMs bound [^{3}H]heparin with a K_D of 0.5 µM and a B_{max} of 109 pmol/mg protein, whereas the homogenate expressed a similar K_D but a lower B_{max} of 38 pmol/mg. Both binding activities were inhibited by most glycosaminoglycans. A proteoglycan-like species was purified from solubilized SPMs on fibronectin-agarose eluted with GRGDSP. It has similar properties as that recognized by anti-keratin and may represent a class of proteoglycan involved in matrix organization in synapses. Supported by AFOSR#890383.

458.14

HIGH YIELD PURIFICATION OF GLYCOPROTEINS FROM SYNAPTIC MEMBRANES OF RAT CNS. D.E. Hurlbut and E. Weber. University of California, Irvine, CA 92717.

The postsynaptic density (PSD) is a structure associated with the postsynaptic membrane (PSM) found in the majority of chemical synapses and is comprised of various proteins including four glycoproteins which have been characterized by their affinity for concanavalin A (Con A; Mena, 1984). Of the four, the 110,000 kD glycoprotein (type III) is the major component (Mena and Cotamn, 1982,Science 216:422-424.). We decided to purify the type III glycoprotein and attempt to sequence it.

An alternative method from the described approach to isolate CON-A glycoproteins was devised. Briefly, rat CNS P2 pellets pretreated with Triton X-100 were solubilized with an SDS/beta-mercaptoethanol solution, centrifuged, and the supernatant was subjected to affinity chromatography on Sepharose 4B beads coupled to Con-A. Bound proteins were eluted from the column with 0.2 M alpha-D-methyl-glucoside. The eluted glycoproteins were dialyzed against water, lyophilyzed and analyzed by SDS electrophoresis.

The 110kD glycoprotein appeared as a doublet on 8.5% SDS-PAGE. This doublet was further resolved by running the Con-A eluted proteins on 5% SDS-PAGE. Of the doublet, the upper band was selected to be further purified and undergo amino-acid analysis. In order to reassure us that the 110kD doublet was a product of PSDs, CON A glycoproteins were also generated by the method of Mena and Cotman, 1982,Science 216:422-424). Once again a 110kD doublet was observed on 8.5% and 5% SDS-PAGE. This suggests that either method produces the same results with the notable exception that our method yields a greater amount of 110kD protein. *Supported by NIDA fellowship DA05393 to DEH and partially by NIDA grant DA06726 EW.*

458.15

SYNAPTOGLYCAN: A KERATAN SULFATE MEMBRANE PROTEOGLYCAN OF SYNAPTIC VESICLES. T.W. Scranton*, M. Iwata, and S.S. Carlson. Dept. of Physiology and Biophysics, Univ. of Washington, Seattle, WA 98195.

Previously a membrane proteoglycan was identified as a specific component of synaptic vesicles from elasmobranch electric organ. This molecule is recognized by two mAbs, Tor 70 and anti-SV1, which bind to the same luminal epitope (SV1). The SV1 epitope is found in high concentrations in the neurons which innervate the electric organ; very little is found elsewhere in the fish nervous system. This proteoglycan was thought to be either a member of the heparin/heparan sulfate or keratan sulfate family. Also shown to be present in synaptic vesicles is a 100kD glycoprotein identified with another mAb, anti-SV2, which recognizes a cytoplasmic epitope. We have now shown that the synaptic vesicle proteoglycan contains both the SV1 and SV2 epitopes. On SDS-PAGE two forms of the SV2 molecule exist in vesicles, a 100kd form with few glycosaminoglycan side chains and a higher molecular weight form (>200kd) with large glycosaminoglycan side chains. These glycosaminoglycan side chains contain keratan sulfate in electric organ vesicles, in fish brain, and in rat brain. In electric organ vesicles these side-chains vary greatly in size. The SV1 epitope is present on the largest keratan sulfate side chains of the electric organ proteoglycan. When chromatographed on Sephacryl S-500 under denaturing and reducing conditions the electric organ proteoglycan behaves like a protein of 250kd; the rat brain proteoglycan behaves like a 200kd protein. When solubilized with non-denaturing detergents, the electric organ proteoglycan exists in a complex with 4 small proteins. We propose the name synaptoglycan for this molecule.

SYNAPTIC STRUCTURE AND FUNCTION: HIPPOCAMPUS

459.1

ANATOMY OF ELECTROPHYSIOLOGICALLY CHARACTERIZED INTERNEURONS IN IMMATURE HIPPOCAMPUS. C.M.Gómez[1], K.L.Smith*[2], J.W.Swann[2]. [1]Dept. of Anatomy, Cell Biology & Neurobiology, Albany Medical College, Albany, N.Y. 12208 and [2]Wadsworth Center for Laboratories and Research, NYS Dept. of Health, Albany, N.Y. 12201.

The morphology of subpopulations of CA3 hippocampal interneurons were examined. Neurons were impaled in Stratum pyramidale (S. pyramidale) of minislices taken from area CA3 of 1 to 2 week old rat hippocampus. Cells were identified as interneurons when they displayed brief action potentials with prominent afterhyperpolarization and high firing rates in response to depolarizing steps of intracellularly injected current. Biocytin was injected via the recording electrode. Approximately 35% of such fills yielded single cells, 65% were dye-coupled with the number of cells filled ranging from 2 to 6. Thus far three classes of interneurons have been identified all of which possess dense basket-like terminal arbors. These include 1) pyramidal basket cells and 2) stellate cells, both with terminal arbors primarily in S. pyramidale; and 3) stellate cells with projections primarily outside S. pyramidale. Of the dye coupled cells, 100% were coupled to at least one other interneuron. In one instance, 6 interneurons were dye coupled. 38% of cells were coupled to spiny pyramidal cells which characteristically have intrinsic burst properties. The density of terminal arbors and attendent fiber swellings and breadth of projection within the CA3 subfield suggest that these immature hippocampal interneurons have many of the morphological features commonly ascribed to interneurons in mature hippocampus.

459.2

DUALISTIC PROPERTIES OF INTERNEURONS IN THE IMMATURE HIPPOCAMPUS. K.L.Smith*, C.M.Gómez and J.W.Swann, Wadsworth Center for Labs & Research, NY State Department of Health, and Albany Medical College, Albany, NY 12208.

Intracellular recordings were obtained from fast spiking cells in the CA3 cell body layer of mini-slices taken from rats 10-16 days of age. Characteristically, these cells have brief action potentials with prominent spike afterhyperpolarizations. They fire repetitively at frequencies of up to 200Hz and show little or no spike accommodation during prolonged depolarizing current steps. In experiments reported here, penicillin was bath applied and spontaneous synchronized population discharges were recorded. Previous studies have shown single bursting cells can initiate population discharges via recurrent excitatory synapses. Our results suggest fast spiking neurons also have the ability to trigger synchronized population discharges. Moreover, dual intracellular recordings reveal fast spiking cells can produce polysynaptic EPSPs in bursting cells. In order to anatomically identify these neurons they were injected with biocytin via an intracellular micropipette. Surprisingly, these cells have features of inhibitory interneurons. (Gómez et. al. this meeting) In order to determine if these interneurons produce IPSPs in bursting cells, penicillin was washed from the bath and CNQX was added to abolish residual synchronized discharges. Thusfar, one interneuron has shown both the ability to initiate the population discharge and to produce monosynaptic IPSPs in a pyramidal cell. The physiological processes underlying the excitatory actions of these interneurons are currently under investigation. (Supported by NIH Grant NS18309)

459.3

GLYCOLYSIS IS NECESSARY FOR NORMAL SYNAPTIC TRANSMISSION IN GUINEA-PIG HIPPOCAMPAL SLICES. Peter Lipton. Dept. of Physiology, Univ. of Wisconsin, Madison, WI 53706.

Synaptic transmission in dentate gyrus (DG) and CA1 of guinea-pig hippocampal slices is measured as the evoked extracellular field epsp and population spike following low frequency (.125Hz.) stimulation of the perforant path and Schaffer collateral/commissural pathway respectively.

The population spike decays rapidly in both regions following removal of glucose from the extracellular medium (9' in CA1; 15' in DG) and is rapidly restored to near normal levels by 5mM lactate. There are no changes in local ATP levels associated with these effects. The mechanism of the failure of transmission is presently unknown. However, there is no associated depletion of the releasable glutamate pool and there is no inhibition of the antidromically evoked population spike in DG.

Treatment of the "lactate-fortified" slices with 2 inhibitors of glycolysis, .2mM iodoacetate and 20mM 2-deoxyglucose, completely abolishes the population-spike in both regions, with no local changes in ATP. Transmission in these slices is also abolished by sodium pyruvate (2.5mM) and by higher concentrations of lactate. These data show that some aspect(s) of the glycolytic pathway is necessary for normal synaptic transmission even when there is adequate mitochondrial substrate; inhibition by pyruvate and elevated lactate is considered due to block of glycolysis by high citrate levels.

It is concluded that the glycolytic pathway is directly and obligatorily coupled to an aspect of synaptic transmission in guinea-pig hippocampus; this may explain the high ratio of local glucose metabolism to oxygen metabolism seen during physiological stimuli (Fox et al. Science 241:462).

459.4

PROPERTIES OF INHIBITORY AND EXCITATORY MINIATURE CURRENTS IN HIPPOCAMPAL NEURONS MAINTAINED IN LOW DENSITY CULTURE. K.S. Wilcox and M.A. Dichter. Depts. Physiology & Neurology, Univ. of Pennsylvania & Graduate Hospital, Phila., PA 19104

Electrophysiological properties of inhibitory and excitatory neurotransmission were studied using the whole cell patch clamp technique in hippocampal neurons maintained at an extremely low density in dissociated cell culture. Neurons were prepared as described (Buchhalter & Dichter, 1991), plated at 3.5-10x10^4 cells/ ml, and media was supplemented at 1-5 days with 20 mM [K^+] (Mattson & Kater,1989). Inhibitory and excitatory spontaneous miniature currents were recorded in the presence of TTX in neurons 2-3 weeks old. Miniature inhibitory currents occurred at lower frequencies than excitatory currents (0.01-3.3 Hz vs 0.75-16.0Hz), had lower rates of decay (>10 msec vs <4 msec) that were prolonged with depolarization, were blocked by $GABA_A$ receptor antagonists and prolonged with pentobarbital. Excitatory currents were blocked by glutamate receptor antagonists and showed NMDA and non-NMDA components. The frequency of currents increased with application of hyperosmotic bath solution. Amplitude distributions exhibited a large degree of variance for both currents. These results provide evidence that neurotransmission at both types of synapse is quantal. This evidence is essential before quantal analysis can be used to study short term changes in synaptic efficacy. Recordings from isolated pairs of neurons have demonstrated that frequency dependent changes in synaptic strength occur at inhibitory and excitatory synapses in this low density culture system. Supported by NS24260 to M.A.D.

459.5

COMPARISON OF TWO ANATOMICALLY DISTINCT EXCITATORY SYNAPTIC INPUTS TO CA3 PYRAMIDAL NEURONS.
S.H. Williams & D. Johnston, Division of Neuroscience, Baylor College of Medicine, Houston, Tx 77030.

The two major excitatory synaptic inputs to area CA3 of the hippocampus, the mossy fibers (MF) and the commissural/associational fibers (C/A), exhibit different forms of long term potentiation. This may in part reflect a differential distribution of NMDA receptors on the CA3 neuron's dendritic tree. These synapses also are likely to terminate at different electrotonic locations. We have investigated the kinetic properties of these two synaptic inputs. Rat hippocampal slices were maintained at 32-34°C. Inhibition was blocked by 10 μM picrotoxin and 10 μM bicuculine, and Ca^{2+} and Mg^{2+} ions were raised to 5 mM to prevent epileptiform activity. Single electrode current- and voltage-clamp recordings were made. The more distal C/A synapses had a slower 10-90% rise time and a slower decay time constant(τ_D) than the MF synapses. The τ_D of the MF EPSC was not voltage dependent. The C/A synapse did show a voltage-dependent prolongation of synaptic current at depolarized potentials (-20 mV or more). This was blocked by APV. The rise time of the EPSP for the two inputs were similarly different. The τ_D of the EPSP was also measured. If the dendritic tree is purely passive one would expect the EPSP to decay with the membrane time constant. We observed that both the MF and C/A EPSPs decayed more slowly than the membrane time constant. This effect was most marked for C/A EPSPs, which decayed with a time constant 1.8 times slower than the membrane time constant (MF decay was 1.3 times slower). This prolongation was voltage-dependent but not affected by APV. These data suggest that the kinetics of these synaptic inputs are affected both by electrotonic location of the synapses and by gated channels in the dendrites.

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459.6

QUANTITATIVE ULTRASTRUCTURAL CHANGES ASSOCIATED WITH REACTIVE SYNAPTOGENESIS IN THE CA1 REGION OF THE HIPPOCAMPUS.
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Following the unilateral kainic acid lesion of the rat hippocampus, the effects of the dennervation on the distribution of asymmetric synapses in the CA1 pyramidals cell area were quantified. Ultrastructural examination of the proximal, mid and distal sublayers of the s. radiatum of the hippocampus ipsilateral to the lesion revealed a substantial loss of synapses from these sublayers, which peaked at between 3 to 7 days post lesion. This was followed by rapid reactive synaptogenesis that was differentially distributed, with the largest number of new synapses concentrated in the sublayer proximal to the CA1 pyramidal cell layer. An interim enhancement of multisynaptic boutons, dendritic shaft and complex synapses was also found in this sublayer.

Measurement of spine parameters revealed significant increases in neck length and width over the same time period. These morphological changes might underlie the apparent recruitment of NMDA receptor involvement in epileptiform activity in this model(Turner & Wheal, Neuroscience in press).

This project was funded by the Wellcome Trust

459.7

AFFERENT INNERVATION REGULATES SYNAPTIC MOLECULAR ARCHITECTURE IN THE HIPPOCAMPUS OF ADULT RATS.
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The synapse plays a pivatol role in neuronal communication and information storage. Nonetheless, little is known about the factors that regulate synaptic structure. Our previous work indicated that synapses in developing and mature superior cervical sympathetic ganglion of rats were regulated by presynaptic innervation. In the present study, we investigated whether presynaptic innervation plays a similar regulatory role in hippocampus to define the regulation of synaptic molecular structure in the brain. Lesion of entorhinal cortex, a major souce of synaptic input to hippocampus, resulted in a dramatic 31% decrease in calmodulin binding to the novel major postsynaptic density protein, mPSDp, one day postoperatively. The maximum effect, a 55% decrease in mPSDp, was obtained 3-5 days after denervation. The lesions did not alter total synaptic membrane protein, suggesting that the denervation specifically affected the mPSDp. Combined lesions of fimbria-fornix and the entorhinal cortex resulted in a 66% decrease in the mPSDp one week after the surgery. The present findings suggest that afferent innervation plays an important role in regulating synaptic molecular architecture in the hippocampus, reproducing the findings obtained in the peripheral nervous system.

459.8

MULTIPLE CONTACTS BETWEEN HIPPOCAMPAL CA3 AXONS AND APICAL DENDRITES OF CA1 PYRAMIDAL CELLS.
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Recent findings from quantal analyses of paired recordings between hippocampal CA3 and CA1 pyramidal cells have prompted this study to determine the frequency of contacts occurring between these cells (Malinow and Tsien, 1990; Bekkers and Stevens, 1990; Friedlander et al., 1990; Foster and McNaughton, 1991). In earlier work (Harris et al., 1980), the rapid Golgi method was used on hippocampal slices from adult rats (50-60 days old) that were maintained *in vitro*. In the present study, these slices were used to visualize the CA3 axons and the CA1 pyramidal cells. Eighty-five well-impregnated axons were located in s. radiatum between 19 and 370 microns from the apical edge of the CA1 pyramidal cell bodies; 42 of these were within 190 and 310 microns. All of these CA3 axons traversed the entire width of at least one dendritic arbor from a single CA1 pyramidal cell. A *contact* was defined as a point where an axon crossing a dendrite touched the dendrite or a dendritic spine in the same focal plane at 1260x magnification. Three classes of contacts were observed: i) single contacts (76%), ii) multiple contacts onto 2 dendrites (17%), 3 dendrites (2%), or 4 dendrites (1%) of a single arbor, and iii) two contacts onto a single dendrite (4%). These results are in accordance with earlier estimates of 1.3 contacts by each CA3 axon (Andersen, 1990, Prog. Brain Res. 83:215-222). Thus, at least 24% of the CA3 axons make multiple contacts with dendrites of a single CA1 pyramidal cell, a rate of occurrence that will need to be considered for a complete interpretation of quantal analyses from paired recordings of these cells.

PRESYNAPTIC MECHANISMS: PHOSPHORYLATION

460.1

EVIDENCE THAT CYCLIC AMP (A-KINASE) REGULATES HIGH AFFINITY SEROTONIN TRANSPORT BY AN INDIRECT MECHANISM.
Steven C. King*, Anita Tiller*, Albert S. Cheng*, Charles Densmore, and Dominic Man-Kit Lee. Baylor College of Medicine, Center for Biotechnology, The Woodlands, TX 77381.

We find that cAMP affects serotonin uptake in 1)PC12, a rat pheochromocytoma; JAR, a human placental choriocarcinoma; and L-14, a transgenic mouse fibroblast line. Transport in all cases was inhibited in medium lacking either Na^+ or Cl^-, and imipramine was a potent inhibitor (IC50<1 μM).

Agents elevating the cellular cAMP (e.g., isobutylmethylxanthine, forskolin, cholera toxin) either stimulated (JAR cells), inhibited (PC12), or had no effect (L-14) on serotonin uptake.

Toxin-mediated inhibition of serotonin transport in PC12 was caused by a two-fold decrease of Vmax with no significant effect on K_m. Stimulation of serotonin transport in JAR was due primarily to a two-fold increase in Vmax in toxin-treated cells. A significant increase in the JAR affinity for serotonin which contributed to the stimulatory effects of cholera toxin with subsaturating serotonin concentrations (i.e., 0.1 μM).

Although cholera toxin acts rapidly to raise the cellular concentration of cAMP, the maximum effect on serotonin transport in either JAR or PC12 occurs at 15 to 20 hours. These results, together with the fundamental observation that JAR, PC12, and L-14 behave differently under the influence of cAMP, suggest that these regulatory phenomena cannot be entirely explained by direct phosphorylation of the serotonin carrier by the A-kinase. The possibility that cAMP regulation of high-affinity serotonin transport occurs at the level of transcription or translation deserves further consideration.

460.2

GLIAL-DERIVED S-100 PROTEIN SELECTIVELY INHIBITS THE NEURON-SPECIFIC PROTEIN F1/GAP-43 PHOSPHORYLATION BY BETA I RECOMBINANT PROTEIN KINASE C: IMPLICATIONS FOR A GLIAL-NEURONAL INTERACTION.
F.-S. Sheu[1], E.C. Azmitia[2,3], D.R. Marshak*[3], P.J. Parker*[4], and A. Routtenberg[1] 1. Northwestern Univ., Evanston, IL 60208. 2. New York Univ., New York, NY 10003. 3. Cold Spring Harbor Lab., CSH, NY 11724. 4. Imperial Cancer Res. Fund, London, WC2A 3PX.

S-100 protein from brain glia exists as an alpha-beta heterodimer (S-100a), or a beta-beta homodimer (S-100b) (Cell Calcium 7:123, 1986). S-100b is elevated in Alzheimer disease and Down syndrome (DS) brain (PNAS 86:7611, 1989) and the S-100b gene is on chromosome 21 (Science 239:1311, 1988) which is trisomic in DS. The overexpressed S-100b suggests a role for S-100 in regulating learning and memory functions. Neuron-specific protein F1/GAP-43 may play a role in neuronal growth (Annu. Rev. Neurosci. 12:127, 1989) and its phosphorylation is increased after long-term potentiation, a physiological model of memory (Brain Res. 399:205, 1986). Since S-100b in cultured serotonergic neurons enhances neurite outgrowth (Brain Res. 516:354, 1990), perhaps it does so by regulating the phosphorylation of protein F1/GAP-43.

Four recombinant PKC subtypes from baculovirus promoter were maximally stimulated to phosphorylate purified rat protein F1/GAP-43. Using a mixture of S-100a and S-100b, a dose-dependent inhibition of phosphorylation occurred. The IC_{50} (uM) values of S-100 were 16, 2, 16, 2 for the alpha, beta I, beta II and the gamma PKC subtype, respectively. When pure S100 beta was used, a subtype-specific inhibition of beta I PKC subtype was observed. 8 uM of S-100 beta inhibited F1/GAP-43 phosphorylation by 5, 60, 5 and 17% for alpha, beta I, beta II and gamma PKC. The inhibitory effect of S-100 on protein F1/GAP-43 phosphorylation may arise from a direct kinase inhibition as indicated by decreased PKC autophosphorylation or binding to substrate (JBC 264:1824, 1989). These data suggest glial-neuronal communication and that S-100 protein may be important for trophic functions by regulating phosphorylation of protein F1/GAP-43. (Supported by NIMH 25283, NSF 88-12892 and AFOSR 90-0240).

460.3

INTERACTIONS BETWEEN ARACHIDONIC ACID AND Ca^{2+} IN THE PHOSPHORYLATION OF GAP-43 (F1, B-50)

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The presynaptic PKC substrate GAP-43 has been implicated in neuronal outgrowth, regeneration, and plasticity[1]. LTP in the mature hippocampus is characterized by correlated increases in GAP-43 phosphorylation[2] and transmitter release[3]. These presynaptic changes may depend on the post-synaptic generation of arachidonic acid[4] (AA; 20:4) and its subsequent stimulation of AA-sensitive isoforms of PKC[5,6]. In the present study, synaptosomal membranes from rat cerebral cortex were used to examine whether AA affects GAP-43 phosphorylation and if it interacts with changes in Ca^{2+} levels. Low concentrations of AA (50 μM) stimulated GAP-43 phosphorylation, and this effect was potentiated by Ca^{2+} (10^{-6} M). High AA concentrations (500 μM) decreased GAP-43 phosphorylation to less than that exhibited in the absence of the fatty acid. This bimodal effect was mimicked by another *cis*-unsaturated fatty acid (18:1*c*), but not by the *trans* stereoisomer (18:1*t*), nor by the saturated isomer of AA (20:0). These results demonstrate that the phosphorylation of GAP-43 is regulated synergistically by free AA and Ca^{2+}. This presynaptic regulation may be important in modifying mature synapses and may also play a role in the initial development of neuronal circuitry.

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460.4

DEPOLARIZATION-DEPENDENT TYROSINE PHOSPHORYLATION IN SYNAPTOSOMES.

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Protein phosphorylation is involved in the regulation of synaptic activity. Although protein tyrosine kinases are present at the synapse [eg. Ellis and Gurd (1988) J. Neurochem. 51 611-620] their relationship to synaptic function is unknown. We therefore assessed the effect of depolarization on synaptosomal phosphotyrosine-containing proteins (ptyr-proteins) by immunoblotting with anti-ptyr antibodies. Freshly prepared synaptosomes from rat forebrain contained several ptyr-proteins and these remained essentially unchanged during incubation in vitro. Incubation for 45 min. in the presence of 0.1 mM Ca^{2+} followed by depolarization with high K^+ (41 mM) led to an increase in the amount of immunodetectable ptyr associated with a protein of Mr 117,000 (ptp117). This increase was apparent within 1 min. of the start of depolarization, reached a maximum between 3 and 5 min. and then decreased during the next 20 min.. Omission of Ca^{2+} from the incubation buffer, or the addition of EGTA 5 min. prior to depolarization prevented the increase in tyrosine phosphorylation of ptp117. The results suggest that the phosphorylation of ptp117 on tyrosine may be involved in the regulation or modulation of synaptic activity.

Supported by the Natural Science and Engineering Research Council.

460.5

DEPHOSPHORYLATION OF B-50 (GAP-43) BY TYPE 1 AND 2A PHOSPHATASES IN SYNAPTIC PLASMA MEMBRANES.

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The neuronal protein kinase C substrate B-50 (GAP-43) is dephosphorylated in rat cortical synaptic plasma membranes (SPM) by phosphatase type 1 and 2A (PP-1 and PP-2A)-like activities. The present studies further demonstrate that B-50 is dephosphorylated not only by the active PP-1-like enzyme, but also by a latent form following pretreatment of SPM with 0.2 mM cobalt/20 μg/ml of trypsin in an inhibitor [1 μM okadaic-acid (OA) and 2.6 μg/ml of inhibitor-2 (I-2)]-sensitive manner. In the presence of an excess of I-2, histone H1 (16-64 μg/ml) and spermine (2 mM) increased B-50 dephosphorylation, an effect which was prevented by 2 nM OA. PP-1 and PP-2A-like activities from SPM were further displayed by using exogenous phosphorylase a and histone H1 as substrates. OA-induced inhibition of B-50 dephosphorylation was also demonstrated in rat cortical synaptosomes by use of an immunoprecipitation assay with affinity-purified anti-B-50 IgG. These results provide further evidence that SPM-bound PP-1 and PP-2A-like enzymes that share considerable similarities with their cytosolic counterparts may act as physiologically important phosphatases for B-50. [Supported by the Ohio Department of Aging and NIH (NS23598)].

460.6

HISTAMINE AND NICOTINE, VIA DISTINCT MECHANISMS, STIMULATE PARALLEL INCREASES IN SYNAPSIN II PHOSPHORYLATION AND NOREPINEPHRINE RELEASE

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Protein phosphorylation is widely recognized as the primary molecular mechanism for regulation of protein function, and a family of phosphoproteins known as the synapsins are thought to be involved in neurosecretion. Indeed, a recent report demonstrated that synapsin II may play a role in production of synaptic vesicles and in synapse formation. (Han et al., *Nature, 349*, 697-700.) We have been examining synapsin II phosphorylation and its relationship to norepinephrine (NE) release from bovine adrenal medullary chromaffin cells (BACC). This is a complex phenomenon modulated by cholinergic and non-cholinergic transmitters. Primary cultures of BACC can be stimulated to release NE by nicotine, mimicking the cholinergic stimulus from the splanchnic nerve. Histamine also stimulates NE release from these cells in a time- and dose-dependent manner. We have previously shown that these two transmitters increase $^{32}PO_4$ incorporation into synapsin II. We report here that maximal doses of nicotine (100 μM) and histamine (100 μM), when combined, produce additive increases in both NE release and synapsin II phosphorylation. These data suggest different mechanisms of activation. We also find that nicotine-stimulated release is abolished in the absence of extracellular calcium, whereas histamine-stimulated release remains significantly elevated in calcium-free media. Nicotine is known to act through nicotinic receptors, stimulating a depolarization-dependent influx of extracellular calcium. Histamine acts through H_1-receptors, stimulating a release of intracellular calcium via IP_3 production. Thus, calcium arising from two separate pools can stimulate additive increases in NE release and synapsin II phosphorylation in BACC. In addition, these data provide evidence for the hypothesis that synapsin II phosphorylation plays a role in NE release from these cells. Supported by PHS grant DK40486 to MDB.

460.7

OPTICAL MEASUREMENT OF SYNAPTIC VESICLE RECYCLING

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We have studied the pattern of dye loading and unloading in frog cutaneous pectoris motor nerve terminals using FM1-43, a derivative of RH414 (Molecular Probes, Inc). FM1-43 stains synaptic vesicle membranes, producing bright spots along the length of nerve terminals, each spot consisting of a cluster of hundreds of stained vesicles. We tested the hypothesis that recycled vesicles mix randomly within a vesicle cluster. Results from 3 experiments support the hypothesis.

1. Partial dye loading gives spots the same size as full dye loading, only dimmer.

2. Stained terminals destain with nerve stimulation. If a terminal is partly destained, and then stimulation is halted for 10 minutes and then resumed, the second destaining rate is slower than the first.

3. As independent measures of transmitter release, summed end plate potentials (epps) were compared with fractional dye release during destaining. The two curves deviated after 1-2 minutes, with dye release falling below summed epps.

These results quantitatively support the hypothesis that recycled vesicles distribute randomly throughout the vesicle cluster.

Supported by NS10207 and NS23466 (to WJB).

460.8

SYNAPTIC VESICLE-ASSOCIATED CaM KINASE II BINDS THE C-TERMINAL REGION OF SYNAPSIN I.

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Synapsin I is a major neuron-specific phosphoprotein which associates with small synaptic vesicles at multiple sites in a phosphorylation-dependent fashion. In an attempt to identify vesicle protein(s) which interact with the C-terminal "tail" region of synapsin I, purified NTCB-cleaved tail fragment was conjugated to the heterobifunctional cleavable reagent SASD and subsequently iodinated in the dark. The ^{125}I-ASD-tail was then incubated with synapsin I-depleted synaptic vesicles, photolyzed and cleaved. Proteins in the mixture were then resolved by SDS-PAGE. A major vesicle protein of 47 kDa was labeled with ^{125}I. The protein was extracted from preparative gels and digested with endoproteinase Asp-N. Three peptides were purified by reverse phase HPLC and sequenced. The amino acid sequences were identical to regions of the α-subunit of CaM kinase II, which is associated with synaptic vesicles (see Rubenstein J. *et al.*, this meeting). We tested whether the binding of the tail fragment is competed by peptides corresponding to the sequences surrounding phosphorylation sites 2 and 3 of synapsin I or by CaM kinase II inhibitory peptides based on the sequence of the autoregulatory domain of the kinase. We found that, under basal conditions, the CaM kinase II peptides [273-302]Ala^{286} and [281-302]Ala^{286} were both effective in displacing the tail fragment from synaptic vesicles (IC_{50} = 10 and 35 μM, respectively), the site 3 peptide was less potent (IC_{50} = 450 μM) and the site 2 peptide was ineffective. The results indicate that CaM kinase II associated with synaptic vesicles is involved in binding the tail region of synapsin I. If synapsin I is present on the vesicles in close association with CaM kinase II, its phosphorylation, stimulated by an increased intraterminal Ca^{2+} concentration, could be an extremely fast process, conceivably involved in mediating exocytosis.

460.9

LABELING OF SYNAPTIC VESICLES IN LIVING NEURONS WITH ANTIBODIES TO THE LUMENAL DOMAIN OF SYNAPTOTAGMIN/P65
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We used polyclonal antibodies (p65-Abs) directed against the intravesicular domain of the intrinsic protein of synaptic vesicles (SVs) synaptotagmin (protein p65) to monitor SV exocytosis and recycling in living hippocampal neurons in culture. Cultures were first incubated with p65-Abs under different experimental conditions and subsequently without the antibodies for various times. Cells were then fixed, permeabilized and stained with rhodamine-conjugated secondary antibodies. Only very weak staining was observed when neurons were incubated with p65-Abs at 0°C, suggesting that the pool of SV proteins present in the plasmalemma at any given time is very low. When cultures rich in synaptic contacts were incubated for 1 hr in the presence of p65-Abs followed by a short wash (15 min) a specific staining of synapses was observed. This indicates a specific uptake of p65-Abs in synaptic terminals. When similar experiments were performed on neurons grown in isolation, intense SV staining was observed throughout the axonal arbour, indicating that SVs undergo exo-endocytosis even in absence of synaptic contacts. The staining pattern for internalized p65-Abs and for synaptic vesicle antigens was found to be identical even when the 1hr incubation with p65-Abs was followed by 2-5 days incubation without antibodies. If during this time synapses were formed, p65-Abs became clustered at synaptic sites in parallel with SVs. These observations indicate that binding of p65-Abs to the lumenal domain of p65 does not significantly affect the localization and traffic of the protein. Our results suggest that p65-Abs may be a powerful marker for SVs and nerve terminals in living neurons.

460.10

LOW pH PREVENTS SYNAPTIC VESICLE RECYCLING IN SNAKE MOTOR NERVE TERMINALS.
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We have previously shown (Searl, T., *et al., Neuroscience*, **35**, 145-156, 1990) that prolonged motor nerve stimulation induces the appearance of two populations of miniature end-plate currents (MEPCs) at the snake neuromuscular junction. The so called "small-mode" MEPCs have been attributed to the release of recycling quanta. It is not clear why these recycling quanta are smaller than the normally released pre-formed quanta. Two possibile explanations are: (i) that the cytoplasmic supply of acetylcholine (ACh) for these recycling quanta is limited or (ii) that the synaptic vesicle proton gradient, required for the translocation of ACh into the synaptic vesicles, is in some way impaired. To determine which of these two possibilities is more likely we have examined the effects of 5 minutes of 10 Hz nerve stimulation on MEPC amplitude distributions in the presence of a reduced pH (5.0). In unstimulated preparations MEPC amplitude distributions were very similar in pH 7.0 (normal) and 5.0. However, in contrast to the effects of stimulation at pH 7.0, stimulation immediately following a switch to pH 5.0 did not lead to the appearance of a bi-modal distribution of MEPC amplitudes, the MEPC amplitude distributions being essentially identical to those seen pre-stimualtion. The inability to detect small-mode MEPCs following stimulation in the presence of a low pH suggests that, in the snake, the ACh content of recycling synaptic vesicles is more critically dependent on the synaptic vesicle proton gradient than the nerve terminal ACh content.

Supported by the Wellcome Trust and the Medical Research Council.

CALCIUM CHANNELS: MULTIPLE TYPES

461.1

PREFERENTIAL RECRUITMENT OF THE DIHYDROPYRIDINE-SENSITIVE COMPONENT OF ACTION-POTENTIAL-INDUCED Ca^{2+} INFLUX.
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Intracellular free calcium concentration $[Ca^{2+}]_i$ transients were elicited by electrical field stimulation and measured by indo-1 based microfluorimetry in single rat hippocampal neurons grown in primary culture. The $[Ca^{2+}]_i$ transients were mediated by action potentials as indicated by a response threshold sharply dependent on stimulus amplitude, and complete block by tetrodotoxin (1 uM). $[Ca^{2+}]_i$ transients elicited by 10 pulses at 10 Hz (peak 546 ± 42 nM from basal 159 ± 16 nM, n=7) were only slightly sensitive to inhibition (11 ± 4%) by nitrendipine (NIT, 1 uM). Application of tetraethylammonium (TEA, 2mM) increased the amplitude of the response by 290 ± 60%. The TEA-recruited response was blocked by NIT (94 ± 9%, n=4). Stimulation at 50 Hz for 30 pulses increased the amplitude of the $[Ca^{2+}]_i$ transient by 199 ± 21% relative to that observed at 2 Hz. NIT did not effect the low-frequency response, but showed a marked inhibition of the high-frequency-recruited response (50 ± 7%, n=3). Previous results have shown that TEA (1) and high-frequency electrical stimulation (2) slow action potential repolarization by decreasing K^+ current. Preferential recruitment of dihydropyridine-sensitive Ca^{2+} influx by increased action potential duration may depend on the slow activation and inactivation kinetics of the dihydropyridine-sensitive, voltage-activated Ca^{2+} channel.

(1) Storm J. F., 1987. J. Physiol., 385, pp. 733-759.
(2) Jackson M. B., et. al., 1991. Proc. Natl. Acad. Sci., 88, pp. 380-384.

461.2

POTENTIATION OF THE LOW-VOLTAGE-ACTIVATED (LVA) CA++ CURRENT BY ALBUMIN.
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Bovine serum albumin (BSA) has been reported to enhance LVA Ca and Na currents in chick and rat dorsal root ganglion neurons. In the present study, we have purified BSA with FPLC techniques, cleaved the molecule enzymatically with trypsin and tested the fragments for biological activity on the LVA Ca current.

Patch clamp recordings (whole cell configuration) were performed on acutely dissociated E_{13} chick dorsal root ganglion (DRG) neurons with 20mM Ca in the external solution. While not affecting HVA Ca currents, BSA enhanced peak amplitudes of the LVA Ca component with little effect on activation or inactivation kinetics. Half maximal enhancement occurred at approximately 1mg/ml BSA.

Upon chromatography on a MonoQ anion exchange column, the biological activity comigrated with the 67kDa protein. Enzymatic cleavage with trypsin yielded prevalent fragments with apparent molecular weights between 20 and 50kDa. On the MonoQ column, the trypsinized products eluted as three major protein peaks. Based on its protein contents, the first peak (primarily containing 7, 10 and 26kDa components) was about 15 times as active as the peaks two and three (containing fragments around 40kDa and albumin, respectively).

Our experiments indicate that the LVA potentiating activity is an integral part of albumin and that low molecular weight fragments retain this biological effect.

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461.3

HIGH- AND LOW-THRESHOLD CALCIUM CURRENTS IN ACUTELY ISOLATED STRIATAL NEURONS
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The neostriatum is intimately involved in the control of locomotion. Ca^{2+} channels are important in determining the firing patterns of a variety of neurons. The nature and possible modulation of voltage-dependent Ca^{2+} channels has not to date been described in mature striatal neurons. The ionic currents in acutely isolated neurons from rat (21 to 28 day postnatal) striatum were investigated using whole-cell voltage clamp. Striatal neurons, isolated in trypsin-hyaluronidase, could be divided morphologically into a population of small neurons with a whole cell capacitance of less than 10pF and a population of large neurons with greater than 12 pF capacitance. A TTX-sensitive Na^+ current as well as K^+ currents with properties similar to the A-current and delayed rectifier current were seen in both types of neurons. To isolate Ca^{2+} currents, the pipette solution contained (in mM): trizma phosphate 70, trizma base 28, TEACl 40, EGTA 11, MgATP 2, NaGTP 0.3 and the extracellular solution contained (in mM): NaCl 90, TEACl 40, HEPES 10, 4-AP 4, TTX 0.0012 and either Ba^{2+} 2.5 or Ca^{2+} 5. In 2.5mM Ba^{2+}, both types of neurons exhibited high-threshold, sustained inward currents. In 5mM Ca^{2+}, distinct high- and low-threshold inward currents were seen in both populations of neurons. At a holding potential of -100 mV, threshold for activation of the low threshold, rapidly inactivating current was about -60 mV and this current reached maximum at -40 to -30 mV. At holding potentials positive to -80mV this current was inactivated. A sustained, high-threshold inward current activated at -40 to -30 mV and reached maximum at -10 to 0 mV. Because dopamine is a major neurotransmitter in the striatum, we examined the possibility that it might modulate Ca^{2+} currents in these neurons. A reduction in high threshold Ca^{2+} current by either dopamine (10μM) or the D_2 agonist, quinpirole (10μM), was seen in only 3 out of 20 neurons. (Supported by the MRC of Canada and AHFMR).

461.4

MULTIPLE CALCIUM CURRENTS RECORDED IN RAT SUBSTANTIA NIGRA ZONA COMPACTA NEURONS *IN VITRO*.
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Numerous studies using intracellular voltage recordings have suggested that dopamine (DA) neurons in the rat substantia nigra zona compacta (SNZC) possess at least 2 distinct calcium currents (I_{Ca}s). To further examine the properties of I_{Ca}s in these cells, whole-cell recordings were obtained from DA neurons in horizontal slices of rat midbrain. Such cells were identified electrophysiologically by their broad action potentials, regular pacemaker firing and strong anomalous rectification.

I_{Ca}s were isolated from other voltage-dependent membrane currents by using cesium chloride (140 mM) in the recording pipette and by superfusion of medium containing TEA (10-20 mM), 4-aminopyridine (2 mM) and TTX (1 μM). When recording in the voltage-clamp mode under these conditions, 3 distinct I_{Ca}s could be elicited . The first, a transient inward current analagous to the T-type current recorded in many other neurons, was activated by relatively small (20-40 mV) depolarizations from a holding potential (V_h) of -80 to -90 mV and peaked between -20 and -30 mV. This low voltage-activated (LVA) current rapidly inactivated during a maintained (250 ms-1 s) voltage step and could not be elicited from a V_h more positive than -60 mV. In some cells, large voltage steps from a V_h of -80 mV activated an I_{Ca} which substantially inactivated during a 250 ms step and may correspond to the N-type I_{Ca} reported elsewhere. Finally, step depolarizations from a V_h of -40 mV activated a large, high voltage-activated (HVA) current which only partially inactivated during a prolonged (1 s) depolarizing step. This current peaked between 0 and -10 mV and its current-voltage relationship resembled that of the previously described L-type current.

Both the LVA and HVA I_{Ca}s were reduced or blocked by cadmium (30 μM- 1 mM) and the dihydropyridine nifedipine (100 nM- 3 μM), although the LVA current was relatively more resistant to these treatments.

461.5

"N"- AND "L"-LIKE CALCIUM CURRENTS IN LUNG CANCER CELLS ARE BLOCKED BY LAMBERT-EATON IgG.

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Lambert-Eaton syndrome (LES), an autoantibody-mediated disorder of presynaptic calcium channels at the neuromuscular junction (NMJ), is often associated with small cell lung cancer (SCLC). We are therefore studying the calcium currents in human SCLC cell lines that express both "N"- and "L"-like calcium currents in an attempt to characterize the type(s) of calcium channels that may exist at the NMJ. Whole-cell and cell-attached patch clamp recordings from the NCI-H345 cell line reveal two types of high-threshold calcium current (carried by barium). The "L"-like current is non-inactivating, 50% steady-state inactivated at -10 mV, sensitive to dihydropyridines, with a single channel conductance of about 21 pS. The "N"-like current significantly inactivates during a 100 ms pulse, is 50% steady-state inactivated at -50 mV, insensitive to dihydropyridines, and has a single channel conductance of about 16 pS. This is the first demonstration of an "N"-like channel in a non-neuronal cell. Both currents are significantly reduced following 72-hour incubation in LES IgG and unaffected by IgG from myasthenia gravis patients. Supported by grants from the MDA, NIH (CA37343†, NS06232) and NSF (BNS8919841).

461.6

A NEURONAL CELL LINE WITH ONLY T-TYPE CALCIUM CHANNELS.

R.T. McCarthy and P.E. TanPiengco. Miles Inst. Preclin. Pharm., West Haven, CT 06514.

Calcium channel currents have been studied using whole-cell and cell-attached patch recording in a cell line derived from N1E-115 (passages 20-40). After 2-5 days in culture these cells have only low threshold current (n=30). Culturing with DMSO (2-10% ;2 days-3 wks.) in an attempt to differentiate these cells failed to enhance neurite formation or induce high threshold currents. Elicitable currents activated near -55 mV, peaked near -15 mV and reversed positive to +40mV (20 Ca++/130 Cs+). Substitution of barium for calcium as the charge carrier did not enhance inward current or alter the voltage dependence of channel inactivation. The midpoint of the steady-state availability curve was -58.0 mV (n=5) with no elicitable current from depolarized holding potentials (Vh = -30 mV) even with strongly depolarizing test potentials (Vt = +20 mV). Tail currents with slow T-type kinetics (V_B=-65; τ=6 msec) were well fit by single exponentials. Cell-attached patch recording (n=6) characterized the underlying conductance as a 7.5 pS channel (110 Ba^{++}). This preparation provides an opportunity to study neuronal T-type calcium channels in isolation from N- or L-type.

461.7

PHARMACOLOGY OF T - TYPE CALCIUM CHANNELS IN A NEURONAL CELL LINE.

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The pharmacology of T-type calcium channel current was studied in a neuronal cell line derived from N1E-115 that expressed only T-type channels. These low threshold currents (activation as negative as -55 mV) could be inhibited by nickel (150 μM) and completely inhibited by cadmium (500 μM). Omega-conotoxin (5 and 10 μM) had no effect on currents. Neither whole-cell peak current nor tail current were enhanced by BAY K 8644 and, instead, appeared to be slightly inhibited by this drug. The agonist enantiomer of BAY K 8644 [BAY R 5417 (100 nM)] had no effect on this low threshold current. Studies with cell-attached patch recording (110 Ba^{++}) in the presence of calcium channel agonist failed to demonstrate large conductance openings or a switch in the mode of gating of the 7.5 pS conductance. Activity of the 7.5 pS channel was retained in excised patches. This calcium channel current shares the pharmacological characterization of T-type channels, providing an opportunity to study the modulation of T-type channel current isolated from other neuronal calcium channel types.

461.8

DIPHENYLBUTYLPIPERIDINE (DPBP) ANTIPSYCHOTICS PREFERENTIALLY BLOCK T-TYPE CALCIUM CHANNELS IN NEURAL CREST DERIVED THYROID C CELLS.

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The inhibition of Ca^{2+} channels by the DPBP penfluridol was studied with whole cell patch clamp in thyroid C-cell lines. Three separate components of Ca^{2+} current, corresponding to T, L, and N channels were identified in rat rMTC 6-23 (clone 6) cells. The low voltage-activated, rapidly inactivating T current was potently blocked by penfluridol. At a concentration of 500 nM, penfluridol inhibited 78.0 $\pm$ 2.3% (n=28) of T current. The slowly inactivating component of Ca^{2+} current, primarily N- and L-type, was less potently inhibited (25.6 $\pm$ 3.5%, n=29). Block of T current by penfluridol was use-dependent and promoted by channel activation. T-type Ca^{2+} channels were also effectively blocked by penfluridol in the human TT C-cell line. Among organic Ca^{2+} antagonists DPBPs are distinctive in their ability to preferentially block T-type Ca^{2+} channels at low concentrations. DPBPs will be useful in determining the function of this Ca^{2+} channel subtype in excitable cells.

461.9

LOW THRESHOLD CALCIUM CURRENT OF RAT LATERODORSAL TEGMENTAL NEURONS STUDIED WITH WHOLE-CELL PATCH CLAMP IN SLICES.

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As an integral part of the ascending reticular activating system, the laterodorsal tegmental (LDT) nucleus plays an important role in the regulation of thalamocortical function. Intracellular recordings have shown that there exist at least three electrophysiologically different classes of neurons in the LDT area. We have used the whole-cell patch clamp technique as applied to the *in vitro* slice preparation to study the characteristics of the transient calcium current, I_T, exhibited by a subpopulation of LDT neurons. The average current amplitude was 300 pA, which became larger in solutions containing elevated [Ca^{2+}], smaller in the presence of 2 mM Co^{2+} , and was unaffected by substitution of choline for sodium. I_T could be evoked by depolarizing voltage steps to -50 mV from a holding potential of -65 mV. Activation of I_T showed extremely steep voltage dependence in the voltage range from -55 mV to -45 mV. The time-to-peak latency became shorter at more depolarized voltages. The decay phase of the current followed a single exponential time course, with an average tau of 70 ms, and did not show strong voltage dependence. Removal of inactivation was both time and voltage dependent, with 300 ms needed for total removal at a membrane potential of -80 mV. The biophysical properties of I_T are largely responsible for the bursting behaviour of this subpopulation of LDT neurons.

Supported by the MRC.

461.10

T-TYPE CA++ CURRENT AND ITS FUNCTION IN TRIGGERING CA++ INFLUX IN EMBRYONIC *XENOPUS* SPINAL NEURONS.

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Previous studies have shown that spontaneous Ca^{++} influx during a critical period plays an important role in development of embryonic *Xenopus* spinal neurons in culture. We have used the whole cell voltage clamp technique to study low voltage activated (LVA) T-type Ca^{++} current and its role in regulating Ca^{++} influx during neuronal development. Blocking high voltage activated (HVA) N- and L-type Ca^{++} currents with intracellular F^- and replacing or removing extracellular Ca^{++} showed that LVA T-type Ca^{++} current is present in these cultured neurons.

This T current is a transient inward current and can be activated at membrane potentials between -60 and -45 mV. It has the lowest threshold among the voltage activated depolarizing currents recorded in young neurons, and reaches its peak at membrane potentials around -35 mV with an amplitude of 50 pA. The rates of both activation and inactivation are voltage dependent. With steps to -35 mV, the time to half maximum of activation and the time constant of inactivation are 3.6±0.2 ms and 17.5±1.8 ms, respectively. The T current is present in both young and mature neurons with no apparent change of its properties. Ni^{++} has been shown to block increases in intracellular Ca^{++} triggered by Ca^{++} influx. In the present study, we found that Ni^{++} can be used to selectively block T current with only a minor effect on HVA currents.

LVA T current may be involved in depolarizing cells and triggering HVA Ca^{++} currents, thus inducing Ca^{++} influx in cultured neurons at early stages in their differentiation. Supported by NIH NS15918 to NCS.

461.11

TRANSIENT AND SLOW CALCIUM CURRENTS IN EMBRYONIC MUSCLE GROWN IN THE PRESENCE AND ABSENCE OF NERVE.
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Skeletal muscle displays two types of inward Ca^{++} currents: a slow, dihydropyridine sensitive current and a transient current which is less sensitive to dihydropyridines. The purpose of the present study was to determine if the developmental appearance of these two currents was influenced by the presence of the nerve. Muscle cells were removed from embryos of the frog Xenopus laevis at early stages (12-14) of development, 4-6 hr prior to innervation, and plated in culture (nerve-free, NF). The Ca^{++} currents in these cells were compared to those in muscle cells derived from older embryos (stage 19-21) and grown in the presence of neural tube cells (nerve-enriched, NE). Ca^{++} currents were examined using the whole cell recording technique in the absence of Na^+ or K^+ (extracelluar solution, in mM: 127.5 tetraethylammonium, 20 $BaCl_2$, 1.2 $MgCl_2$, 10 Hepes; electrode solution, in mM: 140 CsCl, 5 $MgCl_2$, 1 BAPTA, 10 HEPES; both solutions were pH 7.4). There was no difference between the NE and NF cultures in the number of cells with a transient or slow current, the developmental appearance of the two currents, or the current density. The two currents however, differ from each other in their developmental time course. By the first day in culture about 60% of the cells have a slow current compared to 11% with a transient current. By day 2 all cells had a slow current whereas by day 3 only 50% of the cells had a transient current. The results of the present study indicate that although the slow and transient Ca^{++} currents differ from each other in a number of ways the developmental appearance of these currents is not influenced by the presence or absence of the nerve.

461.12

PROPERTIES OF SINGLE VOLTAGE-GATED CALCIUM CHANNELS IN DISSOCIATED MEDIAL SEPTUM/DIAGONAL BAND (MS/DB) NEURONS.
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We have previously identified both low-voltage activated (LVA) and high-voltage activated (HVA) calcium currents in MS/DB neurons using the whole-cell patch-clamp technique (Neurosci Abstr. 16:676,1990). The present study identified the properties of single calcium channels contributing to these macroscopic currents. Calcium channels were recorded in MS/DB neurons acutely dissociated from adult guinea pig (175-700 gms) using the cell-attached recording configuration. Barium (100-110 mM) inside the pipette was used as the charge carrier and isotonic K-aspartate (140 mM) was used to zero the cell's resting potential. From a holding potential of -80 mV, two channel types were recorded most often, first a low conductance channel (6-7 pS) that was activated near -40 mV, and second, a large conductance channel (24-26 pS) activated near -20 mV. These latter channels were recorded in the presence of Bay K 8644 (1-5 μM). A third channel type (12-14 pS) was recorded less frequently and was activated near 0 mV. These three channel types differed in their voltage-dependence, open probability and sensitivity to Bay K 8644 and may resemble T, L and N type channels recorded in hippocampal and ganglion cells.
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CALCIUM CHANNELS: CONOTOXINS AND OTHER LIGANDS

462.1

THE EFFECTS OF SPIDER VENOMS ON ω-CONOTOXIN GVIA (ω-CT)-SENSITIVE AND -INSENSITIVE $^{45}Ca^{++}$ INFLUX AND NEUROTRANSMITTER RELEASE.
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Recent studies have shown that some spider venoms contain toxins that interact with voltage-sensitive calcium channels (VSCC). The present study evaluated the effects of 10 spider venoms (Spider Pharm, Inc.) against K^+-evoked $^{45}Ca^{++}$ influx in chick (ω-CT-sensitive) and rat (ω-CT-insensitive) synaptosomes as a first step to find additional toxins with activity at VSCC. At a concentration of 0.3 ul venom/ml, venoms from Agelenopsis aperta, Hololena curta, Plectreurys tristis and Phidippus ardens inhibited K^+-evoked $^{45}Ca^{++}$-influx in both preparations by greater than 70%. Venoms from Alphonopelma chalcodes and Peucetia viridans inhibited $^{45}Ca^{++}$ influx by 50-70%. Venoms from Delana cancerides, Heteropoda venatoria and Loxosceles deserta inhibited $^{45}Ca^{++}$ influx by less than 50%, whereas Eriophora edax venom had no effect on $^{45}Ca^{++}$ influx. Basal $^{45}Ca^{++}$ influx was slightly inhibited (approximately 20%) by Plectreurys tristis and Peucetia viridans venoms, and slightly enhanced (approximately 20%) by Phidipus ardens venom. None of the active venoms exhibited substantial selectivity for inhibition of chick vs rat synaptosomal $^{45}Ca^{++}$ influx at 0.3 ul/ml. Selected venoms were characterized further against K^+-evoked ^{3}H-norepinephrine release (ω-CT-sensitive) and ^{3}H-D-aspartate release (ω-CT-insensitive) from rat hippocampal slices. For example, Agelenopsis aperta venom inhibited release of both transmitters with virtually identical potencies (IC_{50} ~ 0.03 ul venom/ml). The results suggest that spider venoms may be a rich source of toxins that inhibit ω-CT-sensitive and -insensitive VSCC.

462.2

THE EFFECTS OF VARYING [K^+] ON ω-CONOTOXIN GVIA (ω-CT) INHIBITION OF K^+-EVOKED RELEASE OF ^{3}H-NOREPINEPHRINE (^{3}H-NE) AND SYNAPTOSOMAL $^{45}Ca^{++}$ INFLUX.
T.J. Mangano, T.M. Piser, A.I. Salama and R.A. Keith. Department of Pharmacology, ICI Americas, Inc., Wilmington, DE 19897.

The effects of varying [K^+] on the inhibition of ^{3}H-NE release from rat hippocampal brain slices and evoked synaptosomal $^{45}Ca^{++}$ influx by ω-CT and neomycin (NEO) were examined. K^+ (15-100 mM) caused a concentration-dependent release of ^{3}H-NE that was greater than 90% dependent on extracellular calcium. The ability of ω-CT to inhibit ^{3}H-NE release was optimal at 25 mM K^+ and was substantially reduced at higher concentrations of K^+. ω-CT (0.1 uM) maximally inhibited ^{3}H-NE release by 49% (15 mM K^+), 63% (25 mM K^+), 20% (50 mM K^+), and 8% (75 mM K^+). In contrast, NEO caused a concentration-dependent and complete inhibition of ^{3}H-NE release at all concentrations of K^+, with IC_{50} values: 210 uM (15 mM K^+); 150 uM (25 mM K^+); 450 uM (50 mM K^+); and 1500 uM (75 mM K^+). ω-CT (1 uM) had little effect (less than 10% inhibition) on hippocampal synaptosomal $^{45}Ca^{++}$ influx at any concentration of K^+, whereas NEO could completely inhibit $^{45}Ca^{++}$ influx. The results suggest that inhibition by ω-CT of K^+-evoked ^{3}H-NE release is voltage dependent, or that ω-CT-resistant calcium channels are recruited at higher concentrations of K^+. The apparent absence of ω-CT-sensitive synaptosomal $^{45}Ca^{++}$-influx sites suggests that N-type calcium channels are a small subset of channels in synaptosomes. Dihydropyridines, selective L-channel antagonists, have been shown to have little effect on either ^{3}H-NE release or $^{45}Ca^{++}$-influx. Thus, NEO's inhibition of ω-CT-resistant responses may be due to activity at non-L/non-N voltage-sensitive calcium channels.

462.3

VENOM OF THE SPIDER, HOLOLENA CURTA, BLOCKS A DIHYDROPYRIDINE, ωCgTx RESISTANT VSCC IN MAMMALIAN SYNAPTOSOMES.
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Two major high threshold VSCC's exist in neuronal tissue on nerve terminals (N and L type). Inhibition of the N or L type channel with omega conotoxin GVIA (ωCgTx) and the dihydropyridine (-) 202-791 respectively produces only slight reduction (– 25 percent) of measureable K^+ induced Ca^{2+} influx or the rise in intracellular [Ca^{2+}] in mammalian tissue (Lundy et al. Eur. J. Pharmacol. 206, 61. 1991). These results and others, suggest the existence of another undefined VSCC responsible for about 75 percent of the total measureable influx in rat synaptosomes. Venom from the funnel web spider Hololena curta potently (IC_{50} - 1:10,000 or 4.21 μg venom protein/ml) inhibited the Ca^{2+} influx in an apparently irreversible manner at depolarization times 1-30s. At higher concentrations (1:1,000 or 42.1 μg venom protein/ml) the inhibition was 90 percent complete. Inhibition was unaffected by prior incubation with ωCgTx plus (-) 202-791 (both 1 μM). The majority of this VSCC inhibitory activity was found in fractions with mol wt. > 3,000. The venom also blocked motor responses in a variety of electrically stimulated peripheral tissues and inhibited [^{3}H] noradrenaline release from the rat anococcygeus muscle without significant L channel activity. Hololena venom or the active constituent of hololena venom which inhibits this VSCC should provide useful tools to investigate the role of this novel Ca^{2+} channel in neuronal function.

462.4

ω-Conotoxin block of calcium channels in peripheral neurons: Reversibility and the influence of divalent cation concentration.
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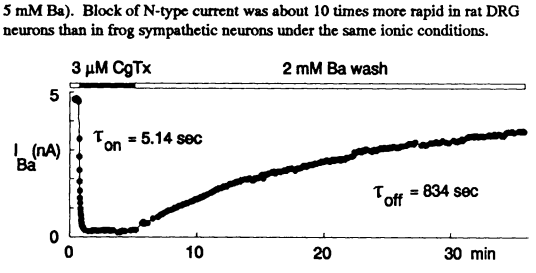
Ca channel block by ω-conotoxin (fraction GVIA) was investigated using whole-cell recordings from freshly dissociated peripheral neurons. ω-Conotoxin blocked about 50% of the high-threshold Ca channel current in rat DRG neurons and about 90% of the current in bullfrog sympathetic ganglion neurons. When frog sympathetic cells were bathed in 3 μM nimodipine, nearly all of the remaining Ba current was blocked by 3 μM ω-conotoxin. In frog sympathetic cells, with 2 mM Ba as charge carrier, block by 3 μM ω-conotoxin developed with a time constant of 7 sec. When the toxin was washed, recovery occurred with a time constant of about 800 sec (in cells with stable currents, see figure). Block was faster in lower external Ba and slower in higher Ba (τ ~3 sec with 0.5 mM Ba and τ ~54 sec with 5 mM Ba). Block of N-type current was about 10 times more rapid in rat DRG neurons than in frog sympathetic neurons under the same ionic conditions.



462.5

STRUCTURE-ACTIVITY ANALYSIS OF OMEGA-CONOTOXIN GVIA INTERACTION WITH NEURONAL CA++ CHANNELS. R.A.Lampe, J.H. Herman*, M.B.Horn*, R.A.Keith, R.Spreen*, M.W.McLane* and M.M.Lo. ICI Pharmaceuticals Group, Wilmington DE 19897.

Venom from the fish hunting snail species, Conus geographus, contains a 27 a.a. peptide known as omega-conotoxin GVIA (ω-GVIA). Preliminary structure-activity relationships (SAR) have demonstrated that this highly basic peptide, containing three intramolecular disulfide bonds, interacts with a subpopulation of mammalian neuronal Ca^{++} channels. To further evaluate the SAR of this peptide, biological profiles (ie, ω-GVIA binding to hippocampal membranes; Ca^{++} influx in chick synaptosomes) were obtained following either chemical modification or primary a.a. alteration of ω-GVIA.

Acetylation of primary amino moieties of Cys-1, Lys-2 and Lys-24, under non-saturating conditions, generated 7 (3 mono-, 3 di- and 1 tri-acetylated) distinct peptides. Positional assignment of acetylation was accomplished using a combination of tryptic digestion, RP-HPLC and MS. Biological assays indicate that successive acetyl additions lead to a loss of activity. From binding analyses, the N-terminal amine of Cys-1 is more significant than the ε-amino moieties of either Lys-2 or Lys-24. Selective modifications of the primary structure of ω-GVIA will be explored to evaluate the physiological relevance of other basic moities and of secondary structural features as a result of disulfide bridging.

462.6

ω–CONOTOXIN EXERTS FUNCTIONALLY DISTINCT LOW AND HIGH AFFINITY EFFECTS IN THE NEURONAL CELL LINE NG 108-15. J.L. Werth, L.D. Hirning, and S.A. Thayer. Dept. of Pharmacology, Univ. of Minnesota, Minneapolis, MN 55455, and Natural Product Sciences, Salt Lake City, UT 84108.

Depolarization-induced intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) transients were recorded in single differentiated NG108-15 cells with the Ca^{2+} indicator indo-1 and a dual emission microfluorimeter. In differentiated NG108-15 cells a 30 second superfusion with 50 mM K^+ increased the $[Ca^{2+}]_i$ from a basal level of 142±10 nM to a peak level of 1655±287 nM. This $[Ca^{2+}]_i$ transient could be blocked completely and reversibly by the dihydropyridine Ca^{2+} channel blocker nitrendipine in a concentration dependent manner (IC 50= 1.9 nM). In contrast, 100nM ω-conotoxin GVIA (ω-CgTx) produced a maximal inhibition of the depolarization-induced rise in $[Ca^{2+}]_i$ of only 52%. The block was irreversible. Repeated applications of, or prolonged incubations with ω-CgTx failed to increase the maximal block. We have demonstrated that ω-CgTx distinguishes two populations of dihydropyridine-sensitive Ca^{2+} channels. Whole cell Ba^{2+} (5mM) current elicited by depolarization to 0 mV from -70 mV was inhibited 48% by 3μM ω-CgTx. Subsequent application of 3 μM nifedipine reduced the current by an additional 45% further indicating that there is a dihydropyridine-sensitve, ω-CgTx insensitive Ca^{2+} channel in these cells. ω-CgTx inhibition of indo-1 measured $[Ca^{2+}]_i$ increase was concentration-dependent but reversed in a graded manner at higher ω-CgTx concentrations. This entire U-shaped dose-response curve could be shifted in parallel fashion by modulation of the extracellular divalent metal concentration without changing the maximal inhibition. When divalents are reduced, treatment with a high (1 μM) concentration of ω-CgTx, which produced a modest (10%) inhibition of $[Ca^{2+}]_i$ increase, protected the cell from the effects of a second exposure to a normally effective concentration of ω-CgTx (10 nM). We conclude that there are two ω-CgTx binding sites on these cells, one to which ω-CgTx binds with high affinity and produces an irreversible Ca^{2+} channel blockade, and a second, lower affinity site to which ω-CgTx binds that does not block the channel but does prevent access to the high affinity site.

462.7

EXPRESSION OF AN [^{125}I] W-CONOTOXIN-SENSITIVE BINDING SITE IN MOUSE CORTICAL ASTROCYTES. M.C. Howell*, M.J. Litzinger, G.A. Skeen and H.S. White. Dept. of Pharmacol. & Toxicol. and Depts. of Biol., Physiol. and Pediatrics, Univ. of Utah, S. L. C. UT 84108

Previous investigations in this laboratory have demonstrated that mouse cortical astrocytes express a physiologically functional *w*-conotoxin-sensitive binding site. These results suggested that astrocytes possess an N-type voltage-sensitive Ca^{2+} channel (VSCC) since *w*-conotoxin is a rather specific marker of this VSCC type. Several reports have suggested that [^{125}I]*w*-conotoxin interacts with the L-type VSCC which is labeled with [^{3}H]nimodipine or [^{3}H]PN200. The present investigation was initiated in an attempt to ascertain whether nimodipine and *w*-conotoxin do interact with the same binding site. Results obtained thus far have demonstrated that [^{125}I]*w*-conotoxin binding increases pari passu with [^{3}H]nimodipine binding through the fourth culture week. After the fourth week in culture, [^{3}H]nimodipine binding decreases with culture age; whereas, [^{125}I]*w*-conotoxin binding plateaus. These results suggest that the ligands for the N- and L-type VSCC's are not interacting with the same binding site. Furthermore, physiological studies conducted with the Ca^{2+}-sensitive fluorescent probe Indo-1 have demonstrated that *w*-conotoxin completely blocks KCl (55 mM)-induced $[Ca^{2+}]_i$ transients, whereas nimodipine only attenuates KCl-induced $[Ca^{2+}]_i$ transients. The expression of distinctly different VSCC types on astrocytes may provide important pharmacological targets for drugs useful in the treatment of seizure disorders and stroke. Supported by NIH grant 2-RO1-NS22200 from the NINDS (HSW).

462.8

UNIQUE STRUCTURE OF THE PURIFIED ω-CONOPEPTIDE RECEPTOR FROM ELECTRIC RAY ELECTRIC ORGAN NERVE TERMINALS. M. Tsubokawa*, C. Kiraly*, A. Woppmann*, N. Liu*, G. Miljanich, and J. Ramachandran*, NEUREX Corporation, Menlo Park, CA 94025 and Biological Sciences, USC, Los Angeles, CA 98009.

Synthetic ω-conopeptides such as SNX-111 from *Conus magus* (see abstract by Miljanich, et al.) specifically block neuronal calcium channels. An SNX-111 receptor and putative calcium channel has been localized to the presynaptic membrane of nerve terminals of the electric organ of the electric ray, *Narcine brasiliensis*. The detergent solubilized receptor was purified 2,000-fold to near homogeneity using wheat germ agglutinin chromatography and SNX-111 affinity chromatography. In solution, the native receptor appears to be a multi-subunit complex (Mr=1,500,000-2,000,000) as shown by SDS-PAGE under nonreducing conditions. The molecular weight of the receptor shifted in two steps upon exposure to reducing agent. A single band of Mr=350,000 was observed with 0.1mM DTT and a doublet of Mr=200,000 and Mr=180,000 was observed with 25mM DTT. Cross-linking experiments with intact synaptosomes provided further evidence that the purified protein is the presynaptic conopeptide receptor. That is, ^{125}I-SNX-111 specifically labeled a band of Mr>1,000,000 under nonreducing conditions and a single band of approximately 210,000 under reducing conditions. Thus, it is likely that one of the affinity purified polypeptides contains the conopeptide binding domain and that it is disulfide-linked to the other polypeptide to form a heterodimer. Moreover, several heterodimers are linked through disulfide bonds to form a multi-channel complex.

462.9

NOVEL ω-CONOPEPTIDES REVEAL CALCIUM CHANNEL SUBTYPES: BINDING. G. Miljanich, K. Gohil*, R. Kristipati*, A. Woppmann*, S. Bowersox*, K. Tarczy-Hornoch*, L. Nadasdi*, J. Fox, J. Bell*, and J. Ramachandran*. NEUREX Corporation, Menlo Park, CA 94025 and Biological Sciences, USC, Los Angeles, CA 90089.

Synthetic ω-conopeptides, SNX-111 ("111") from *Conus magus* and SNX-183 ("183") from *C. striatus*, appear to define at least four subclasses of brain voltage-sensitive calcium channels (VSCC's). In rat brain synaptosomal membranes, 111 was more potent than 183 in displacing binding of [125I]-111 ("*111"; IC_{50}'s of 10pM and 2nM, respectively) whereas 183 was more potent than 111 at displacing *183 (IC_{50}'s of 4nM and 94nM), suggesting two binding sites. Thus, 111 displays high affinity for site 1 only, whereas 183 has about equal affinity for site 1 and site 2. These sites are likely to be associated with VSCC's, since binding behavior correlates with inhibition of calcium-dependent transmitter release (see abstract by Rivnay, *et al.*). Covalent cross-linking of *111 to its receptor and subsequent SDS-PAGE specifically labeled only a 210KDal polypeptide band representing the site 1 VSCC. Displacement by unlabeled 111 and 183 showed single site binding. *183 specifically cross-linked to polypeptide bands of 210, 172, 150, and 138KDal. Displacement by 111 and 183 revealed two co-migrating 210KDal polypeptides representing two binding sites and presumably reflecting site 1 and 2 VSCC's. Receptor autoradiography in brain slices with *111 showed relatively dense, localized binding in cortex, caudate, and hippocampus, for example, while large areas of midbrain exhibited much less labeling. As expected, *183 labeled regions that were labeled by *111 plus additional regions (some thalamic nuclei, for example). Surprisingly, some areas, such as substantia nigra, were labeled by *111 but not by *183. Thus, four subclasses of VSCC's may be defined as those binding 111 and 183, 183 only, 111 only, and neither.

462.10

NOVEL ω-CONOPEPTIDES REVEAL CALCIUM CHANNEL SUBTYPES: TRANSMITTER RELEASE. B. Rivnay*, J. Fox, R. Newcomb, K. Gohil*, S. Cain*, A. Palma*, P. Adriaenssens*, L. Nadasdi*, G. Miljanich, J. Bell*, J. Ramachandran*. NEUREX Corp., Menlo Park, CA, 94025.

Synthetic ω-conopeptides (see abstract by Miljanich et al) block the potassium induced release of radiolabeled norepinephrine (NE) or dopamine (DA) from rat hippocampal slices. SNX-111 blocked NE release partially (50-70%) with an IC_{50} of 1 nM whereas SNX-183 inhibited K^+ induced release completely with an IC_{50} of 400 nM. In contrast, SNX-183 was much more effective in blocking DA release (IC_{50} 160 nM) than was SNX-111 (IC_{50} > 1000 nM).

Two sets of experiments confirm that the conopeptides block neurotransmission in a more physiological setting. EPSP field potentials elicited by electrical stimulation in rat hippocampal slices *in vitro* were reversibly blocked by a variety of Neurex conopeptides and again were more completely blocked by SNX-183 (~100%) than SNX-111 (~50%). The compounds also blocked neurotransmitter release *in vivo* in the rat. Extracellular concentrations of Glu and GABA as monitored by *in vivo* microdialysis during potassium infusion through the probe were inhibited in the hippocampus and thalamus by about 50% by SNX-111 as compared to the levels of the nontransmitter amino acid serine.

These techniques provide functional evidence for the presence of multiple subtypes of voltage gated calcium channels and thus extend the binding data presented in the abstract by Miljanich et al.

462.11

METABOLISM OF VOLTAGE-OPERATED CALCIUM CHANNELS IN HUMAN NEUROBLASTOMA CELLS. E. Sher*, M. Passafaro*, P. Strata° and F. Clementi*, CNR Ctr. of Cytopharmacology, Dept. of Medical Pharmacology, Univ. of Milano, Milano, and °Dept. of Anatomy & Human Physiology, Univ. of Torino, Torino, Italy.

ω-Conotoxin (ωCTx) binds and blocks a particular subtype (ω) of voltage-operated calcium channels (VOCCs) present in both neuronal and endocrine cells. We have used human neuroblastoma cell lines (IMR32 and SH-SY5Y) to study the metabolism of the ω-type VOCC, and its modulation. We have determined the half-life of the channel with two procedures by measuring the rate of disappearance from the cell surface of ^{125}I-ωCTx labeled VOCCs, and by determination of the number of VOCCs present at the cell surface at different time points after treatment of the cells with cycloheximide. In both cases the measured half-life of ω-type VOCCs was between 16 and 20 hours (average 18±1). Toxin binding *per se* therefore did not affect channel turnover. By using an "acid-washing" procedure, we measured both the rates of channel internalization and degradation. At 37°C the two rates were parallel and at 4°C they were both blocked. At 20%C, however, channel internalization proceeded normally, but the channels were not degraded, and were intracellularly accumulated. The 20°C effects were mimicked by chloroquine and leupeptin in a dose-dependent manner. ω-type VOCC turnover could be modulated in neuroblastoma cells after differentiation induced by several drugs. The half-life was significatively increased leading to an increase of membrane VOCCs. On the other hand, anti-channel autoantibodies from Lambert-Eaton myasthenic syndrome patients induced an increase in the rates of channels internalization and degradation, leading to a reduction in surface channels.

462.12

DOSE RESPONSE RELATION FOR SYNTHETIC FTX BLOCK OF VOLTAGE-DEPENDENT CALCIUM CURRENTS IN DISSOCIATED PURKINJE CELLS M. Sugimori, B. Cherksey and R. Llinas
Dept. of Physiology and Biophysics, NYU Medical Center, New York, N.Y. 10016

Dissociated cerebellar Purkinje cells from guinea pig were studied using whole cell voltage clamp technique (Kay 1989, Dissection of tissue culture, Alan R. Liss Inc.). The properties of of synthetic arginine-polyamines derived as possible analogs of the naturally occurring FTX (Cherksey et al 1989, Biol. Bull., 177: 321) were tested as potential calcium channel blockers. Three structures were studied FTX(3:3), FTX(4:3) and FTX(3:4) (see Cherksey et al. this meeting).

The results indicate that of these three polyamines FTX(3:3), FTX(4:3) and FTX(3:4) have decreasing abilities of blocking. The most potent FTX(3:3) is capable of reducing calcium currents at the initial dose at the 100 nanomolar level. This block increases in magnitude such that at close to ten micromolar level calcium current is almost totally blocked. A similar set of experiments in which FTX(4:3) and FTX(3:4) permutations of polyamine were tested demonstrate that the FTX(4:3) was approximately one order of magnitude less potent than FTX(3:3) and that the FTX(3:4) permutation was basically not effective.

These results indicate that FTX action-like polyamines, which have a dose response curve suggesting a powerful binding to these channels, have been shown to be specific blockers for the so-called P-channel (Llinas et al 1989, PNAS 86:1689). Moreover these polyamines, which do not block the L, T or N type calcium channels (Fox et al 1987, J. Physiol. 394: 149) or the sodium or potassium currents at the levels utilized, may ultimately represent an element of a family of polyamines, normally present in the brain, that regulate channel activity.
NS13742

462.13

EFFECTS OF (+) AND (-) ENANTIOMERS OF SDZ 202-791 ON 15mM K$^+$-STIMULATED [^{3}H]5-HT RELEASE IN RAT SPINAL CORD SYNAPTOSOMES. V.C. Gandhi and D.J. Jones. Depts. Anesth. & Pharm., Univ. Texas Hlth. Sci. Ctr., San Antonio, TX 78284-7838

Previous studies from this laboratory established that L-type calcium channels activated by the dihydropyridine (DHP) agonist Bay K 8644 modulate the K$^+$-stimulated release of [^{3}H]monoamines from spinal cord synaptosomes (Eur. J. Pharmacol. 187:271-281, 1990). Under similar conditions, activation of protein kinase C (PKC) by phorbol 12-myristate 13-acetate (PMA)also enhanced 15mM K$^+$-stimulated release. The enhancement due to either PMA or Bay K 8644 was blocked by DHP antagonists as well as inhibitors of PKC suggesting a coupling of these two systems. The present studies further evaluated this relationship by examining the effects of enantiomers of the DHP SDZ 202-791 on K$^+$-stimulated release of [^{3}H]5-HT from spinal cord synaptosomes.

Bay K 8644 (1uM) typically produced a 40-60% enhancement of 15mM K$^+$-stimulated [^{3}H]5-HT release. Whereas (+) SDZ 202-791 (1uM) increased release 38 ± 4.8% over control, (-) SDZ 202-791 was inactive. The effects of (+) SDZ 202-791 were blocked by nifedipine (1uM). Activation of PKC with PMA (0.2uM) in the presence of 15mM K$^+$ increased release of [^{3}H]5-HT 30-40%. This effect is blocked by PKC inhibitors and also the DHP antagonist nifedipine. PKC inhibitors also antagonized the enhanced release of [^{3}H]5-HT due to Bay K 8644 or (+) SDZ 202-791. (-) SDZ 202-791 antagonized the PMA-induced enhancement in K$^+$-stimulated release. The present results suggest a common pathway linking the L-type voltage dependent Ca^{++}-channel and PKC in enhancing K$^+$-stimulated release of [^{3}H]5-HT from spinal cord synaptosomes. The L-type channel site demonstrates stereospecificity for DHP binding, activation, Ca^{++} influx and enhanced [^{3}H]5-HT release. Supported by NSF BNS-8820008.

462.14

HIGH AFFINITY [^{3}H]RYANODINE BINDING SITES IN POSTMORTEM HUMAN BRAIN. M.B. Stein*, R.A. Padua, J.I. Nagy, and J.D. Geiger, Depts. Pharmacology and Psychiatry, Univ. Manitoba. Winnipeg, Manitoba R3E 0W3.

[^{3}H]Ryanodine binding sites have been characterized pharmacologically and localized autoradiographically in rat brain [Padua et al., Brain Res. 542 (1991): 135-140; Soc. Neurosci. Abs. 1991]. Here we examined high affinity [^{3}H]ryanodine binding sites in crude membrane preparations of postmortem human brain. Saturation analyses revealed a single class of high affinity sites with a K_D value of 3.6 nM and a B_{max} value of 99 fmol/mg protein. In competition studies, unlabelled ryanodine displaced its radiolabelled counterpart with a K_i value of 7 nM. Binding was found to be dependent on the concentration of free Ca^{2+} with an EC_{50} value of 139 μM. Binding was reduced by 25% in the presence of 5 mM Mg^{2+} and was restored with the addition of 10 mM caffeine. Caffeine alone did not significantly alter the levels of binding. The distribution of [^{3}H]ryanodine binding sites in human brain was heterogeneous with high levels observed in putamen and caudate nucleus, intermediate levels in cortex, hippocampus and globus pallidus, and low levels in cerebellum. The high levels of binding seen in basal ganglia suggest that ryanodine receptors may be particularly important here in the regulation of intracellular calcium and may be relevant to such disorders as neuroleptic malignant syndrome, Huntington's disease, and Parkinson's disease.

462.15

PHARMACOLOGY AND CELLULAR LOCALIZATION OF [^{3}H]RYANODINE RECEPTORS IN RAT BRAIN. R.A. Padua, W. Wan, D. Fyda, T. Yamamoto, J.D. Geiger and J.I. Nagy. Depts. Physiol. and Pharmacol., Univ. Manitoba. Winnipeg, Manitoba R3E OW3.

[^{3}H]Ryanodine binding sites were previously found in various crude subcellular fractions of rat brain (Padua et al., Brain Res. 542 (1991): 135-140). Here we examined the pharmacological characteristics of these sites, their localization in purified subcellular fractions, and their distribution throughout rat brain. Saturation analyses of binding in crude membrane preparations revealed a single class of high affinity sites with a K_D value of 7.6 nM and a B_{max} value of 193 fmol/mg protein. Competition studies with unlabeled ryanodine showed the presence of both high and low affinity sites with K_D and B_{max} values of 1.6 nM and 24 μM, and 86 fmol/mg protein and 23 pmol/mg protein, respectively. Association and dissociation rate constants were $3x10^7$ $M^{-1}min^{-1}$ and 0.001 min^{-1}, respectively. [^{3}H]Ryanodine binding was Ca^{2+}-dependent; EC_{50} 119 μM. Binding was inhibited by Mg^{2+}, ADP, dB-cAMP, nifedipine, verapamil, trifluoperazine and various benzodiazepines, but not by IP_3 or caffeine. Synaptosomes isolated from crude mitochondrial/synaptosomal fractions, displayed high affinity (K_D 6.1 nM) and a 4 fold greater level of [^{3}H]ryanodine binding (B_{max} 67 fmol/mg protein) compared with that found in purified mitochondria. Binding was enriched in a subfraction of microsomes that contained low activities of enzyme markers for plasma membrane and endoplasmic reticulum. Autoradiographically, the highest levels of [^{3}H]ryanodine binding sites were found in regions of the cortex, hippocampus and olfactory bulb. Lesions in the hippocampus revealed the presence of these sites in neurons. In addition, evidence was found for their localization in mossy fiber terminals suggesting that ryanodine receptors in these terminals may be involved in the regulation of Ca^{2+} mobilization and/or neurotransmitter release.

462.16

DISTRIBUTION OF RYANODINE RECEPTORS IN THE CNS Y. Ouyang*+, T. J. Deerinck*+, P. D. Walton*‡, J. A. Airey*‡, S. J. Young+, J. L. Sutko*‡, and M. H. Ellisman+. San Diego Microscopy and Imaging Resource, + Dept. of Neurosciences, Univ. of Calif. San Diego, La Jolla, Calif.92093; ‡Pharmacology, Univ. of Nevada, Reno, Nevada, 89557.

We recently identified two ryanodine binding proteins (α and β ryanodine receptors, RR's) in the chicken CNS using antibodies against the purified skeletal muscle RR (Ellisman et al., Neuron, 5:135-146, 1990). In skeletal muscle, RR's are associated with Ca release from the intracellular SR membrane system. A similar function is probable in neurons as these proteins are most concentrated in intracellular membranes of cerebellar Purkinje cells, known to manifest fluctuations in cytosolic Ca. In order to gain a better understanding of the functional significance of neuronal RR's we have studied the distribution of these receptors throughout the chicken CNS with a monoclonal antibody, 34C, that recognizes both isoforms. Nuclei demonstrating the strongest RR-immunoreactivity include those containing very large neurons such as: nucleus ruber, nucleus reticularis pontis oralis, nucleus cerebellaris, paleostriatum primitivum (globus pallidus), nucleus vestibularis lateralis, nucleus laminaris, and the nucleus magnocellularis cochlearis. Prominent staining was also found in spinal motor neurons, the olfactory bulb, and in spinal ganglia. Staining was observed in perikarya, dendrites and axons. The subcellular pattern of staining appears similar to that previously noted for cerebellar Purkinje neurons in that no labeling of dendritic spines was detected.

We also determined whether RR-like immunoreactivity outside the cerebellum was a consequence of the presence of the α, β, or both forms. Monoclonal antibodies 110F and 110E, which are specific for the α and β forms, respectively (Airey et al., J. Biol. Chem. 265:14187-14194, 1990), were used to probe alternate sections of the same brains. As in the Purkinje neurons, immunoreactivity to both α or β forms occurred in other brain regions. However, in contrast to the Purkinje neuron where the α form predominates, we observed the strongest immunoreactivity for the β form in neurons outside the cerebellum. Differences in the distributions of these distinct RR isoforms will be investigated further.

463.1

MULTIPLE BRAINSTEM PROJECTION TARGETS OF LATERODORSAL TEGMENTAL (LDT) AND PEDUNCULOPONTINE TEGMENTAL (PPT) NUCLEUS CHOLINERGIC NEURONS IN THE RAT. M. Yanagihara, K. Ito*, L. Dauphin*, D. Fuchs*, & R. W. McCarley. Lab. Neurosci., Dept. Psychiatry, Harvard Med. Sch./VAMC, Brockton Ma 02401.

Our laboratory has shown cholinergic LDT/PPT neurons have projections to both ipsilateral and contralateral giant cell field (here abbreviated GCF, = FTG in cat) of pontine reticular formation (PRF); these projections have been implicated in the production of REM sleep phenomena. The present study used triple-labeling techniques to examine whether the same LDT/PPT neuron projects to brainstem GCF and also to other brainstem regions. Projection targets were identified by retrograde transport of either rhodamine- or fluorescein-conjugated latex beads stereotaxically placed in GCF and, contralaterally, in other brainstem sites. After 2-3 days survival, rats were sacrificed and ChAT-immunoreactive neurons were labeled with aminomethylcoumarin (AMCA). RESULTS: Double-labeling data confirmed PPT/LDT cholinergic projections to pontine GCF, with PPT density > LDT. Triple labeling studies showed that approximately 10% of cholinergic PPT neurons projecting to pontine GCF also projected to the small cell PRF zone just ventral to locus coeruleus (often termed LC_α) that is implicated in the production of muscle atonia of REM sleep. Cholinergic ipsi- and contra-lateral PPT/LDT projections to Motor V nucleus were present, and many of these neurons also projected to pontine GCF. Finally, cholinergic PPT projections to bulbar GCF were present (less dense than PPT-->pontine GCF), and some cells also projected to a small cell bulbar reticular zone. We conclude that this innervation of multiple brainstem targets may provide a substrate for coordination of REM sleep phenomena.

463.2

CHOLINERGIC NEURONS IN THE LATERODORSAL TEGMENTAL NUCLEUS RECEIVE SYNAPTIC INPUT FROM CATECHOLAMINERGIC AXON TERMINALS IN THE RAT. Y. Kubota and S.R. Vincent. Kinsmen Lab. of Neurol. Res., Dep. of Psychiatry, Univ. of British Columbia, Vancouver, BC V6T1Z3, & Neural Systems Lab., Frontier Research Program, RIKEN, Wako, Saitama 351-01, Japan

The cholinergic neurons of the laterodorsal tegmental nucleus (TLD) are located just medial to the locus coeruleus in the pontine and have connections with cortex, septum, thalamus, hypothalamus, basal ganglia, tectum, and spinal cord. We studied the ultrastructure of choline acetyltransferase (ChAT) immunoreactive neurons and the synaptic relationship between them and tyrosine hydroxylase (TH) immunoreactive axon terminals in TLD. Many large ChAT immunoreactive neurons were present in TLD. They have a few processes, sometimes closely apposed to the blood vessel and received many synaptic contacts from non-immunoreactive axon terminals on their cell soma, dendrites and spines. Using double immunohistochemistry, ChAT-immunoreactive cells stained with β-galactosidase, showed blue colored reaction product in the light microscope and dot like immunoreactivity in the electron microscope, while TH immunoreactivity detected with a glucose-oxydase DAB reaction was dark brown in the light microscope and showed difuse electron dense material in the electron microscope. Therefore we could distinguish the difference between these two different kinds of immunoreactivity quite easily at both light and electron microscopic level. Using this double staining technique, ChAT immunoreactive neurons were found to receive synaptic contacts from TH immunoreactive axon terminals on their soma and proximal dendrites in TLD.

463.3

SEROTONIN HYPERPOLARIZES CHOLINERGIC LATERODORSAL TEGMENTAL NUCLEUS NEURONS IN THE RAT BRAIN SLICE

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Intracellular and whole cell patch clamp techniques in the rat brainstem slice were employed to examine the response to serotonin (5-HT) and carboxamidotryptamine maleate (5-CT) of identified cholinergic and non-cholinergic low threshold burst (LTB) and non-LTB neurons in the laterodorsal tegmental nucleus (LDT). Two-thirds of the neurons were LTB and one-third were non-LTB. The majority (83%) of the LTB neurons were identified as cholinergic, while the minority (33%) of the non-LTB neurons were cholinergic. The principal finding was that most **cholinergic** LDT neurons responded to 5-HT and 5-CT with a membrane hyperpolarization and decrease in membrane resistance. Voltage and patch clamp recordings revealed that the hyperpolarizing response was the result of an inwardly rectifying current having a reversal potential of -90mV and associated with an increase in membrane conductance. Serotonin altered the firing pattern of LTB neurons in response to hyperpolarizing and depolarizing steps. In control conditions, when the membrane potential was relatively depolarized, LTB neurons responded to hyperpolarizing but not depolarizing current steps with a low threshold burst. In the presence of serotonin the LTB neurons were relatively hyperpolarized and responded to depolarizing but not hyperpolarizing current steps with a burst firing pattern. Thus the effect of serotonin can be to alter the firing pattern of cholinergic LTB neurons in the LDT in response to hyperpolarizing and depolarizing inputs.

463.4

5-HT AND MUSCARINE HYPERPOLARIZE A SUBPOPULATION OF NEURONS IN RAT NUCLEUS RAPHE MAGNUS IN VITRO. Z.Z. Pan* and J.T. Williams. *Vollum Institute, Oregon Health Sciences University, Portland OR 97201*

Both 5-HT and Ach have been implicated in the antinociceptive function of nucleus raphe magnus (NRM). Intracellular recordings were made from NRM cells in a slice preparation. 5-HT and 5-HT_1 receptor agonists hyperpolarized most NRM neurons. The hyperpolarization (X_{max} =12 mV) was blocked by spiperone (1 μM), but not dopamine receptor antagonists, suggesting that a 5-HT_{1A} receptor mediated the response. In a proportion of neurons that were hyperpolarized by 5-HT, muscarine also caused a dose-dependent hyperpolarization (X_{max}=10 mV). The dose response curve for muscarine was shifted to the right by pirenzepine (300 nM - 3 μM) and methoctramine (50 nM - 200 nM). Schild regression analysis indicated that a non-M_1 (possibly M_2) muscarinic receptor is probably involved. Both 5-HT and muscarine caused an outward current with similar voltage dependance, showing inward rectification that was abolished by $BaCl_2$. The reversal potentials were about -90 mV in normal potassium and shifted to less negative potentials when extracellular K^+ was increased. In addition, the currents induced by 5-HT and muscarine were not additive. The results suggest that both 5-HT and muscarine inhibit a population of the NRM neurons by increasing the same inwardly rectifying K^+ conductance, an effect that may be involved in the modulation of opioid analgesia in NRM. Supported by NIH grants DA04523 and MH45003.

463.5

EFFECTS OF TETRAHYDROACRIDINE (THA) ON SYNAPTIC TRANSMISSION AND SENSITIVITY TO ACH OR GLUTAMATE OF VISUAL RELAY NEURONES. C. Berti*, R. Levy and A. Nistri[1]. Dept. Old Age Psychiatry, Inst. Psychiat., Univ. London; [1]Pharmacol. Dept., Queen Mary and Westfield College, London E1 4NS, U.K.

In lower vertebrates neurotransmission between the optic nerve and relay neurones is probably glutamatergic and upregulated by nicotinic receptors. The action of the antiChE agent THA (currently studied for treating Alzheimer's disease) on glutamatergic and cholinergic responses of the isolated frog optic tectum was investigated with electrophysiological recordings. THA (0.1-1 μM) increased by up to 30% the half amplitude of population EPSPs elicited by optic nerve stimulation but it failed to do so in the presence of 100 μM neostigmine. No change in afferent volley spikes was noted. The EPSP-enhancing action of ACh was potentiated by 1 μM THA (without changing the maximum response) whereas it was antagonized by 10 μM THA. In neostigmine solution 1 μM THA increased responses to low doses of ACh but depressed those to large ones. No change in responses to 100 μM carbachol was found. The EPSP-enhancing action of low doses of glutamate was augmented by 0.1 - 1 μM THA, while no change in responses to 0.5 mM GABA was seen. These data suggest that THA possesses cholinomimetic activity partly due to its antiChE properties. The enhancement of responses to exogenous glutamate by THA indicates an additional postsynaptic action of this compound leading to facilitation of excitatory synaptic transmission.

463.6

DENDRITIC MORPHOLOGY OF CHOLINERGIC AND NON-CHOLINERGIC RETICULAR FORMATION NEURONS PROJECTING TO THE THALAMUS IN THE RAT. Kazue Semba. Dept. of Anatomy, Dalhousie Univ., Halifax, Nova Scotia, Canada B3H 4H7.

Neurons in the brainstem reticular formation (RF) are known to play important roles in the activation of the thalamus during waking and REM sleep. Many of RF neurons projecting to the thalamus are cholinergic, and acetylcholine has been shown to facilitate sensory transmission in the thalamus. However, there are also non-cholinergic RF neurons with thalamic innervation, and current data correlating physiology, transmitter phenotype, projection, and morphology of RF neurons are discrepant. To clarify the relationship between transmitter phenotype and morphology of RF neurons innervating the thalamus, we used a combination of retrograde fluorogold tracing, immunofluorescence for choline acetyltransferase, single cell injection with lucifer yellow in fixed slices, and/or immunohistochemistry with an antibody to fluorogold. Cholinergic RF neurons projecting to the thalamus had large somata with 4-5 thick primary dendrites. Secondary and tertiary dendrites tended to have a wavy appearance and extended in several directions up to 500 μm from the somata, sometimes beyond the boundary of the nucleus. Spines were frequent, particularly on distal dendrites. At least some non-cholinergic RF neurons with thalamic projection that were intermixed with cholinergic neurons displayed similar morphology. In contrast, non-cholinergic neurons in the central tegmental field with thalamic projection had smaller, round somata with 3-4 thinner, aspinous dendrites oriented perpendicular to the central tegmental tract. These findings indicate that cholinergic RF neurons projecting to the thalamus have morphology similar to cortically projecting basal forebrain neurons. The morphological differences of RF neurons innervating the thalamus may reflect their distinct roles in the control of waking and REM sleep. Supported by the MRC, Alzheimer Society, and Scottish Rite Charitable Foundation.

463.7

DUAL PROJECTIONS OF SINGLE CHOLINERGIC AND AMINERGIC BRAINSTEM NEURONS TO THE BASAL FOREBRAIN AND THALAMUS IN THE RAT. Bruno J. Losier and Kazue Semba. Departments of Psychology and Anatomy, Dalhousie University, Halifax, N.S., Canada B3H 4H7.

Recent evidence indicates that during waking cholinergic basal forebrain neurons are strongly activated, whereas in the thalamus spindle activity is suppressed and sensory transmission facilitated. Both of these subcortical structures receive projections from the brainstem cholinergic and aminergic neurons, and these neurons are known to be active during waking. However, it remained unclear whether single transmitter-specific brainstem neurons contributed to both of these ascending projections. In the present study we examined this possibility of dual projections by injecting two fluorescent retrograde tracers into the thalamus and basal forebrain, followed by immunohistochemistry with antibodies to choline acetyltransferase, tyrosine hydroxylase, serotonin, and histidine decarboxylase. Cholinergic neurons projecting to both the basal forebrain and thalamus were found in the pedunculopontine and laterodorsal tegmental nuclei, and noradrenergic dually projecting neurons were seen in the locus coeruleus. However, few serotonergic neurons in the brainstem appeared to have the dual projection. In conclusion, subpopulations of cholinergic and aminergic neurons in the brainstem have branching axons that innervate both the basal forebrain and the thalamus. Through these dual projections, these single transmitter-specific neurons can concurrently modulate the activity of both subcortical structures during cortical arousal. Supported by the MRC, Alzheimer Society, and Scottish Rite Charitable Foundation of Canada.

463.8

CORTICAL CHOLINERGIC FIBERS IN THE HUMAN BRAIN: AN IMMUNOCYTOCHEMICAL STUDY OF THE HIPPOCAMPAL FORMATION. C. Lim, S. de Lacalle and C.B. Saper. Department of Pharmacological and Physiological Sciences, University of Chicago, Chicago, IL 60637, USA.

The cholinergic innervation of the hippocampal formation is thought to play an important role in regulating memory consolidation. We investigated the organization of the cholinergic innervation of the human hippocampal formation by using polyclonal antisera against human placental choline acetyltransferase (ChAT - gift of Dr. L. Hersh) and acetylcholinesterase (AChE - gift of Dr. S. Younkin) in a series of normal individuals. Staining in selected sections was intensified by a modification of the Fontana-Masson method. Fine, varicose ChAT-immunoreactive (ir) fibers were found densely innervating the different hippocampal fields in a laminar pattern that was similar to that previously reported in other species. In the dentate gyrus, there was dense ChAT-ir innervation of the granular, and the infra- and supra-granular layers. Diffuse ChAT-ir innervtion was seen in the CA3 field and mainly in the infrapyramidal layer of CA1, but little innervation was seen in CA2. A much larger number of fine, smooth AChE-ir fibers were seen in the hippocampal formation providing a more extensive and somewhat different pattern of innervation than was revealed by the ChAT staining. For example, the CA2 field was the most densely innervated in AChE-stained material. These observations indicate that cholinergic innervation plays a major role in the function of the human hippocampus, but that the AChE-stained fiber distribution does not provide an accurate estimate of the cholinergic innervation.

463.9

PROJECTIONS OF THE BASAL FOREBRAIN TO VISUAL CORTICAL AREAS IN THE CAT. Christopher C. Nabors* and Kenneth E. Kratz, Department of Anatomy, Louisiana State University Medical Center, New Orleans, LA 70112.

Cells of the basal forebrain provide cholinergic input to the entire neocortex. In the present study retrograde tracer techniques (rhodamine labelled latex microspheres and WGA-HRP) combined with ChAT immunocytochemistry were used to examine the topography of the forebrain projections to functionally defined visual cortical areas in the cat. Our results indicate that primary visual cortex (area 17) is heavily innervated by the nuclei of the diagonal band with a less dense innervation from the more lateral and caudal cell groups of the basal forebrain. On the other hand areas 18 and 19 along with the visual association areas of the lateral suprasylvian gyrus and areas 20 and 21 receive a substantial input from the diagonal band nuclei but receive their most dense projection from the substantia innominata, neurons within the putamen and globus pallidus, and neurons scattered within the internal capsule. It appears that the processing of visual information in functionally distinct areas of cortex may be subject to different regulatory inputs from the cell groups of the basal forebrain. The diagonal band nuclei may play an important role in the gating of sensory information into the primary areas of visual cortex while the more lateral cell groups may have a greater influence over higher order processing of visual information.

463.10

PRIMATE NUCLEUS BASALIS NEURONS DEVELOP RESPONSES TO STIMULI ASSOCIATED WITH WATER REWARD. R.T. Richardson Dept. Neurology, Johns Hopkins Univ., Baltimore, MD 21205

We have previously reported that a large proportion of neurons of the nucleus basalis in naive monkeys had altered discharge rates following arousing stimuli (water reward, air puff, hypertonic saline). In contrast, far fewer basalis neurons responded to neutral stimuli such as tones and perturbations of the forearm. We now report that many more basalis neurons respond to neutral stimuli that have become associated with a water reward.

Conditioning procedures have been completed in one juvenile male rhesus monkey. In the first phase of testing, one of two torques (150 g to the left or right applied to the forearm) was a CS+ which preceded a water reward by 1.5 to 2 s, and the other stimulus of the same modality was a CS- which never preceded a water reward. After several months, one of two tones (500 Hz and 1000 Hz) became the CS+ and the other was the CS-. The CS+ and CS- were periodically reversed.

In the naive monkey, 21% of 141 basalis neurons responded to the somatosensory stimuli, but in the conditioned animal, 72% of 68 neurons responded to torques. Of the 49 responsive neurons, 47% had noticeably larger responses to the CS+ than to the CS-, and 8% had larger responses to the CS- than to the CS+. Similar results were obtained for the auditory stimuli. Only 3% of 74 basalis neurons responded to tones in the naive animal, compared to 55% of 78 neurons in the conditioned animal. Of the 43 neurons responding to tones, 42% had larger responses to the CS+, and 5% had larger responses to the CS-. These findings provide further evidence that many basalis neurons are primarily responsive to stimuli that are inherently arousing to the animal (aversive or appetitive stimuli) or that have become arousing through conditioning procedures.

463.11

CORTICAL AND BASAL FOREBRAIN NEURONAL ACTIVITY FOLLOWING PERIPHERAL NERVE TRANSECTION. H.H. Webster and R.W.Dykes. Dept. of Physiology, University of Montreal, Montreal, Canada. H3C-3J7.

Recent reports have shown that acetylcholine (ACh) acts as a neromodulator to influence cortical reorganization after damage to the peripheral sensory system. some of these conclusions have been deduced from a depletion of the transmitter following destruction of cells in the basal forebrain or of the fibers projecting from those cells. However, other reports have also suggested that ACh may not be the main neuromodulatory agent involved in this process or that the basal forebrain cholinergic neurons may not be the source of the ACh. In order to further investigate the role these cells may be playing in response to peripheral nerve lesions we recorded spontaneous, extracellular single unit activity from the hindpaw somatosensory cortex and from in the basal forebrain before and 4 days after transection of the sciatic nerve in adult rats. Typically, one penetration was made with a tungsten-in-glass microelectrode over the hindpaw cortex at AP -1.8; and ML 3.0, sampling continuously from the surface of the cortex to 8.0 ml below the surface where cholinergic cells of the basal forebrain are located. The location of the electrode tract and tip was confirmed in sections stained for Nissl substance. These results show that in the cortex and basal forebrain, in general spontaneous activity is higher and spike duration is longer in transected than in controls, and support idea that the basal forebrain participates in the reoganization process following peripheral nerve lesions.

463.12

MEMBRANE PHOSPHOLIPIDS IN FRONTAL CORTEX ARE DECREASED FOLLOWING NUCLEUS BASALIS LESIONS IN THE RAT. T.C. Holmes, R. Nitsch, and R.J. Wurtman. Dept. of Brain and Cognitive Sciences, MIT, Cambridge, MA 02139.

Membrane phospholipid (PL) metabolism is abnormal in Alzheimer's disease (AD) brain. Levels of glycerophosphocholine and glycerophosphoethanolamine are increased by 50-100 % whereas free choline and ethanolamine are reduced by 25-50 %. In order to develop a rat model for these changes, we examined the effects on PL metabolism in frontal cortex (FC) by lesioning the cholinergic nucleus basalis (nBM). Rats received ibotenic acid (10 mg/ml in PBS, 0.5 ul) in one nBM, and FC sections were obtained after 1 week. Choline acetyltransferase (ChAT, nmol acetylcholine/μg protein/hr) activity in FC was lower ($P<0.01$, n=9) on the lesioned side (275±17) than the contralateral side (371±12). Total membrane PL (nmol/mg protein) in adjacent FC sections exhibited concomitant decreases in total membrane PL ($p<0.01$, n=9, Newman-Keuls test, 417±9 vs. 435±9). These data show that impaired cholinergic neurotransmission results in depletion of membrane phospholipids in cholinergic target regions. Supported by NIMH grant MH-28783.

463.13

THE EFFECT OF SOMAN ON THE VISUAL EVOKED RESPONSE OF THE CAT. A.W. Kirby, A.T. Townsend*, C.D. Pope*, B.A. Lanoue*, T.A. Tapia* and J.B. Lopez*. USAARL, Ft. Rucker, Al 36362.

Both diisopropylfluorophosphate (DFP) and soman are irreversible inhibitors of cholinesterase (ChE). DFP is used widely to investigate cholinergic function, while soman is a chemical warfare agent. Last year at this meeting we presented evidence suggesting that DFP has some noncholinergic effects. Soman was used in this study to validate the previous findings with DFP.

All experiments were done on fully anesthetized adult cats. Following collection of baseline visual evoked responses (VER) and measurement of blood ChE activity, i.v. soman was administered. VERs then were recorded and ChE activity measured periodically.

Preliminary results from eight cats show a preferential loss of low spatial frequency information similar to that following DFP. The VER loss can be reversed, at least partially, with atropine. At similar levels of blood acetylcholinesterase activity, blood pseudocholinesterase inhibition, ChE inhibition in visual cortex, and VER reduction is less after soman. Neurochemical changes in tissue from visual cortex also will be discussed.

463.14

BRAIN ACETYLCHOLINE AND CHOLINE LEVELS IN ANIMALS SUBJECTED TO CROSS COUPLED MOTION DURING THE DARK PHASE OF A LIGHT-DARK CYCLE. **J.O. Owasoyo and C.A. Walker. UAPB Research Center, Univ. of Arkansas at Pine Bluff, Pine Bluff, AR 71601.**

Land, sea or air travel sometimes causes motion sickness in humans. The mechanisms responsible for motion sickness are not entirely clear. Results from recent studies in our laboratory suggest that brain biogenic amines are involved in the mechanism of motion sickness. The present study examined the effect of cross-coupled motion on brain acetylcholine-choline in animals exposed to the motion during the dark phase of a light-dark cycle. Adult, male Fisher rats were used in this study. Control and sham animals, as well as animals (n=6) subjected for 20 min. to cross-coupled motion during the dark phase of a light-dark cycle were sacrificed at 30, 60 and 120 min. after exposure to motion. The brain was removed and dissected into cerebral cortex, medulla and cerebellum for HPLC analysis of acetylcholine and choline. Exposure to cross-coupled motion resulted in a significant decrease in the choline level of the cortex and medulla with no change in acetylcholine level of either brain area. No changes in the acetylcholine and choline levels were observed in the cerebellum. These findings suggest an involvement of brain cholinergic system in motion sickness. (Performed at NCTR, Jefferson, AR and supported by NASA grant #NAG 2-427).

463.15

DIRECT CYTOTOXIC EFFECT OF THE CHOLINERGIC TOXIN AF64A ON CEREBRAL CAPILLARY ENDOTHELIAL CELLS IN CULTURE. C. Gómez*, C. Martin* and C. Estrada. Dep of Physiology. Sch of Medicine. UAM, Madrid.

The ethylcholine mustard aziridinium ion (AF64A)has been reported to selectively destroy cholinergic neurons, and is currently used to provide animal models of cholinergic hipofunction. A previous study in the rat retina showed that, in addition to cholinergic neuron damage, ultrastructural alterations were also observed in endothelial cells. The purpose of the present investigation has been to evaluate and compare the direct cytotoxic effect of AF64A on cultured cerebral capillary endothelial cells (CCE) , on a choline acetyltransferase-containing neuroblastoma cell line (IMR32), and on cultured cerebrovascular smooth muscle cells (CVSM).

Cells grown in 24 well plates were washed with PBS, and incubated with DMEM containing different concentrations of AF64A (1-100 μM) for 2 or 24 hours. Control and 0.1% Triton X100-treated cultures were run in parallel. After the incubation period, media were collected, centrifuged and the supernatant was used for lactate dehydrogenase (LDH) measurements. Cell injury index was calculated as (A-C/T-C) x 100, where C,A and T represent LDH activity in the media from control, AF64A-treated, and TX100-treated cultures respectively.

Two hour incubation with AF64A did not increase LDH release in CCE or CVSM; however, a slight enhancement in LDH concentration was observed in IMR32 supernatants after exposure to 100μM AF64A. After 24 hour, morphological alterations and floating cells were observed in CCE and IMR32 cultures, but not in CVSM. Accordingly, LDH release increased in a concentration-dependent manner in CCE and IMR32 treated with AF64A, but no changes were observed in CVSM cultures. Half of the maximal cell injury was obtained with 30 μM AF64A in CCE , and with 15 μM AF64A in IMR32.

These results show that a) AF64A has a direct toxic effect on cerebral endothelial cells, b) the sensitivity of cultured endothelial cells is close to that found in neuroblastoma cells, and c) such cytotoxic effect is not nonspecific, because it was not observed in cerebrovascular smooth muscle cells.

EXCITATORY AMINO ACIDS: RECEPTORS IV

464.1

REGULATION OF NMDA RECEPTORS BY GLYCINE AT SYNAPSES AND IN OUTSIDE-OUT PATCHES. G. Tong, R. A. J. Lester* and C. E. Jahr.
Vollum Institute, Oregon Health Sciences University, Portland OR 97201

Although glycine is a required co-agonist for the activation of NMDA receptors, its modulation of synaptic NMDA function is less clear. The actions of glycine agonists and antagonists on postsynaptic NMDA currents recorded between pairs of hippocampal neurons in culture have been studied. Additionally we have examined these interactions on NMDA receptors in outside-out patches. Synaptic stimulation of NMDA receptors is mimicked by brief application (~ 5 ms) of L-glutamate (200 μM) to outside-out patches, which in the presence of saturating concentrations of glycine (10 μM) produces a long-lasting response. In the absence of glycine and/or the presence of glycine antagonists an NMDA response is barely detectable Subsequent addition of glycine at various intervals following brief activation by transmitter/L-glutamate allows NMDA channels to open and produces a current with a time course and amplitude dependent on the interval. Following synaptic stimulation in the presence of glycine, rapid removal of glycine has little effect on the decay of the NMDA current, indicating that glycine binds with high affinity, whereas removal of a lower affinity ligand, L-alanine, rapidly curtails the synaptic NMDA current. These data are consistent with the requirement for occupation of the glycine site to allow NMDA channels to open.

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464.2

GLUTAMATE OPENS NMDA CHANNELS WITH A HIGH PROBABILITY C.E. Jahr, Vollum Institute, Portland, OR 97201.

NMDA receptor-channels require both glutamate and glycine binding sites to be occupied for channel opening to occur. If free glutamate is present only briefly, as is probable at excitatory synapses, then the agonists could unbind before the channel has the chance to open. Outside-out patches containing many NMDA channels were used to determine the probability of a liganded channel opening. Four ms pulses of a saturating concentration of glutamate (200 μM) were applied to patches in the continued presence of 20 μM glycine every 10 or 15 s. After stable responses were obtained, one pulse of glutamate was applied in the continued presence of the channel blocker, MK-801 (10 μM), which at -60 mV will block open, but not closed, NMDA channels in about 2-3 ms, on average. These channels will remained blocked for prolonged periods if held at -60 mV. Responses to subsequent glutamate pulses (in the absence of MK-801) resulted in currents about 30% of control. These results indicate that once NMDA channels are fully liganded, the probability that they will open is about 0.7. The currents activated in the presence of MK-801 describe a first-latency distribution and indicate that of those channels destined to open, over 80% open for the first time within about 30 ms of glutamate binding. If NMDA channels in outside-out patches mimic those at the synapse and spontaneous synaptic release results in receptor saturation, then there must be very few NMDA receptors present adjacent to release sites as spontaneous NMDA minis are very small (Bekkers and Stevens, Nature 341:230, 1989). Supported by the McKnight Endowment Fund for Neuroscience and NIH grant NS21419.

464.3

EFFECTS OF ANIRACETAM AND WHEAT-GERM AGGLUTININ ON FAST EXCITATORY SYNAPTIC TRANSMISSION IN CULTURED HIPPOCAMPAL NEURONS. L. Vyklicky, Jr.*, D. K. Patneau and M. L. Mayer. Section on Neurophysiology and Biophysics, LDN, NICHD, NIH, Bethesda, MD 20892.

The rapid time course of desensitization at AMPA/kainate receptors suggests that desensitization may determine the rate of decay of excitatory synaptic responses (Trussell and Fischbach, 1989). Concanavalin-A, a lectin which decreases desensitization, fails to slow the decay of EPSCs (Mayer & Vyklicky, 1989). To reassess the role of desensitization in fast excitatory synaptic transmission, we have examined the action of two other drugs which modulate responses at non-NMDA receptors.

Monosynaptic EPSCs were evoked between pairs of neurons in low-density cultures of rat hippocampal neurons. The nootropic drug aniracetam (5 mM) increased the amplitude and slowed the decay time constant of EPSCs by 1.8 and > 2-fold, respectively. Variance analysis indicated a postsynaptic action. The lectin wheat-germ agglutinin (WGA; 300 µg/ml) also slowed decay of the EPSC (1.3-fold). In the presence of both drugs τ_{EPSC} increased from 4.1 ± 1.0 ms to 10.1 ± 1.7 ms.

Both drugs markedly reduced desensitization of whole-cell responses to fast application of L-glutamate (2 mM): The amplitude of steady-state responses was increased 8.2 ± 3.5-fold by aniracetam, and 9.4 ± 6.0-fold by WGA. The time constant of the fast component of desensitization (τ_f) was slowed from 7.5 ± 2.0 ms to 34.4 ± 8.2 ms by aniracetam and to 18.0 ± 2.6 ms by WGA. In the presence of both drugs τ_f was increased to 47.7 ± 5.9 ms.

Aniracetam's effects on EPSCs suggest that desensitization at AMPA/kainate receptors may affect both the amplitude and time course of synaptic currents. The smaller effect of WGA is consistent with its effect on L-glutamate induced desensitization. It is also possible that these drugs modulate ion channel gating independent of desensitization. Brief applications of L-glutamate and single channel recordings can provide additional information on the specific mechanism of action of these drugs, and by inference, whether the time course and amplitude of synaptic currents can indeed be modulated by desensitization.

464.4

NOVEL WILLARDIINE DERIVATIVES EXHIBIT VARYING DEGREES OF DESENSITIZATION AT AMPA/KAINATE RECEPTORS. D. K. Patneau, M. L. Mayer, D. E. Jane* and J. C. Watkins*. Sect. of Neurophysiology and Biophysics, LDN, NICHD, NIH, Bethesda, MD 20892; and Dept. of Pharmacology, School of Medical Sciences, University of Bristol, Bristol BS8 1TD, England.

AMPA/Kainate receptors on CNS neurons are activated by two broad classes of agonists. Quisqualate, AMPA and L-glutamate produce strongly desensitizing responses, while kainate and domoate evoke little or no desensitization. Willardiine, a heterocyclic amino acid derived from *Acacia willardinia*, and its derivative 5-bromowillardiine, have previously been characterized as quisqualate-like and kainate-like agonists, respectively (Evans et al., 1980; Agrawal & Evans,1986).

The biologically active S enantiomers of willardiine and five derivatives, formed by substitution of fluorine, chlorine, bromine, iodine or NO_2 at the 5-position carbon atom of the willardiine uracil ring, were synthesized. Their action at AMPA/ kainate receptors was measured using fast perfusion techniques during whole-cell recording from cultured mouse hippocampal neurons voltage-clamped at -60 mV. The equilibrium potency sequence was F > NO_2 > Cl > Br > I > Will. (S)-5-fluorowillardiine (EC_{50}, 1.5±0.4 µM) is considerably more potent than (R,S)-AMPA (EC_{50}, 11±1.5 µM).

Willardiine and its derivatives all produced rapidly desensitizing responses at AMPA/kainate receptors, but exhibited varying degrees of desensitization. The amplitude of the equilibrium response to a maximally effective dose of willardiine was similar to that of AMPA and L-glutamate (<10% of the response to 6 mM kainate). Responses to 5-substituted willardiine derivatives were larger, and ranged from 15±6% (fluorowillardiine) to 89±8% (iodowillardiine) of the kainate response, reflecting different degrees of desensitization. The desensitization sequence was I < Br < Cl < NO_2 < F. Cross-desensitization experiments confirmed that willardiine derivatives activate the same receptor(s) as AMPA and kainate: Responses to kainate and AMPA were inhibited by 5-nitrowillardiine, while the steady-state response to the weakly desensitizing agonist 5-iodowillardiine was inhibited by the more strongly desensitizing agonists 5-fluorowillardiine and willardiine.

464.5

MAGNESIUM DEPENDENCE OF CALCIUM FLUXES EVOKED BY GLUTAMATE AGONISTS IN CULTURED CEREBELLAR GRANULE CELLS. S.S. Oja, V. Varga*, R. Janáky*, I. Holopainen* and P. Saransaari. Tampere Brain Res. Ctr, Dept. Biomed. Sci., Univ. Tampere, Finland.

Glutamate activates at least three subtypes of membrane receptors defined by their agonists N-methyl-D-aspartate (NMDA), kainate and quisqualate. The activation results in enhanced influx of calcium ions into neurons. The responses to glutamate and NMDA are known to be blocked by magnesium whereas the effects of Mg on the actions of kainate and quisqualate are less well characterized. We studied now the effects of all these agonists on the influx of Ca into glutamatergic cerebellar granule cells in culture. Kainate was most potent of the selective agonists tested. Glutamate, a mixed agonist, evoked responses which were largely similar to those of kainate. These results thus demonstrate the dominance of kainate receptors in cerebellar granule cells. The NMDA and quisqualate-evoked influx of Ca was totally and the kainate and glutamate-evoked influx partially blocked in 1.3 mM extracellular Mg. NMDA was most effective in totally Mg-free medium whereas the maximal effects of glutamate, kainate and quisqualate were seen at 0.1 mM Mg. The mechanism of Mg sensitivity of kainate responses seems to be complex and apparently involves the kainate-evoked release of endogenous excitatory amino acids from the cells. D-2-Amino-5-phosphonovalerate blocked partially and phencyclidine completely the enhancement of Ca influx by quisqualate in 0.1-mM Mg medium. The biphasic dose response curve for quisqualate also indicates that this agonist apparently acts at two receptors, activating at high concentrations NMDA receptors as well. (Supported by the Emil Aaltonen Foundation, Finland.)

464.6

GLUTAMATE RECEPTOR RESPONSES IN NEURONS OF THE CHICK NUCLEUS MAGNOCELLULARIS. I.M. Raman & L.O. Trussell. Neuroscience Training Program & Dept. of Neurophysiology. University of Wisconsin, Madison 53706.

Neurons of the nucleus magnocellularis of the chick receive strong, somatic excitatory innervation from the auditory nerve. While this synapse is believed to use glutamate receptors, the detailed properties of these receptors are unknown. We have therefore explored the action of glutamate receptor agonists on neurons acutely isolated from day 17-20 embryos. Neurons were voltage clamped with the patch electrode technique and solutions were applied rapidly (<6 ms exchange) from an array of 400 µm diameter glass tubes.

Glutamate (19 of 19 cells), kainate (12/12), quisqualate (13/13), and AMPA (8/8) activated inward currents at the resting potential. CNQX (10 µM) blocked quisqualate-evoked currents (N=3). Responses to these agonists reversed near 0 mV and often showed a striking degree of outward rectification in their steady-state current: the ratio of slope conductance between voltages of 30 & 90 mV to the slope conductance between -40 & -90 mV was 4.0±2.4 (±s.d., N=19 cells). Glutamate-, quisqualate-, and AMPA-induced currents showed rapid and profound desensitization. For example, the average peak response to 1 mM glutamate at -60 mV was 4.3±1.8 nA (N=8) while the steady-state current was 119±17 pA. The decay could be fit with two exponentials having time constants of 4.1±1.4 ms (83% of total current) and 14.5±5.6 ms (N=6). The faster decay rate may be an underestimate, since its measurement is limited by the exchange rate of our drug application system for whole cells. Desensitization was therefore measured in excised patches, where the solution exchange occurs within 1 ms. A single exponential fit to patch responses decayed with a τ of 1.5±0.2 ms (N=4). These rapid kinetics may be reflected in the fast time course of magnocellularis synaptic currents (see Trussell, L.O., Neurosci. Abstr., 1991).

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464.7

SYNAPTIC CURRENTS RECORDED IN VOLTAGE CLAMPED NEURONS OF THE CHICK NUCLEUS MAGNOCELLULARIS. L.O. Trussell. Dept. of Neurophysiology, University of Wisconsin, Madison, 53706.

The adendritic neurons of the nucleus magnocellularis of the chick receive auditory nerve input directly onto their cell bodies. Thus, the preparation offers the opportunity to study the postsynaptic electrical properties of a putatively glutamatergic CNS synapse under nearly ideal voltage clamp conditions. Tight-seal whole-cell recordings were made from 100-200 µm thick slices maintained at 31°C on the stage of an upright microscope equipped with differential interference contrast optics.

During voltage clamp recordings (V_{hold}=-60 mV) spontaneous, small inward synaptic currents were apparent. In the presence of 1 mM bath Mg^{++}, these currents reversed near 0 mV and were blocked by 5 µM CNQX or by 5 mM kynurenic acid. Their variable amplitude (5 to 150 pA) and random nature suggest that they may correspond to "miniature" synaptic currents observed in other neuronal preparations. A striking feature of the synaptic currents was their rapid kinetics. Their mean rise time was 220±20 µsec (10-90% rise, N=7 cells) and their exponential decay constant was 450±70 µsec. Many synaptic currents had a slower secondary phase of decay which was probably due to activation of NMDA receptors as it was blocked by 500 µM 2-APV or 1 mM Mg^{++} and was resistant to 5 µM CNQX.

The very rapid time course of the EPSC's non-NMDA component may reflect a novel form of non-NMDA receptor and is consistent with the role of this synapse in precise encoding of auditory input. However, the presence of an NMDA component is unexpected, and may play a role in the trophic maintenance of these neurons by synaptic activity or in the slow regulation of excitability. Current experiments are exploring the properties of the nerve-evoked rapid non-NMDA synaptic current and the possible roles of the NMDA component.

Supported by the Deafness Research and Sloan Foundations.

464.8

A SIMPLE MODEL DESCRIBES RAPIDLY DESENSITIZING QUISQUALATE-ACTIVATED CURRENTS. D.O. Smith and W.T. Cumming*. Depts. of Physiology and of Electrical and Computer Engineering, Univ. of Wisconsin, Madison, WI 53706.

Rapid desensitization of post-synaptic currents in quisqualate receptors has been observed in numerous preparations. To understand the kinetics of this reaction, we developed a sequential model where 2 transmitter molecules bind to the receptor followed by a rate-limiting transition to the conducting state and where desensitization occurs from the closed state with 2 bound agonist molecules. Using rate constants for desensitization, resensitization, and channel opening and closing from our quisqualate data and binding rate constants from ACh data (Wathey et al., 1979), the model achieves a reasonable fit to our experimental results in most respects. To improve the fit, we needed only to increase the forward desensitization rate constant and the inverse rate constant for the second binding step by factors of 2 and 5, respectively. These results imply that the connection between our experimental data and the rate constants of the model may be slightly more complex than we predicted. Supported by NIH grant NS13600.

464.9

EFFECTS OF SPERMINE ON NMDA-INDUCED CURRENTS IN RAT HIPPOCAMPAL NEURONS: A WHOLE CELL AND SINGLE CHANNEL STUDY. R. C. Araneda, R.S. Zukin and M.V.L. Bennett. Dept. Neuroscience, Albert Einstein Coll. of Med., Bronx, NY 10461.

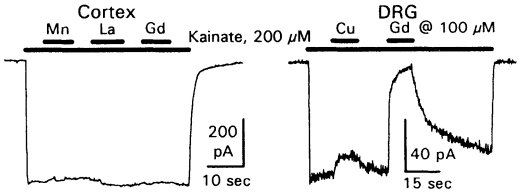
Recent studies have shown that polyamines potentiate NMDA-induced currents in *Xenopus* oocytes injected with rat brain mRNA (McGurk *et al.*, PNAS 87: 9971, 1990), as well as increasing the binding of [^{3}H]-glycine and [^{3}H]-MK-801 to the NMDA receptor complex. These observations suggest a physiological role for polyamines in modulating NMDA-evoked responses. To gain further insight into the actions of polyamines on NMDA receptors, we studied the effects of the polyamine, spermine, on NMDA-induced currents in rat hippocampal neurons. At the whole-cell level, application of 100 μM NMDA in the presence of low glycine (0.3 μM) and absence of external Mg^{2+} induced an inward current which reversed near 0 mV. Co-application of spermine (100-300 μM) increased the NMDA-induced current by about two-fold. Single NMDA-activated channels in outside-out patches were characterized by unitary conductance of 50 pS and an open time distribution that could be fit by a single exponential with a mean open time of 4.5 ms. Perfusion of the patches with 3 μM NMDA (plus 0.3 μM glycine) in the presence of spermine (100-300 μM) increased the open probability of the channels by about 50%, primarily due to an increase in the duration of the openings of the channel. These studies should clarify the mode of action of polyamines on NMDA-activated channels.

464.10

GLUTAMATE RECEPTOR CHANNELS IN RAT DRG NEURONS: SELECTIVE INHIBITION BY LANTHANUM AND GADOLINIUM. J. E. Huettner. Department of Neurobiology, Harvard Medical School, 220 Longwood Ave., Boston, MA 02115

Rat sensory neurons express a novel form of non-NMDA receptor that exhibits strong desensitization to the agonists kainate and domoate (*NEURON* 5:255, 1990). In a search for selective antagonists able to distinguish the receptor in DRG cells from conventional non-NMDA receptors in CNS neurons, the inorganic cations La and Gd were found to inhibit kainate current in DRG cells at 100 μM, a concentration that had little effect on kainate current in neurons from cerebral cortex. Blockade of kainate current in DRG cells by 100 μM La or Gd was rapid and complete at holding potentials up to +60 mV. Lower concentrations produced partial block. Full recovery took 60 to 90 sec.

The divalent cations Cu, Mn, Zn, Cd, Co, and Ni produced slight inhibition (5-20% at 100 μM) of kainate current in DRG neurons but did not reduce the current gated by kainate in cortical neurons.



464.11

NMDA RECEPTOR BLOCK BY H^+, Mg^{2+}, AND Zn^{2+} FOLLOWING ALKYLATION OF THE REDOX MODULATORY SITE. Lian-Hong Tang and Elias Aizenman, Dept. of Physiology, Univ. of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

We have used the alkylating agent N-ethylmaleimide (NEM) to permanently modify the NMDA receptor via its redox modulatory site (Aizenman et al., *Neuron*, 2:1257; 1989). In addition, we have examined the actions of known endogenous blockers of the NMDA receptor, H^+, Mg^{2+}, and Zn^{2+}, following alkylation. The purpose of these experiments was to study any possible relation between the redox site and the sites responsible for block by these various agents. In cultured rat cortical neurons, NEM (300 μM; 2 min) can effectively "lock" the enhancement of whole-cell voltage-clamped NMDA-induced responses produced by the reducing agent dithiothreitol (DTT, 4 mM; 2-4 min). Under these conditions, the oxidizing agent 5-5-dithio-*bis*-nitrobenzoic acid (DTNB, 500 μM; 1-2 min) can no longer reverse the potentiating effects of DTT. When we measured the block produced by H^+ (pH 6.8), Mg^{2+} (1 mM), and Zn^{2+} (20 μM) on NMDA responses before and following alkylation of the redox site (DTT treatment always preceded NEM application), we observed that all three agents could effectively block NMDA responses under both conditions, except that the inhibition produced by Mg^{2+} and Zn^{2+} decreased slightly after alkylation, while the proton block increased (%block alkylated/%block control): 1.3, H^+; 0.8, Mg^{2+}; 0.7, Zn^{2+}. Supported by NIH grant NS29365.

464.12

EVIDENCE FOR KAINATE RECEPTOR SUBTYPES BASED ON DIFFERENTIAL ETHANOL SENSITIVITY OF BRAIN AND GLUR3 RECEPTORS EXPRESSED IN XENOPUS OOCYTES. J. E. Dildy-Mayfield*, J. M. Sikela, and R. A. Harris. VAMC & Dept. of Pharmacology, Univ. of CO Health Sci. Ctr., Denver, CO 80262.

Ethanol's (EtOH) effect on kainate (KA) receptor-operated channels was initially examined in *Xenopus laevis* oocytes expressing mRNA from rat hippocampus (E_m clamped at -70 mV). EtOH (50, 100 mM) significantly inhibited KA-induced currents in the presence of 400, 200, 100, 50, 25, or 12.5 μM KA but did not significantly alter the EC_{50} for KA (~78 μM). DNQX inhibited maximum KA responses (400 μM) with an IC_{50} of ~1 μM; EtOH (50, 100 mM) did not alter the IC_{50} for DNQX but did produce further inhibition of KA-induced currents. Despite the apparent noncompetitive inhibition produced by EtOH on KA receptor-mediated responses, the EtOH inhibition of KA-stimulated current increased as the KA concentration decreased. For example, 50 and 100 mM EtOH inhibited 400 μM KA responses by 15 & 25%, respectively, compared to 45 & 51% inhibition of 12.5 μM KA responses. This differential inhibition was not due to different current amplitude stimulated by low vs. high KA concentrations. Oocytes expressing rat cerebellar mRNA also demonstrated greater ethanol inhibition of low vs. high KA responses (e.g. 100 mM EtOH inhibited 12.5 μM KA-stimulated current by 60% compared with 31% inhibition of 400 μM KA responses). However, in oocytes injected with the rat glutamate receptor subunit clone, GluR3, 100 mM EtOH produced similar inhibition of 400 and 12.5 μM KA-induced currents (46 & 52%, respectively). Thus, EtOH's differential inhibition of KA responses in oocytes may reflect selective actions of EtOH on individual KA receptor subtypes.

464.13

ENHANCEMENT OF NMDA-MEDIATED RESPONSES BY CYANIDE. M.N. Patel, R.W. Peoples, G.K.W. Yim and G.E. Isom.* Dept. of Pharmacol. & Toxicol., Purdue Univ., W. Lafayette, IN 47907 and Section of Electrophysiology, National Institute of Alcohol Abuse and Alcoholism, Rockville, MD 20852.

The effect of cyanide on NMDA-induced Ca^{+2} influx and inward current was studied. $[Ca^{+2}]_i$ was measured in cultured rat hippocampal neurons using fura-2 microfluorimetry. Removal of extracellular Mg^{+2} resulted in a five-fold increase in NMDA-induced peak $[Ca^{+2}]_i$. NaCN (1-10 mM) enhanced the peak responses obtained with NMDA in the presence, but not in the absence of extracellular Mg^{+2}. Similar results were obtained in whole-cell patch clamp recordings from cultured rat hippocampal neurons. Cyanide enhanced NMDA-activated current in the presence, but not the absence of extracellular Mg^{+2}. Enhancement of NMDA-activated current was observed within 1 sec of cyanide application. From these results it appears that cyanide alters the Mg^{+2} block of the NMDA receptor. (Supported by PHS Grant ES04140)

464.14

Ca IONS CHANGE THE AFFINITY OF GLYCINE TO NMDA RECEPTOR COMPLEX. Yanping Gu[1] and Li-yen Mae Huang[1,2]. Marine Biomedical Institute[1] and Department of Physiology and Biophysics[2], University of Texas Medical Branch, Galveston, TX 77550.

Ca ion play an important role in regulating the function of NMDA receptor channels. To better understand the interaction between Ca and NMDA-receptor channels, we examined the properties of NMDA-activated currents in various external Ca solutions. The whole cell currents were recorded from isolated trigeminal neurons using the patch clamp technique. We found the effects of Ca ions on NMDA-activated currents depend on the concentration of glycine. In the low external glycine, the NMDA-activated currents were increased as we raised the Ca concentration from 2 mM to 20 mM. But in the high external glycine, the NMDA-activated currents were decreased as we increased the external Ca. To study the mechanism of the actions of Ca, we determined the dose response for the action of glycine on NMDA-activated currents in different Ca solutions. Ca was found to have dual effects on NMDA-activated responses: It inceased the binding of glycine to the NMDA receptor complex, and it blocked monovalent cation permeation through NMDA receptor channels. Supported by grants: John Sealy Memorial Endowment Foundation, RCDA NS01050 and NS23061.

464.15

SPERMINE ENHANCES NMDA RECEPTOR CURRENTS IN CULTURED RAT CORTICAL NEURONS. D.M. Rock# and R.L. Macdonald+. Neuroscience Program# and Depts. of Neurology and Physiology+, Univ. of Michigan, Ann Arbor, MI 48109.

Spermine modulates the activity of NMDA in a variety of receptor binding and electrophysiological studies. At μM concentrations, spermine enhances the action of NMDA but at higher concentrations spermine is less effective. We studied the effect of spermine on NMDA receptor currents using whole cell and single channel recording techniques.

Whole cell NMDA currents in cultured rat cortical neurons were enhanced by spermine (0.1 μM - 1 mM) in a concentration-dependent manner. At concentrations above 10 μM, this enhancement was limited at -75 mV but not at +75 mV. The percent enhancement of NMDA current by spermine varied among preparations and in some cases no enhancement was observed.

In excised outside-out patches, spermine (1 μM - 1 mM) produced a concentration-dependent increase in channel opening frequency with a 40% increase over control at 1 μM. The effect of spermine on opening frequency was independent of external glycine concentration.

These results demonstrate that spermine enhances NMDA current by increasing channel opening frequency. The limitation of enhancement may be due to the other actions of spermine. Spermine is found in high concentrations in the nervous system and appears to act at an important regulatory site on the NMDA receptor-ion channel complex.

This research was supported by the USPHS grant NS19613 (RLM).

464.16

***trans*-ACPD BLOCKS HIGH THRESHOLD CALCIUM CURRENTS ON CULTURED HIPPOCAMPAL NEURONS.** Y. Sahara & G.L Westbrook. Vollum Institute, Oregon Health Sciences University, Portland, OR.

The metabotropic receptor (mGluR), the best characterized G protein coupled glutamate receptor, triggers inositol trisphosphate production and the release of intracellular calcium in mRNA-injected *Xenopus* oocytes and in hippocampal neurons. Although quisqualate and AP4 (2-amino-4-phosphonobutyrate) both inhibit high threshold calcium currents (Lester & Jahr, Neuron, 1990; Trombley & Westbrook, Soc.Neurosci.Abst.,1990), the receptor and coupling mechanism involved are unclear. For example, L-AP4 does not activate mGluR and appears to be a membrane-delimited interaction of a G protein with the calcium channel. These data suggest either multiple G protein coupled glutamate receptors or coupling mechanisms. We have examined whether the selective mGluR agonist, (1S,3R)-1-aminocyclopentane-1,3-dicarboxylate (*trans*-ACPD) can also inhibit high threshold calcium currents on cultured hippocampal neurons. Whole-cell voltage clamp recordings were made with patch pipettes containing GTP or a 5:1 ratio of GTP/GTPγS with calcium buffered to pCa = 7. High threshold calcium currents were evoked by 30 ms voltage jumps to 0 mV from a holding potential of -60 mV using 2.5 mM barium as the charge carrier. *trans*-ACPD (50-300 μM) or L-AP4 (50 μM) were delivered by flow pipes. *trans*-ACPD produced reversible and dose-dependent decreases in the barium current; at 200 μM the reduction was 29.5 ± 4.5 % (n=10). Decreasing the GTP/GTPγS ratio resulted in irreversible reduction of the barium current by *trans*-ACPD. L-AP3 (500 μM), a reported mGluR antagonist, partially antagonized the action of *trans*-ACPD. Under the same conditions, L-AP4 produced a 24.4 ± 3.1 % inhibition (n=6) of the barium current. Repeated applications of *trans*-ACPD in barium/ 0 calcium solutions caused similar percent inhibition, suggesting that release of intracellular calcium was not responsible for block of the current. Supported by NIH grant #NS26494 and the Klingenstein Foundation.

464.17

L-TRANS 1-AMINO-1,3-CYCLOPENTANE DICARBOXYLIC ACID (ACPD) STIMULATES INTRACELLULAR CA^{++} MOBILIZATION IN A MG^{++} AND DAPV SENSITIVE MANNER IN CULTURED CA1 PYRAMIDAL NEURONS. K. Curry and K.G. Baimbridge. Department of Physiology, University of British Columbia, Vancouver, B.C. V6T 1Z3 Canada.

Trans ACPD has been said to activate a glutamate G-protein linked (Glu_G) receptor which is metabotropic rather than ionotropic in nature. This action mobilizes intracellular Ca^{++} and we have studied this effect using the D and L isomers of trans ACPD and monitoring the Ca^{++} levels intracellularly with the dye Fura 2.

Application of the D-isomer produced no effect on neurons at concentrations of up to 1 mM when applied to the bathing medium while the L-isomer produced elevated intracellular Ca^{++} levels at 200 μM. This action of the L-isomer was found to be sensitive to Mg^{++} and D-2-amino 5-phosphonovaleric acid (DAPV). The results indicate that L-trans ACPD operates through the N-methyl-D-aspartate rather than the Glu_G receptor and we conclude that the action of the compound may be sensitive to different areas of the CNS and to the age of the neurons being studied.

464.18

NMDA BUT NOT AMPA/KAINATE ANTAGONISTS BLOCK SPONTANEOUS EPILEPTIFORM DISCHARGES (SED) IN THE RAT CORTICAL WEDGE. L.J. Robichaud, T. Malone' and P.A. Boxer, Parke-Davis Pharm. Res. Div., Warner-Lambert Co., Ann Arbor, MI 48105.

We examined functional responses to glutamate antagonists (30 min treatment) in the low Mg^{++} rat cortical wedge preparation. As previously described, SED was inhibited by the NMDA recognition site antagonist CPP (IC_{50} = 0.35 μM), the NMDA/ion channel antagonist MK-801 (IC_{50} = 0.5 μM). Similarly, the novel NMDA/ion channel antagonist PD137889 [(+)-1,2,3,4,9,9a-hexahydro-N-methyl-4aH-fluoren-4a-amine monohydrochloride] inhibited SED with an IC_{50} = 0.6 μM. Antagonists of the strychnine-insensitive glycine site also inhibited SED: 7-chlorokynurenic acid (IC_{50} = 10 μM), 5,7-dichlorokynurenic acid (IC_{50} = 5 μM), and (±) HA-966 (IC_{50} = 120 μM). This inhibition was completely reversed by glycine at concentrations that had no effect on SED alone. NBQX, a selective antagonist of AMPA/kainate (vs NMDA) receptors, blocked AMPA-induced depolarizations at 10 μM, but at concentrations up to 100 μM did not inhibit SED. DNQX, which is both an AMPA/kainate and glycine site receptor antagonist inhibited SED (IC_{50} = 12 μM), however glycine completely reversed the inhibitory actions of DNQX. Glycine did not reverse the inhibition of SED by NMDA recognition site or ion channel blockers. These data demonstrate that glycine can only reverse the inhibition produced by glycine-site antagonists and therefore that the inhibitory effect of DNQX on SED can be attributed to its glycine antagonism alone. In summary, spontaneous epileptiform discharges in the rat cortical wedge are not inhibited by AMPA/kainate antagonists, but are sensitive to modulation by antagonists of several sites of the NMDA-receptor ion channel complex.

464.19

INCREASED REQUIREMENT FOR HIGH-ENERGY PHOSPHATES BY NMDA-RECEPTORS IN ACUTELY DISSOCIATED KINDLED GRANULE CELLS. G. Köhr and I. Mody. Dept. of Neurology & Neurological Sciences, Stanford Univ. Sch. of Med., Stanford, CA.

Previous studies in hippocampal slices have shown that kindling increases synaptic activation of NMDA receptors in the dentate gyrus. Furthermore, whole-cell dialysis in cultured neurons has uncovered the requirement for intracellular high-energy phosphates (P_i) for the full expression of NMDA channel activity. We have examined if alterations in the P_i requirement of NMDA channels may be present following kindling-induced epilepsy.

Whole-cell NMDA-currents were evoked in acutely dissociated control and kindled dentate gyrus granule cells in Mg^{2+}-free extracellular medium containing 3 μM glycine using a solenoid-operated double-barrelled θ-tubing (200 μM NMDA/4-20 s). In the presence of P_i (Mg-ATP + phosphocreatine + creatine-phosphokinase) the amplitudes of NMDA currents were comparable in control and kindled preparations (range: 90-100 pA) and did not wash-out over 20 min of whole-cell recordings. Fluctuation analysis revealed mean open times of 3.1 to 7.9 ms and single channel conductances of 20 to 49 pS which were not significantly different between control and kindled neurons, and did not change in the absence of P_i. However, in the absence of P_i the control and kindled NMDA responses were reduced by 27% and 45% respectively. The significantly diminished kindled NMDA currents recorded without P_i are consistent with an altered phosphorylation state of NMDA receptors after kindling, but other mechanisms cannot be excluded. Presently no estimate can be made about the *in situ* phosphorylation level of NMDA receptors, but our data show that a covalent modification of NMDA receptors may have occurred during kindling.

Supported by the NIH grant NS 12151 (I.M.) and DFG (G.K.).

464.20

L-ASPARTATE RECEPTORS IN MULLER CELLS: TROPHIC INTERACTIONS. M. Romo-de-Vivar* and A.M. López-Colomé. Instituto de Fisiología Celular, UNAM. Apartado Postal 70-600, 04510 México, D.F. México.

Receptors for L-^{3}H-aspartate have been biochemically characterized in Muller cells from the chick retina in primary culture. Two binding sites with affinity in the nM and uM range were detected, showing pharmacological profiles similar to those for neurotransmitter (QA) and high-affinity-uptake systems, respectively. Since these receptors became apparent at different stages of development in vitro, we tried to determine if culture conditions could influence their expression and properties. Glial cells were maintained in medium containing 10% FCS. The effect of low serum (1%), diBcAMP, L-glutamate, quisqualate and NMDA on ^{3}H-L-asp binding was studied at days 1,5,8 and 12 in vitro. At concentrations 50-200 uM, neither compound showed effect; diBcAMP was tried up to 1 mM without effect. In cells cultured in low serum, ^{3}H-L-asp binding was increased at all stages: 0.497 to 0.762 pmole/mg protein at 1 DIV, 0.123 to 1.05 at 5 DIV, 0.190 to 1.0 at 8 DIV and 0.24 to 0.82 at 12 DIV. Pharmacologically these sites show the properties of high affinity transport sites. These results suggest that in contrast with cells cultured in the presence of FCS, Muller cells in low serum do not express high affinity binding sites with properties similar to QA transmitter receptors. Partially supported by CONACyT P228CCOX891617.

465.1

$GABA_B$ RECEPTOR-MEDIATED PAIRED PULSE DISINHIBITION REVEALS AN NMDA COMPONENT OF THE EPSP. D. D. Mott, C. W. Xie, R. A. Morrisett, H. S. Swartzwelder, W. A. Wilson and D. V. Lewis. Depts. of Pharmacology, Pediatrics (Neurology), Medicine (Neurology) and Neurobiology, Duke Univ. and V. A. Med. Ctrs, Durham, N.C. 27710.

We have previously reported that, during paired stimulation of the molecular layer in the rat dentate gyrus, the NMDA component of the second response is enhanced by an underlying $GABA_B$ receptor-mediated depression of inhibition. During 5 Hz stimulation, this enhancement of the NMDA component can cause development of long term potentiation (Mott and Lewis, Science, in press). In this study we sought to further investigate the stimulus parameters necessary for expression of this paired pulse disinhibition.

Paired pulse disinhibitionwas studied in the dentate gyrus and area CA1 of the rat hippocampal slice. In the dentate gyrus, paired stimulation in the inner two-thirds of the molecular layer caused the EPSP of the second response to be prolonged and display 1-3 additional population spikes. This enhancement was maximal when stimuli were delivered 150-250 ms apart and was blocked by D-APV (50 uM) or by 2-OH saclofen (400 uM). Enhancement of the second response was most evident with higher intensity stimuli and when the stimulating electrode was placed in the molecular layer within 100 um of the recording electrode. As the stimulating electrode was moved away from the recording electrode, the degree of enhancement of the second response diminished.

Similarly, in area CA1 the EPSP of the second response to paired stimuli delivered to *stratum radiatum* within 100 um of the recording electrode was prolonged. This enhancement diminished as the stimulating electrode was moved away from the recording electrode and was also blocked by D-APV (50 uM) or 2-OH saclofen (400 uM).

Supported by NIH grant NS27488 and by the Veterans Administration.

465.2

EVIDENCE THAT ETHANOL REDUCES $GABA_B$-MEDIATED DISINHIBITION AND SYNAPTIC PLASTICITY INDUCED BY A PRIMING PARADIGM IN RAT DENTATE GYRUS.

R.A.Morrisett, W.A.Wilson, H.S.Swartzwelder. Neurology Research, Duke Univ. and the Veterans Admin. Med. Ctrs., Durham, NC, 27705.

Priming stimulation paradigms (5 Hz) delivered to the molecular layer of the rat dentate gyrus result in hyperexcitability and enhancement of NMDA-mediated synaptic potentials. In this case, the activation of $GABA_B$ receptors leads to a reduction of synaptic inhibition. If at least 10 pulses are delivered at 5 Hz, the resultant excitatory effect is of sufficient magnitude to elicit long-term potentiation of synaptic transmission (Mott and Lewis, Science, in press).

We hypothesized that ethanol would inhibit the the NMDA component of synaptic transmission induced by a priming paradigm since ethanol inhibits NMDA population epsps (Lovinger et al., *J Neurosci.*, 1990; Morrisett et al., *Neuropharmacol.*, in press). Orthodromic test stimuli were applied to the dentate gyrus 200 msec after an identical conditioning stimulus. When slices were treated with ethanol, the disinhibitory effect of paired stimulation was markedly reduced in a concentration-dependent manner (25-100 mM). We also examined the effects of ethanol on long-term potentiation of synaptic transmission. In the presence of 75 mM ethanol, 5 Hz-2 sec trains induced only the decremental form of synaptic plasticity. However, after ethanol washout, identical trains elicited non-decremental potentiation of both epsp slope and population spike.

We conclude that inhibition of priming by ethanol may represent one mechanism responsible for its amnesic effects. (This work was supported by AA 07207, MH 15177-13, NS 17701 and NS 27488).

465.3

NON-SYNAPTIC MODULATION OF AXONAL CONDUCTION BY GABA-B RECEPTORS IN THE NEONATAL RAT OPTIC NERVE: BACLOFEN IMPROVES HIGH-FREQUENCY IMPULSE CONDUCTION IN THE PRE-MYELINATED OPTIC NERVE. A.Z. Hassan*, W. Ching*, and K. Sakatani Dept. of Neurosurgery, NYU Medical Center, New York NY 10016

Recent studies have shown GABA-A receptor sensitivities in a number of white matter preparations, including rat optic nerve. However, the presence of GABA-B receptors in axonal preparations remained unknown. We have addressed this issue by comparing the effects of GABA-A and -B receptors on axonal conduction in the neonatal (1 to 12 days of age) rat optic nerve *in vitro*. The compound action potential (CAP) of isolated optic nerves was elicited by submaximal stimulation (50-60% of maximal responses, 0.2 Hz), and recorded with glass micropipettes.

GABA (100 μM), at first, decreased the latency of the CAP. This initial effect was followed by progressive decrease in the amplitude and increase in the latency. The later effect of GABA was blocked by the GABA-A blockers, picrotoxin and bicuculline, and mimicked by the GABA-A agonist, isoguvacine (100 μM). However, the initial effect of GABA on the latency was resistant to the GABA-A blockers. The GABA-B agonist, baclofen (1-100 μM), mimicked the initial effects of GABA. The GABA-A blockers failed to block the effect of baclofen. Furthermore, baclofen increased the population conduction velocity, estimated from two points recordings, suggesting that the effects of baclofen were along the length of the axons. To evaluate the effects of baclofen on high-frequency impulse conduction, stimulus trains were delivered at frequencies of 10-33Hz, for 103 pulses. At 33 Hz, the control stimulus train depressed the amplitude by 78.6 ± 17% (mean ± SD) and increased the latency by 26.3 ± 12.9% (n=7) by the 103rd test pulse. In the presence of baclofen (100 μM), the amplitude of the 103rd response was decreased by only 27.8 ± 13.0% and the latency was increased by only 20.1 ± 4.1% (n=4).

Our results demonstrate that: (1) functional GABA-A and -B receptors coexist in the neonatal rat optic nerve; (2) baclofen improves high-frequency impulse conduction of the pre-myelinated axons. Supported in part by NIH grant NS10164.

465.4

A NOVEL $GABA_B$ RECEPTOR-MEDIATED IPSP ELICITED IN CA1 PYRAMIDAL CELLS BY MICROAPPLICATION OF GLUTAMATE IN *STRATUM LACUNOSUM-MOLECULARE* OF THE RAT HIPPOCAMPUS *IN VITRO*. S. Williams and J.-C. Lacaille. Département de physiologie, Centre de recherche en sciences neurologiques, Université de Montréal, Qué., Canada H3C 3J7.

Two types of GABAergic responses ($GABA_A$ and $GABA_B$) are present in the hippocampus. Interneurons located in str. lacunosum-moleculare (L-M) may mediate the electrically-evoked late ($GABA_B$) inhibitory postsynaptic potential (IPSP). To verify this hypothesis, IPSPs were elicited in CA1 pyramidal cells (n=88) by glutamate (500 μM) applications to str. L-M (glut-IPSPs) in hippocampal slices. For comparison, early and late IPSPs were evoked by stimulation of the Schaffer collaterals. In 20 cells, the mean amplitude of the glut-IPSPs was -2.0 mV and the mean equilibrium potential was -70 mV. Glut-IPSPs showed little or no response reversal (13/14 cells). Perfusion with TTX (1 μM; n=3) blocked the glut-IPSPs, suggesting that they were generated by local interneurons. Local application of the $GABA_A$ antagonist bicuculline (100 μM; n=2) or perfusion with picrotoxin (10-20 μM; n=6) reduced the early IPSP but not the late IPSP and glut-IPSP. Local applications of the $GABA_B$ antagonist 2-OH-saclofen (1-4 mM; n=7) reduced the late IPSP (46% of control) and the glut-IPSP (47% of control). However, the $GABA_B$ antagonist phaclofen (20 mM; n=4) did not antagonize the glut-IPSP (101% of control) at doses that reduced the late IPSP (47% of control). The K^+ channel blocker barium (1 mM; n=4) diminished the late IPSP (64% of control) but increased the glut-IPSP (116% of control). Repetitive electrical stimulation (3 Hz for 10 sec; n=6) depressed the early, late and glut-IPSP, suggesting that interneurons activated with glutamate were also activated electrically. It is concluded that the late IPSP may be composed of an heterogeneity of $GABA_B$ receptor-mediated mechanisms and that IPSPs elicited presumably from interneurons in str. L-M may mediate one of the $GABA_B$ components. [Supported by MRC, FRSQ, and Savoy Foundation].

465.5

GABA RECEPTOR-MEDIATED SYNAPTIC POTENTIALS IN RAT HIPPOCAMPAL CA1 PYRAMIDAL CELLS ELICITED BY GLUTAMATE MICROAPPLICATION AT THE ORIENS/ALVEUS BORDER OR IN STRATUM PYRAMIDALE. D.D. Samulack and J.-C. Lacaille. Département de physiologie and Centre de recherche en science neurologiques, Université de Montréal, QC, Canada H3C 3J7.

Inhibitory postsynaptic potentials (IPSPs) in hippocampal pyramidal cells arise from a combination of $GABA_A$ and $GABA_B$ receptor-mediated mechanisms. In contrast to interneurons in stratum (str.) lacunosum-moleculare, which may mediate a $GABA_B$ component, interneurons at the str. oriens/alveus border (O/A) or in str. pyramidale (PYR) may mediate portions of the $GABA_A$ component. This hypothesis was tested through the micropressure application of glutamate (500 μM; drop diameter 30-80 μm) at O/A or in PYR, while recording intracellularly from different CA1 pyramidal cells in rat hippocampal slices. The pyramidal cell IPSPs evoked by glutamate application in O/A (peak: -4.0 mV; peak latency: 126 ms; decay: 398 ms; n=8), were very similar to those elicited from PYR (peak: -4.1 mV; peak latency: 104 ms; decay: 404 ms; n=12). Electrical stimulation in str. oriens or radiatum of the CA1 region elicited IPSPs with little difference in early and late components between experimental groups. The glutamate evoked IPSPs from O/A or PYR were both sensitive to the local application of the $GABA_A$ antagonist bicuculline (100-200 μM). However, the reduction in the O/A evoked IPSP (58%; n=5) was less than that of the PYR IPSP (84%; n=5). The glutamate evoked IPSP from PYR showed little sensitivity (6%, n=2) to the $GABA_B$ antagonist 2-OH-saclofen (1 mM). In one cell tested, the IPSP evoked from O/A was reduced by saclofen (33%). These results indicate that interneurons activated from both O/A and PYR may be responsible for the $GABA_A$ mediated component of the electrically evoked IPSP. O/A interneurons may also contribute to the $GABA_B$ component of the late IPSP.

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465.6

CALCIUM CURRENTS IN GOLDFISH RETINAL GANGLION CELLS ARE BLOCKED BY BACLOFEN. V.P.Bindokas and A.T.Ishida. Dept. Animal Physiology, University of California, Davis CA 95616.

Goldfish (*Carassius auratus*) retinal ganglion cells receive synaptic input from GABAergic amacrine cells *in situ* [Muller & Marc (1990) J Comp Neurol 291:281], and have a $GABA_A$-receptor-coupled anionic conductance [e.g. Ishida & Cohen (1988) J Neurophysiol 60:381]. We have investigated whether these cells also possess $GABA_B$-type receptors by using whole-cell patch-clamp recording methods. Calcium currents were isolated as previously described [Ishida (1991) Vision Res 31:477]. Calcium currents activated by voltage steps (250 msec) from -90 to 0 mV display at least two components: transient and sustained. All concentrations of (-)baclofen tested (0.3 to 100 μM), preferentially blocked the sustained component; 100 μM baclofen produced roughly 70% reduction in sustained current (n=10). The residual voltage-activated inward current in the presence of baclofen was fully blocked by replacement of bath Ca^{2+} (2.5 mM) by 2.4 mM Co^{2+} plus 100 μM Ca^{2+}; no outward current appeared under these conditions. Calcium currents activated by voltage steps to -30 mV were reduced less by baclofen than were currents activated by jumps to 0 mV, and peak (transient) currents were less affected than sustained currents at all voltages. The action of baclofen (all doses tested) was at least partially reversible with wash. 750 μM phaclofen did not antagonize the block produced by 300 nM or 30 μM baclofen. Baclofen sensitivity was observed in over 95% of all ganglion cells sampled (n=27). These results indicate that at least some of the voltage-gated calcium current in retinal ganglion cells is sensitive to $GABA_B$-receptor agonists.

Supported by NIH grant EY 08120.

465.7

THE ACTION OF $GABA_B$ ANTAGONISTS IN THE TRIGEMINAL NUCLEUS. G.H. Fromm, K. Sato* and M. Nakata*. Dept. of Neurology, Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA 15261.

D-baclofen blocks the action of L-baclofen in the trigeminal nucleus and spinal cord but not in neocortical or hippocampal slices. This discrepancy could be due to a difference in baclofen concentration used or a difference in receptor type.

The iontophoretic administration of the $GABA_B$ antagonists 2-hydroxy-saclofen and CGP 35348 blocked the action of iontophoretically applied L-baclofen on neurons in the trigeminal nucleus of rats anesthetized with halothane, with CGP 35348 being 3-6 times as potent as saclofen.

The iontophoretic administration of GABA resembled L-baclofen in depressing excitatory transmission and facilitating segmental inhibition in the trigeminal nucleus. CGP 35348 also blocked the depression of excitatory transmission and partially blocked the facilitation of segmental inhibition produced by GABA.

Our observations indicate that CGP 35348 is not only a baclofen antagonist but actually a $GABA_B$ receptor antagonist, and that baclofen is acting at $GABA_B$ receptors in the trigeminal nucleus. The portion of the GABA effect not blocked by CGP 35348 is probably mediated by $GABA_A$ receptors, since we have previously found that segmental inhibition in the trigeminal nucleus can be modulated by $GABA_A$ agonists and antagonists. (Supported by NS-19889)

465.8

DISCRIMINATIVE-STIMULUS EFFECTS OF THE $GABA_A$ AGONIST THIP AND THE $GABA_B$ AGONIST BACLOFEN. N. A. Ator. Div. Behav. Biol., Dept. of Psychiat. and Behav. Sci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205

The discriminative-stimulus effects of specific agonists for the two major GABA receptors were studied in rats trained to discriminate either the $GABA_A$ agonist THIP (gaboxadol, an analogue of the naturally occuring $GABA_A$ agonist muscimol) or the $GABA_B$ agonist baclofen (a central muscle relaxant) from the no-drug condition. Criterion performance was achieved more rapidly under the THIP (5.6 mg/kg, i.p.) than under the baclofen (3.2 mg/kg, i.p.) training condition. In tests, there was dose-dependent generalization to novel doses of the training drugs within each training drug condition, but discriminative control by each training drug was specific in that baclofen did not occasion drug-lever responding reliably in rats trained to discriminate THIP and vice versa. Administration of the specific benzodiazepine-receptor antagonist flumazenil dose-dependently decreased drug lever responding in combination with THIP but not in combination with baclofen. The baclofen training condition was less specific than the THIP training condition, because rats trained to discriminate baclofen were more likely to generalize to midazolam (MDZ) and to pentobarbital (PB) than were rats trained to discriminate THIP. The effects of MDZ and PB are mediated by the $GABA_A$ receptor. Thus, a major part of the baclofen/$GABA_B$ discriminative-stimulus complex may be a generalized muscle-relaxant effect. (Supported by NIDA Grant DA04133)

465.9

THE DEVELOPMENT OF $GABA_b$ ACTIVITY IN THE RAT DENTATE GYRUS: EVOKED POTENTIAL STUDIES IN THE HIPPOCAMPAL SLICE PREPARATION. P.G. DiScenna & T.J. Teyler. Neuroscience Program, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272.

The development of $GABA_b$ activity in the dentate gyrus was studied by examining $GABA_b$-mediated effects on orthodromically-evoked field potentials. The effects of the $GABA_b$ agonist, baclofen (5-10uM: 0.5ml/min), were examined in 8 slices from PN6-PN8 (PN6 was the first day that a population spike was elicited). The population EPSP slope was reduced by an average of 36% and population spike amplitude by 44%. Baclofen did not produce overt multiple spiking, but rather a low amplitude ripple after the primary spike. Short-latency (10-20ms IPI) paired-pulse spike inhibition converted to spike facilitation within 5min. of baclofen perfusion in all slices. This reversal was not caused by decreased pop. spike amplitude of the conditioning pulse response. All effects were reversed, but not totally eliminated by perfusion with the $GABA_b$ antagonist, phaclofen (1mM).

The results suggest that at least a subpopulation of $GABA_b$ receptors matures quickly in DG, the effects being present before the peak generation of DG's primary neurons, the granule cells, occurs.

465.10

THE EFFECTS OF A BENZODIAZEPINE PARTIAL AGONIST, RO16-6028, ON RAT EEG. K.L. Skinkle and C.M. Sinton. Neurogen Corp., Branford, CT 06405.

Recently developed partial agonists at the benzodiazepine receptor may be less sedating than the classical full benzodiazepine agonists. This study was therefore designed to evaluate the changes produced in the frequency spectrum of the cortical electroencephalogram (EEG) in the rat as a way of quantifying the sedative properties of these compounds. RO16-6028 (1.0, 0.25, 0.03 mg/kg iv) and CGS 9896 (1.0, 0.2 mg/kg iv) were thus compared with Diazepam (0.5, 0.04 mg/kg iv) and Alprazolam (0.125, 0.02 mg/kg iv). Rats were chronically implanted with cortical electrodes, and ipsilateral frontal-occipital EEG was recorded in all cases. Drugs were administered via a cannulated tail vein. Spectral analysis of the EEG signals was performed on-line using a microcomputer, and drug effects were evaluated for up to an hour following compound administration. Vigilance was maintained throughout the experiment by using a randomized disturbing noise source. All compounds at all doses increased power in the 8 to 30 Hz frequency band, which has been suggested to represent idling in thalamo-cortical circuits. This EEG change is characteristic of this type of compound: similar EEG effects are seen in man. Interestingly, the highest dose of RO16-6028 modified the EEG in other ways which were not observed with CGS 9896. These changes included a reduction in the 1 to 5 Hz band and an increase in theta frequencies (5-7 Hz). RO16-6028 thus modifies the EEG in the rat in a way which might reflect such underlying processes as recruitment of attentional systems. These processes may partially compensate for the sedative properties of the benzodiazepines, especially as higher doses of RO16-6028 are administered. The results may have implications for the design of novel non-sedating anxiolytics.

465.11

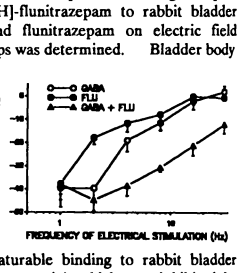
BOTH DOPAMINE AND GABA INHIBIT MELANOTROPH POMC GENE EXPRESSION AND BETA-ENDORPHIN RELEASE VIA A SIMILAR PERTUSSIS TOXIN SENSITIVE MECHANISM. Dominic J. Autelitano[1,2], Joel Parker*[3], Richard Allen*[3] and James L. Roberts[2]. Baker Medical Research Inst., Prahran, Australia[1], Mt.Sinai Med. Ctr., N Y., USA[2] and CROET, Oregon Health Sci. Univ., OR, USA[3]

The melanotroph cells of the rat intermediate lobe (IL) are directly innervated by tuberohypophyseal neurons that contain the two neurotransmitters dopamine (DA) and GABA. Although the long term effects of both DA and GABA on IL pro-opiomelanocortin (POMC) gene expression and peptide release are inhibitory, GABA has been shown to have an acute biphasic action on peptide release from either intact neurointermediate lobes (NIL) or cultured NIL cells. Previous studies have shown that the rapid stimulatory effects of GABA on alpha-MSH release can be mimicked by the GABA-A agonist isoguvacine, while the more prolonged inhibitory action is baclofen sensitive, suggesting a GABA-B receptor mediated effect. Using isolated rat NIL in short term culture (2h or 18h) we have demonstrated that 10μM GABA or the mixed GABA-A/B receptor agonist muscimol (100μM) inhibit ß-endorphin (ßEP) release by around 50%. Both these agents have similar inhibitory effects on POMC cytoplasmic mRNA and nuclear primary RNA transcripts in 18h cultures. In addition, both baclofen and isoguvacine inhibit ßEP release in 2h or 18h cultures, indicating that both GABA-A and -B receptors may have long-term inhibitory actions. Neither the GABA-A antagonist bicuculline nor the GABA-B antagonist 2-hydroxysaclofen completely block the inhibitory actions of GABA on ßEP release. A dose dependent inhibition of ßEP release from NIL is seen with increasing doses of the DA-D2 receptor agonist quinpirole. GABA (10μM) or muscimol (100μM) when given together with sub-maximal doses of quinpirole give additive effects on the inhibition of ßEP release, though no additivity is seen with maximal inhibitory doses of the DA agonist. Similarly, 100μM muscimol, or the DA agonist bromocriptine (0.1μM) each lead to a 50% inhibition of POMC mRNA in 18h cultures but showed no additive effects when administered together. In NIL cultures pretreated for 18h with 100 ng / ml pertussis toxin (PTX), these inhibitory effects of quinpirole, GABA and muscimol are abolished. These data suggest that in the rat IL, GABA can inhibit POMC expression via a complex mechanism that involves both GABA-A and GABA-B receptors. The PTX sensitivity, and lack of additivity of DA and GABA responses suggests that a common or similar G-protein may mediate the inhibitory effects of these receptors.

465.12

PERIPHERIAL TYPE GABA-A RECEPTORS INHIBIT FIELD STIMULATED CONTRACTIONS OF RABBIT URINARY BLADDER. M.R.Ruggieri. Urology Research Laboratories, Temple University, Phila, PA 19140.

Gamma amino butyric acid (GABA) is an established inhibitory neurotransmitter in the central nervous system, and may also be involved in inhibitory circuits of the autonomic nervous system and peripheral organs. GABA-A receptors in different regions exhibit differential sensitivity to GABA and GABA receptor modulators such as benzodiazepines indicating receptor heterogeneity. The specific binding of [3H]-flunitrazepam to rabbit bladder homogenates and the effect of GABA and flunitrazepam on electric field stimulated contractions of in vitro bladder strips was determined. Bladder body strips (1 X 0.2 cm) were suspended in oxygenated Tyrode's solution and stretched to within 10% of the length for maximum tension. Three frequency response curves to electric field stimulation (60 V, 1 msec duration, 1 - 32 Hz) were generated - control, 30 min following exposure to 10^{-4} M GABA or flunitrazepam, and 30 minutes after adding both compounds. Results are displayed at the right. [3H]-flunitrazepam (0.5 - 125 nM) demonstrated specific, saturable binding to rabbit bladder membranes (Kd=21nM, Bmax=2.7pMol/mg protein) which was inhibited by unlabeled diazepam (10^{-11} - 10^{-5} M), but not by unlabeled clonazepam, a "central" type benzodiazepine receptor agonist. The Kd for diazepam was determined by Hill plot to be 28 nM, demonstrating a similar binding affinity of these two ligands. This suggests a role for GABA in inhibitory regulation of bladder function at the end organ.



465.13

$GABA_A$ RECEPTORS: SUB-TYPES AND ALTERATIONS IN CHRONIC HYPERAMMONEMIA. B.A. McLaughlin, M.B. Robinson and D.B. Pritchett. Children's Seashore House; Depts. Ped. and Pharm., Univ. of PA; Philadelphia, PA, 19104.

Multiple subtypes of $GABA_A$ receptors have been identified. Through the use of molecular biology, it has been possible to demonstrate that the diversity of the heteromeric $GABA_A$ receptors may be due to variations in the subunit composition. It was recently shown that the type of α subunit confers specificity for benzodiazepine interactions with the receptor. Transfection of cells with cDNAs encoding the α1, ß3, and γ2 subunit results in a [^{3}H]RO-15-1788 binding site that is potently displaced by zolpidem (IC_{50} = 40 nM). If an α5 subunit is substituted, binding is less potently inhibited by zolpidem (IC_{50} = 30 μM). This raises the possibility that each of the subunits may be independently regulated. Previous studies using [^{3}H]GABA have shown that the density of the $GABA_A$ receptors is increased during acute hyperammonemia induced by hepatic encephalopathy. The binding of [^{3}H]RO-15-1788, a non-selective α1–α5 antagonist, was examined in the sparse fur mouse (*spf/*Y), a model of congenital hyperammonemia. The (*spf/*Y) has an X-linked deficiency of liver ornithine carbamoyltransferase which leads to 2-3 fold elevations in plasma ammonia levels. No differences were observed in cortical, cerebellar or hippocampal homogenate binding of *spf/*Y mice compared to controls. Therefore, we were unable to confirm previously reported alterations in GABA receptors in hyperammonemia. This result could be due to the use of [^{3}H]RO-15-1788 which is non selective. To begin to elucidate subunit alterations, zolpidem displacement of [^{3}H]RO-15-1788 was examined. In normal animals, homogenate binding was consistent with two sites in cortex and hippocampus (Hill Coefficients < 0.7).

465.14

REGIONAL DEVELOPMENTAL SWITCHES IN mRNA ENCODING TYPE I AND TYPE II $GABA_A$ RECEPTORS. R.E. Williamson*, M.A. Dichter, & D.B. Pritchett.Children's Seashore House, Depts Pediat & Pharm Univ Pennsylvania, Phila PA 19104.

Molecular biological studies of the $GABA_A$ receptor reveal pharmacologically distinct receptor subunits consisting of α, β, and γ components. Photoaffinity labelling of certain combinations of subunits with tritiated benzodiazepines generates 2 functional $GABA_A$ complexes that display type I (α1) and type II (α2, 3, & 5) receptor pharmacologies. Previous studies suggest a developmental switch in rat cortex and cerebellum from type II to type I receptors. To investigate this switch, we used the ribonuclease protection assay to hybridize rat brain mRNA with anti-sense mRNA synthesized from probes specific for the α1, α2, α3, and α5 subunits. In *in vivo* experiments using rat cerebellum and cortex, α3 and α5 mRNAs were most abundant at birth and decreased over time. α1 mRNA, which was virtually non-existent at birth, became most abundant by day 15 in cerebellum and day 9 in cortex. The same switch in receptor subtypes did not occur in *in vitro* experiments where mRNA was extracted from rat fetal cortical neurons grown in culture. The cultured neurons harvested at 1 week intervals (7 to 54 days) were the same age as or older than the brain tissue used in the *in vivo* experiments. The same shift in receptor subtypes was observed in mutant reeler mice that have Purkinje cells malpositioned in the granule cell layer of the cerebellum but that still express nearly intact synapses. When the DNA synthesis inhibitor 5-Fluorodeoxyuridine was injected into 2-day old rats, interfering with postnatal neurogenesis of cerebellar granule neurons, the same switch was seen. Further experiments are needed to establish if this developmental shift is a result of synaptogenesis or if it is an intrinsic property of the neuron.

465.15

SEX DIFFERENCES IN TRANSPUBERTAL HYPOTHALAMIC AND HIPPOCAMPAL $GABA_A$ RECEPTOR BINDING OF [^{3}H]-SR-95531 IN THE RAT. W. Jacobson and G.A. Cottrell. Division of Reproductive Science, Dept. of Obstetrics and Gynecology, and Dept. of Pharmacology, University of Toronto, Toronto, Canada M5G 1L7.

The inhibitory neurotransmitter γ-amino benzoic acid (GABA) has been implicated in the control of LH release in the rat. Numerous studies suggest that during the pubertal transition, inhibitory influences on LH release are diminished. Using the technique of in vitro autoradiography, and the arylaminopyridazine derivative SR-95531 (SR), a specific $GABA_A$ antagonist, we investigated the ontogeny of these binding sites in selected mediobasal-hypothalamic nuclei and in the rostral hippocampus in both male and female rats. In males, significant changes in hypothalamic binding were evident only in the medial preoptic area, where the binding on d25 and d32, but not on d37 was increased compared to d15. No changes were seen in other hypothalalmic regions examined, including the Bed Nucleus Stria Terminalis, the anterior hypothalamic area, or the ventromedial nucleus. Hippocampal binding increased sharply in the dentate gyrus, CA1 and CA3 regions transpubertally, while in the CA2 region it returned to d15 levels by d37. In females, significant differences in the amount of [^{3}H]-SR bound to various regions of the hypothalamus were not found transpubertally, while the hippocampus showed large increases with development. Interestingly, significant levels of SR binding were not seen in the BNST in either sex at D15. Thus, it appears that $GABA_A$ receptor patterns demonstrate sex-specific regional heterogeneity with development. The significance of these findings with regard to the pubertal transition are currently under investigation. Supported by the Canadian MRC, MA-10911.

465.16

DEVELOPMENTAL CHANGES IN 35S-TBPS BINDING SITES IN THE CEREBRAL CORTEX OF THE RAT. M.G. Corda, E. Cancedda*, M. Orlandi* and O. Giorgi.

Department of Experimental Biology, Neuroscience Section, Cagliari University, Italy.

The postnatal development of the GABA-gated chloride ionophore was analyzed in the cerebral cortex of the rat by measuring the binding parameters of 35S-t-butylbicyclophosphorothionate (35S-TBPS) at intervals (1 to 90 days) after birth. The density (Bmax) of 35S-TBPS binding sites at birth was only 25% of the adult (90-day-old) value and, after a rapid increase up to 30% above the adult value by postnatal day 15, it decreased to adult levels by postnatal day 45. In contrast, the binding affinity (Kd) of 35S-TBPS remained constant from birth through adulthood. It is well known that the binding of 35S-TBPS to the GABA-gated chloride channel is allosterically modulated by drugs acting on different sites of the GABA-A receptor complex. Thus, GABA, benzodiazepines and barbiturates decrease 35S-TBPS binding whereas an opposite effect is induced by the GABA receptor antagonist, bicuculline, and by convulsant ligands for benzodiazepine receptors, such as methyl 6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM). The allosteric modulation of 35S-TBPS binding sites was already evident at early stages of postnatal development. In fact, the magnitude of the effects of positive and negative modulators of the GABA-A receptor complex on 35S-TBPS binding did not change significantly from birth through adulthood. These results reflect the early development of the functional link between the different components of the GABA-A receptor.

OPIOIDS; ANATOMY AND PHYSIOLOGY II

466.1

ESTRADIOL AND PROGESTERONE MODULATION OF BETA-ENDORPHIN SECRETION IN HYPOTHALAMIC CULTURES L.P. Kapcala and C.F. Weng* Dept. of Med., Univ. of Maryland, SOM Baltimore, MD 21201.

Hypothalamus is the major source of brain β-endorphin (β-E), a neuropeptide involved in the regulation of a variety of brain functions. Considering previous work showing sex steroid (estrogen, progestogen, androgen) alterations of levels of hypothalamic β-E and/or pro-opiomelanocortin (POMC-precursor of β-E) mRNA, it is believed that sex steroids modulate synthesis of POMC and secretion of its derivatives. Despite conflicting results regarding sex steroid effects on POMC mRNA, indirect studies have suggested that estrogen stimulates and progesterone inhibits hypothalamic β-E secretion. To address the issue directly, we tested the hypothesis that estrogen stimulates and progesterone inhibits hypothalamic β-E secretion. We studied β-E (RIA) secretion and concentration in dissociated fetal rat hypothalamic cells (11-20 days in culture) exposed to estradiol (E_2) and progesterone (P_4). Although basal β-E release was not altered by 48 hr pre-incubation with E_2 (10^{-12}-10^{-9}M), depolarization (60 mM KCl)-induced β-E secretion was potentiated variably, up to 2 fold over control. There was no potentiation of secretion following 24 hr E_2 pre-incubation. Low P_4 concentrations ($2x10^{-8}$M) did not alter E_2 (10^{-11}M) potentiated β-E release. However, high P_4 concentrations ($2x10^{-7}$M) significantly blunted the % increment of KCl-induced β-E release/basal release by 55% and 64% respectively vs that produced by pre-incubation with E_2 alone and E_2+P_4 (low dose). E_2 (10^{-11}, 10^{-9}M) treatment (48 hr) increased hypothalamic β-E concentrations in cells by 28 and 56% over that of control. These data are compatible with the hypotheses that E_2 potentiates stimulated hypothalamic β-E secretion by increasing the releasable β-E pool and that P_4 blunts E_2-potentiated release.

CONCLUSION: E_2 and P_4 modulate the secretion of hypothalamic β-E secretion in a counterregulatory fashion.

466.2

ONTOGENY OF MU OPIATE TOLERANCE: EFFECTS ON THE HYPOTHALAMIC PITUITARY-ADRENAL AXIS. P.J. Little and C.M. Kuhn. Dept. Pharmacol. Duke Medical Center, Durham, NC 27710.

Our laboratory has reported two disparate responses of developing rats to chronic opiate treatment. Tolerence has been demonstrated in both pups and adults for stimulation of corticosterone secretion by morphine. In contrast, no tolerance was observed in pups to the antinociceptive effects of morphine, while robust tolerance was demonstrated in weanlings. In order to help resolve these differences, dose-response curves for morphine-induced stimulation of ACTH were determined in developing rats following a chronic morphine treatment which did not cause tolerance to morphine antinociception. Rats were treated between days 4-8 (pups) or 21-25 (weanlings) with morphine (5-25 mg/kg, sc, b.i.d.)and 48 h later dose-response curves for morphine-induced stimulation of ACTH were determined. Morphine (1-10 mg/kg, sc) produced dose-dependent increases in serum ACTH in saline treated pups and weanlings. The dose-response curve for chronically treated morphine pups was similar to that of the saline treated pups. In contrast, there was a significant shift to the right of the morphine dose-response curve in weanlings treated chronically with morphine. This study supports the hypothesis that mu receptor are relatively refractory to the development of tolerance in the neonatal rat. Supported by DA 02739.

466.3

NEONATAL OPIATE ABSTINENCE IN THE ABSENCE OF TOLERANCE. R. T. Windh, P.J. Little and C. M. Kuhn. Dept. of Pharmacol. Duke Medical Center, Durham, NC 27710.

We have demonstrated that neonatal rats are more refractory to developing opiate tolerance to morphine analgesia than are weanling rats. Therefore, neonatal rats provide a useful model for addressing the question of the obligate association of opiate tolerance and abstinence. The following work was performed to determine whether the same chronic morphine regimen which did not cause neonatal tolerance to analgesia can result in abstinence. Rats were treated with 5-25 mg/kg morphine (2x/day, s.c.)on days 4-8 or 21-25, given naloxone 12 hours after the last morphine injection, and then scored for spontaneous behavior for 30 min. or killed for analysis of serum corticosterone. Weanling rats displayed an abstinence syndrome that consisted of ptosis, lacrimation, piloerection, abnormal posture, forepaw treading, and hostility on handling. Neonatal rats also showed an abstinence syndrome, characterized by abnormal posture, spontaneous vocalizations, locomotor hyperactivity, and tremor. Furthermore, morphine-treated pups and weanlings that received naloxone had significantly elevated corticosterone levels compared to animals that did not. Thus, abstinence is observed in pups which show no tolerance to morphine analgesia. Supported by DA 02739.

466.4

MU OPIOID AGONISTS STIMULATE GROWTH HORMONE SECRETION IN IMMATURE RATS. M. Eason and C. Kuhn. Dep't of Pharmacology, Duke Medical Center, Durham, NC 27710.

It has been shown that *d*-opioid receptors mediate the stimulation of growth hormone (GH) release in rats. However, in suckling rats, *d*-opioid receptors are not abundant, but opioid agonists still stimulate GH secretion. Therefore, we chose to investigate the possibility that *u*-opioid receptors are involved in GH release. In this study it was shown that *u*-opioid agonists stimulated GH release in twenty day-old rats. Sufentanil and [D-Ala^2, *N*-methyl-Phe^4, Gly^5-ol]enkephalin (DAMGO), selective *u*-opioid agonists, each stimulated GH release in a dose-dependent manner. [D-Pen^{2,5}]enkephalin, a *d* selective agonist, did not stimulate GH release when given in the same doses as DAMGO. Morphine (5 mg/kg), a non-selective opioid agonist, also stimulated GH release. Naloxone (1 mg/kg) completely blocked the GH response to morphine (5 mg/kg) and sufentanil (2.5 ug/kg). Beta-funaltrexamine (B-FNA, 10 mg/kg), an irreversible and selective *u*-opioid antagonist, diminished the GH response to morphine (5 mg/kg) and sufentanil (2.5 ug/kg). Naltrindole (NTI, 1 mg/kg), a relatively selective *d*-opioid antagonist, blocked neither the response to morphine nor sufentanil. These results suggest that *u*-opioid receptors are involved in the mediation of GH release in developing rats while *d*-opioid receptor-mediated GH secretion might play a more important role later in ontogeny. Supported by DA 02739.

466.5

SPINAL CORD OPIOID PEPTIDE LEVELS ARE ELEVATED DURING GESTATION. V.M. Medina* and A.R. Gintzler. Dept. of Biochem. SUNY Health Science Ctr., Brooklyn, NY. 11203.

In laboratory animals and humans, pregnancy is associated with an opioid receptor-mediated increase in the threshold for maternal responsiveness to aversive stimuli. Pharmacological and behavioral experiments indicate that, at least in part, this analgesia results from the activation of a spinal cord dynorphin/kappa opioid receptor system. We now demonstrate that during late pregnancy there is a significant elevation in the content of opioid peptides in specific regions of the rat spinal cord. Opioid content was assessed by RIA using antibodies selective for either dynorphin (1-17 or 1-8) or met-enkephalin. On day 22 of gestation, in 5 out of 5 rats, the content of dynorphin (1-17) and dynorphin (1-8) increased by 34 and 48%, respectively in the lumbar spinal cord ($p < .01$). In contrast, levels of both these opioid peptides did not show any consistent change in the cervical or thoracic spinal cord at this stage of pregnancy. Levels of met-enkephalin increased in the lumbar and thoracic cord (66% and 32%, respectively; $p<.05$) in 4 out of 5 pregnant (day 20) rats. In addition, there was a numeric, but not statistical, increase in met-enkephalin content in the cervical region. These results provide the first biochemical evidence that during gestation, there is a positive modulation of spinal cord opioid systems.

466.6

ENDOGENOUS OPIOID PEPTIDES (EOP) ARE INVOLVED IN THE SUCKLING INDUCED PROLACTIN INCREASE IN FEMALE RATS. P. Callahan, M. Stanik and J. Janik. Miami University, Oxford, OH 45056.

The purpose of these experiments was to identify the specific EOP involved in the suckling induced prolactin secretory response. Post-partum, lactating female Sprague-Dawley rats were used for all experiments. Animals were implanted with chronic intraventricular (ivt) cannulae into the lateral ventricle of the brain. Following a 5 - 7 day recovery period and one day prior to an experiment, each animal was implanted with a chronic jugular cannulae. On the day of the experiment, pups were removed from the dams. After 6 hours of separation, saline or antiserum to β-endorphin, met-enkephalin or leu-enkephalin (0.75 or 1.5 µg, ivt in 5ul) was administered. Immediately following administration of antiserum, dams were replaced with the pups and monitored for suckling response. Blood samples were withdrawn immediately prior to antiserum administration and 15, 30, 45 and 60 minutes after the onset of suckling.

Antiserum to leu-enkephalin most potently inhibited the suckling induced prolactin increase. This antiserum effectively blocked prolactin release at both concentrations administered. Neither β-endorphin nor met-enkephalin antiserum significantly attenuated the prolactin release at the two concentrations administered. However, a 10 fold increase in the dose of anti-β-endorphin totally abolished the suckling induced prolactin increase. These results clearly indicate that the EOP are involved in the suckling induced prolactin increase.

466.7

EFFECTS ON NEUROHYPOPHYSIAL HORMONE RELEASE, DIURESIS AND ELECTROLYTE OUTPUT OF μ- AND κ-OPIOIDS. B. van de Heijning & T.B. van Wimersma Greidanus*. Rudolf Magnus Institute, Dept. of Pharmacology, Utrecht, the Netherlands.

Opioids alter fluid metabolism and electrolyte balance. The mechanism of action is assumed to involve opioid modulation of neurohypophysial hormone output. The effects of the κ-agonist U69,593 and the μ-agonist DALDA on urinary output and renal electrolyte handling were investigated in normally-hydrated, non fluid-loaded, male rats. Using a protocol of hourly urine sampling up to 6 h, it was found that the s.c. applied κ-agonist (dose range 0.01-5 mg/kg) induced a prominent diuresis up to 3 h post injection. The diuretic effects of the μ-ligand DALDA (same dose range) were less pronounced. Both agonists had no effect on diuresis when given I.c.v. (0.2-10 µg/2µl). No major effects were found on renal sodium or potassium output of either drug administered by either route.

The μ-agonist DALDA (dose range 0.01-5 mg/kg) strongly inhibited the release of both vasopressin (VP) and oxytocin (OT), an effect that was maximal 30-60 min after s.c. injection. The same was found for the s.c. applied κ-agonist U-69,593. I.c.v. applied (0.5 and 5 µg/kg), only DALDA (μ) and not U69,593 (κ) suppressed plasma hormone levels 30 min after injection.

Thus, although both agonists suppress VP and OT release upon s.c. application, the μ-induced diuresis is only marginally compared to the κ-agonist. I.c.v. applied, the μ-agonist did not affect urination but inhibited VP and OT release. We conclude with respect to the data on suppressed VP and OT plasma levels, that the profound κ-induced diuresis cannot be explained entirely by an inhibition of neurohypophysial hormone output, and we suggest that additional mechanisms and sites of action (e.g. in the kidney cortex) are involved.

467.1

EXPRESSION AND PHARMACOLOGICAL CHARACTERIZATION OF A CANINE 5-HT_{1D} RECEPTOR SUBTYPE. J.M. Zgombick, R.L. Weinshank*, M.Macchi*, L.E. Schechter, T.A. Branchek, and P.R. Hartig. Neurogenetic Corporation, Paramus, N.J. 07652

RDC4, a guanine nucleotide-binding protein- (G protein) coupled receptor originally isolated in a canine thyroid cDNA library by Libert and colleagues, was identified as the canine homolog of the human 5-HT_{1D} receptor. Clone RDC4 is an intronless gene encoding a protein of 377 amino acids and containing three consensus sequences for N-linked glycosylation in its amino terminus. The deduced amino acid sequence of clone RDC4 exhibits greatest sequence identity (43%) with the 5-HT_{1A} receptor and lower overall homology to other serotonergic and catecholaminergic receptors. This sequence information suggested that clone RDC4 encoded a novel serotonergic receptor. In order to determine the pharmacological identity of clone RDC4, murine LM (tk$^-$) fibroblasts were stably transfected with the DNA encoding this gene and radioligand binding studies were conducted using [^{3}H]5-HT. Membranes prepared from stable transfectants displayed an apparently homogeneous population of high affinity (K_d = 3.6 nM), saturable (B_{max} = 275 fmol/mg protein) [^{3}H]5-HT binding sites. High affinity [^{3}H]5-HT binding was unchanged using assay conditions (1 μM pindolol and 1 μM SCH 23390) to pharmacologically mask 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{1C} receptors. Serotonergic ligands displaced specific [^{3}H]5-HT binding with a rank order of potency expected of a 5-HT_{1D} receptor subtype: 5CT > 5HT > Yohimbine > DPAT > Ketanserin = Spiperone > Zacopride. These binding properties are supportive of the designation of clone RDC4 as encoding a 5-HT_{1D} receptor subtype.

467.2

IDENTIFICATION OF A NOVEL G PROTEIN-COUPLED RECEPTOR WHICH EXHIBITS HIGH HOMOLOGY TO CLONED SEROTONIN RECEPTORS. Y. Shen, F.J. Monsma, Jr., C.R. Gerfen, L.C. Mahan*, P.A. Jose, M.M. Mouradian & D.R. Sibley. Experimental Therapeutics Branch, NINDS and Laboratory of Cell Biology, NIMH, NIH, Bethesda, MD 20892.

We have utilized the polymerase chain reaction (PCR) technique to selectively amplify G protein-coupled receptor cDNA sequences from rat kidney proximal convoluted tubule (PCT) mRNA. Poly (A)$^+$ RNA was used to synthesize cDNA by reverse transcription followed by PCR amplification using sets of highly degenerate primers derived from the transmembrane sequences of previously cloned dopaminergic, adrenergic, and serotoninergic receptors. This process resulted in the amplification of a number of discrete cDNA fragments. Sequence analysis of some of these fragments revealed several previously identified receptors as well as some putatively novel receptor cDNA sequences. One cDNA was identified which, when compared to all G protein linked receptors cloned thus far, appears to be most closely related to the serotonin receptor family. Within the transmembrane domains, the rank order of homology is 5HT_{1A}>5HT_{DRO}>5HT_{1D}>>5HT_2>5HT_{1C}. Northern blot analysis reveals a ~3.6 kb transcript with the following rank order of abundance in CNS tissues: hypothalamus>hippocampus=mesencephalon>olfactory bulb>cerebral cortex>olfactory tubercle>striatum. In peripheral tissues, this mRNA is most abundant in the spleen with lesser amounts seen in the kidney, ovary, lung and uterus. In contrast, this transcript is not detectable in the cerebellum, pituitary, retina, testes, stomach, prostate, skeletal muscle, liver, gut or heart. *In situ* hybridization analysis confirms the Northern blot data and also reveals a high level of transcript in the thalamic reticular nucleus. Full length cDNA clones have been isolated and are being expressed to establish the specific pharmacology and function of this novel receptor subtype.

467.3

5-HYDROXYTRYPTAMINE BLOCKS $GABA_B$ BUT NOT $GABA_A$ SYNAPTIC POTENTIALS IN DOPAMINE NEURONS. S.W. Johnson, N.B. Mercuri and R.A. North. Vollum Institute, O.H.S.U., Portland, OR 97201.

Intracellular recordings were made from presumed dopamine neurons in slices cut from the midbrain of the rat. Focal electrical stimulation produced a long-lasting hyperpolarization (1 - 1.5 s) which was blocked by the $GABA_B$-receptor antagonist 2-hydroxy-saclofen (300 μM). Serotonin (5-HT; 3 - 100 μM) reduced the amplitude of this synaptic potential 20 - 74% with an EC_{50} of 10 μM, but did not reduce the amplitude of synaptic potentials mediated by $GABA_A$ receptors. The effect of 5-HT was mimicked by the 5-HT_{1B} agonist 1-[3-(trifluoromethyl)-phenyl]-piperazine (300 nM), but not by the 5-HT_{1A} agonist N,N-dipropyl-5-carboxamidotryptamine (1 μM) or the 5-HT_2 agonist (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-amino-propane (10 μM). Cyano-pindolol (30 nM), a 5-HT_{1B} antagonist, blocked the effect of 5-HT. Hyperpolarizations produced by superfused GABA (1 mM) were not affected by 5-HT (30 μM), suggesting that 5-HT does not act post-synaptically. We conclude that 5-HT activates presynaptic 5-HT_{1B} receptors which inhibit the release of GABA onto $GABA_B$ but not $GABA_A$ receptors.

467.4

FUNCTIONAL SUBSENSITIVITY OF SEROTONIN RECEPTORS MEDIATING CLONIDINE-INDUCED INCREASES IN GROWTH HORMONE IN FAWN-HOODED RAT STRAIN RELATIVE TO WISTAR RAT STRAIN. C. S. Aulakh[a], J. L. Hill[b], C. C. Chiueh and D. L. Murphy[a] Lab. of Clinical Science, National Institute of Mental Health, Bethesda, MD 20892.

Brain serotonin changes have been implicated in the etiology of affective illness and the mode of action of antidepressant and antimanic drugs (Meltzer and Lowy, 1987). Various investigators have demonstrated altered responses to serotonin agonists in the Fawn-Hooded (FH) rat strain relative to Wistar and Sprague-Dawley rat strains (Gudelsky et al., 1985; Aulakh et al., 1988, 1989). In the present study, we investigated the effects of various serotonin receptor subtype selective antagonists (mesulergine, metergoline and others) on clonidine-induced hypoactivity and increases in growth hormone levels in male Wistar rats. In addition, we compared the effects of various doses of clonidine on growth hormone levels and locomotor activity in male FH and Wistar rats. Pretreatment with mesulergine and metergoline, did not affect clonidine-induced hypoactivity but completely blocked clonidine-induced increases in growth hormone levels. Furthermore, clonidine-induced increases in growth hormone levels were significantly less in the FH rats relative to Wistar rats. On the other hand, clonidine-induced hypoactivity was not significantly different between these two rat strains. These findings support other evidence indicating that FH rat strain may prove to be a useful genetic model for some brain disorders with possible abnormalities in serotonergic function such as depression, obsessive-compulsive disorder, eating disorders and other neuropsychiatric disorders.

467.5

ANALYSIS OF SEROTONIN BRAIN RECEPTORS IN FAWN-HOODED, SPRAGUE-DAWLEY AND WISTAR MALE RATS.

R.A. Hullihan-Gibilin, Y. Park*, C. S. Aulakh*, and D. Goldman. NIAAA/LCS Bethesda, Maryland 20892

Rats of the Fawn-Hooded (FH) strain exhibit a hemorrhagic disorder known as platelet storage pool deficiency. Behavioral studies have demonstrated that serotonergic function in the CNS is altered in this rat strain relative to Wistar and Sprague-Dawley (SD) animals. In addition, FH rats drink excessive amounts of EtOH and display symptoms related to an animal model of depression compared to control animals. In the present study, specific brain regions from FH, SD and Wistar rats were examined for differences in 5-HT brain receptors. [^{3}H]8-OH-DPAT was used to label 5-HT_{1a} receptor sites and the kD values for frontal cortex, hippocampus, striatum, hypothalamus and brainstem were similar in all three rat strains. However, the Bmax values for [^{3}H]8-OH-DPAT binding in the striatum of FH rats were significantly lower than those of the SD and Wistar rats. [^{3}H]8-OH-DPAT may be labelling presynaptic autoreceptors in the striatum. Preliminary results from 5-HT_2 receptor studies using [^{3}H]Ketanserin to label the binding sites indicate that there are no significant differences in kD values in these brain regions comparing the three strains, but the Bmax values for the cortex and striatum were significantly higher in FH compared to SD rats. Ongoing autoradiographic studies and comparison of mRNA levels will provide further information on these 5-HT receptor differences.

467.6

CHARACTERIZATION OF A 5-HT_4 RECEPTOR ANTAGONIST OF THE AZABICYCLOALKYL BENZIMIDAZOLONE CLASS: DAU 6285. H. Ladinsky[o], A. Dumuis[δ*], H. Gozlan[∞*], M. Sebben[δ*], M. Turconi[o*], C.A. Rizzi[o*], H. Ansanay[∞*], E. Giraldo[o*], P. Schiantarelli[o*] and J. Bockaert[δ*]. [o]Istituto De Angeli, Boehringer Ingelheim Italia 20139 Milan, Italy, [δ]Centre CNRS-INSERM 34094 Montepellier, France and [∞]INSERM 75634 Paris, France.

The benzimidazolone derivative, DAU 6285 (endo-6-methoxy-8-methyl-8-azabicyclo [3.2.1] oct-3-yl-2,3-dihydro-2-oxo-1H-benzimidazole-1-carboxylate HCl) was found to be 3-5 times more potent than ICS 205-930 in blocking adenylate cyclase activity in mouse embryo colliculi neurons stimulated by different chemical classes of 5-HT_4 agonists (5-HT, renzapride and BIMU 8), with respective Ki's of 220, 181 and 158 nM, as calculated from Schild plots. DAU 6285 showed poor activity on 5-HT_3 receptors with respect to ICS 205-930 as determined in vitro, in binding studies (Ki, 332 vs 2.8 nM) and in vivo, on the Bezold-Jarisch reflex (ID_{50}, 231 vs 0.5 μg/kg, i.v.). No significant binding of DAU 6285 to 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_{1D}, 5-HT_2, α_1, α_2, D_1, D_2, or M1-M3 receptor subtypes (Ki >10 μM) was found. The data show that DAU 6285 has somewhat higher affinity for 5-HT_4 receptors, and much lower affinity for 5-HT_3 receptors, than does ICS 205-930. The compound thus represents a new tool for investigating the 5-HT_4 receptors.

467.7

CALMODULIN ANTAGONISTS INHIBIT TRITIATED IMIPRAMINE BINDING TO THE SEROTONIN UPTAKE APPARATUS. D.M. Helmeste and S.W. Tang. Veterans Administration Medical Center, Long Beach, CA 90822. Dept. Psychiatry, University of California, Irvine CA 92715, USA.

In an effort to evaluate potential ligands for the tritiated imipramine (IMI) binding site, we recently observed that calmodulin antagonists inhibit ^{3}H-IMI binding to the 5HT uptake complex. It has previously been found that calmodulin antagonists may regulate uptake and release of precursors or neurotransmitter themselves. Specifically we found that calmodulin antagonists with naphthalenesulfonamide type structures inhibited ^{3}H-IMI binding to either calf cerebral cortex or human platelets with affinities equal to or greater than those reported for calmodulin itself. However, calmodulin antagonists calmidazolium and calmodulin binding domain did not inhibit ^{3}H-IMI binding. Binding inhibition was not affected by the presence or absence of EDTA or calcium.This is inconsistent with an action on calmodulin since these antagonists require calcium for their interaction with this protein. Scatchard analysis of the interaction of W-7 (N-6-Aminohexyl-5-chloro-1-naphthalenesulfonamide) with ^{3}H-IMI binding suggested direct competition rather than an allosteric interaction (as shown by increased Kd rather than Bmax decrease). These results suggest that naphthalenesulfonamide derivatives may directly interact with the ^{3}H-IMI binding site, rather than modulate its binding through calmodulin.

IC_{50} (Human Platelets, 2nM ^{3}H-IMI):

N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide (W-7) = 15μM; N-(6-aminohexyl)-1-naphthalene sulfonamide (W-5) = 75μM; N-(6-aminohexyl)-5-chloro-2-naphthalenesulfonamide = 18μM; N-(6-aminohexyl)-2-naphthalenesulfonamide = 40μM; N-(5-aminopentyl)-5-chloro-1-naphthalenesulfonamide = 5μM ; N-(5-aminopentyl)-1-naphthalenesulfonamide = 40μM; Calmidazolium (R24571) no activity at 1μM. Calmodulin binding domain (Calbiochem 208734), no activity at 100μM.

467.8

IDENTIFICATION OF A NOVEL PHOSPHOINOSITIDE-LINKED INDOLEAMINE BINDING SITE WITH [3H]NOSCAPINE. A. Khan, R.J. Mourey, T.M. Dawson and S.H. Snyder. Depts. of Neuroscience and Pharmacology, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Noscapine, a clinical antitussive phthalideisoquinoline alkaloid isolated from opium, has been used to identify a high affinity binding site in guinea pig brain (Karlsson, M.O. et. al. Eur. J. Pharm., 145:195, 1988). Based on the clinical specificity of noscapine, i.e., cough suppression while lacking analgesic, sedative, respiratory depressant, euphoric and addictive properties, we were interested in further characterizing the pharmacological and neurophysiological properties of the noscapine binding site.

Approximately 1200 drugs, encompassing most drug classes, were screened by a filtration binding assay for the ability to compete for [3H]Noscapine binding. Only drugs which contained an indoleamine or isoquinoline structure had affinity: the vasodilators, papaverine (13nM), hydrastine (32 nM); beta-carbolines (60nM-500nM), and serotonin analogs (100nM-10uM). GABA and serotonin receptor antagonists failed to compete. Structure-binding studies with beta-carboline and serotonin analogs were conducted to learn about chemical structure requirements for binding. The noscapine binding site was also shown to be brain-specific and insensitive to ions.

Using [3H]cytidine as a measure of phosphoinositide (PI) turnover (Hwang, P.M. et. al. Science, 249:802, 1990.), noscapine was shown to potently inhibit carbachol-stimulated PI turnover in guinea pig striatal slices. Noscapine by itself had no affect. More importantly, this effect displayed the proper pharmacology. Drugs which competed for [3H]noscapine binding also inhibited carbachol-stimulated PI turnover in the proper rank order. Drugs which resembled noscapine and papaverine, but were inactive at binding, were either inactive or weakly potent at inhibiting PI turnover.

467.9

EVIDENCE THAT THE SEROTONIN RELEASER P-CHLOROAMPETAMINE ELEVATES BLOOD PRESSURE, PLASMA RENIN AND PLASMA CORTICOSTERONE THROUGH CENTRAL MECHANISMS. Rittenhouse PA, Levy AD, Li Q, Yracheta JM, Kunimoto K, Van de Kar LD. Loyola University Chicago, Maywood, IL 60153.

PCA is a serotonin (5-HT) releaser that reliably increases both plasma corticosterone (CORT) and plasma renin concentration (PRC) when injected peripherally. The aim of these studies was to determine the role of central 5-HT receptors in mediating this hormonal response. PCA was injected intracerbroventricularly (ICV) in conscious male rats at doses (50, 500, 1000 μg/kg) below those peripherally effective. Trunk blood was collected 1 hour later. CORT was elevated in a dose dependent manner, while PRC did not change. Pretreatment with the 5-HT_2 antagonist LY53857 (20 μg/kg, ICV) did not affect either the CORT or PRC response. Since an increase in blood pressure (BP) due to PCA could inhibit the PRC response, femoral arterial catheters were implanted to record BP. We found that 1.0 mg/kg PCA significantly raised BP ($p<.01$) at 2 and 5 minutes after ICV injection, but not after peripheral intra-arterial injection. An additional experiment showed that a dose of 500 μg/kg PCA (ICV) significantly increased PRC ($p<.01$) if preceded by the α_1 antagonist prazosin (1.0 mg/kg, sc). Thus, by masking the cardiovascular effects with prazosin, PCA's stimulatory effect on renin was exposed. Finally, ibotenic acid lesions were made in the hypothalamic paraventricular nucleus (PVN). Ibotenic acid destroys cell bodies, but leaves fibers of passage intact. Two weeks after surgery rats were injected with 8 mg/kg PCA (ip). Rats with histologically verified PVN ibotenic acid lesions had significantly attenuated CORT ($p<.01$) and PRC ($p<.05$) responses compared to vehicle treated controls. Thus, cell bodies in the PVN mediate the CORT and renin response to PCA. Supported by AHA, Chicago.

467.10

IDENTIFICATION OF 5-HT RECEPTORS IN THE DOG DENTAL PULP. M. LIU*, S. KIM*, A.L. KIRCHGESSNER, P.R. WADE & M.D. GERSHON. Dept. of Endodont., Dept. of Anat. & Cell Biol., Columbia Univ., N.Y., N.Y.

5-Hydroxytryptamine (5-HT) causes vasoconstriction of dental pulp blood vessels (Kim et al. 1985) and excitation of intradental nerves (Olgart 1974). We tested the hypothesis that the dog dental pulp contains 5-HT receptors and 5-HT-containing nerve fibers. [^{3}H] 5-HT was used as a radioligand. Binding of [^{3}H] 5-HT to membranes derived from dental pulp was studied. An excess of 5-HT (10 μM) was used to define non-specific binding. 5-HT-containing nerve fibers were detected by immunocytochemistry. Saturable binding of [^{3}H] 5-HT was observed, which could best be described by a 2-site model (K_{d1} = 1.3 nM, B_{max1} = 162.5 fmol/mg protein; K_{d2} = 7.7 μM, B_{max2} = 23.5 nmol/mg protein). The binding of [^{3}H] 5-HT to dental pulp membranes was inhibited only by compounds, such as N-acetyl-hydroxytryptophyl-5-hydroxytryptophan amide (5-HTP-DP), that act at enteric 5-HT_{1P} receptors (Gershon et al. 1990). Radioautography revealed [^{3}H] 5-HT binding sites on intradental nerve bundles. Immunocytochemistry demonstrated 5-HT-immunoreactive (IR) non-varicose nerve bundles running throughout the dental pulp. 5-HT-IR fibers were less common than those which showed neuropeptide immunoreactivity (NYP, CGRP, SP). These preliminary results suggest that 5-HT_{1P} receptors play a role in the regulation of pain and blood flow in the dental pulp. Supported by NIDR DEO-5605 and NS 12969.

467.11

NOVEL HIGH AFFINITY ^{3}H-SEROTONIN BINDING SITES IN BRAIN TISSUE:EVIDENCE FOR FURTHER 5-HT_1 RECEPTOR SUB-TYPES. E. Weisberg and M. Teitler. Dept. Pharmacology and Toxicology, Albany Medical College, 47 New Scotland Avenue, Albany, New York 12208

We report herein that high affinity ^{3}H-5HT binding to homogenates of bovine and rat caudate and cortical tissues cannot be interpreted without including additional "5-HT_1-like" receptors other than the well-characterized $5HT_{1A}$, $5HT_{1B}$, $5HT_{1C}$ and $5HT_{1D}$ receptors. Competition studies were performed in rat and bovine frontal cortex and caudate tissues, using pharmacological blockade of $5HT_{1A}$, $5HT_{1B}$, and $5HT_{1C}$ receptors to facilitate examination of other possible sites. Our hypothesis was that if one other site exists in rat or bovine brain tissue, then the affinity of 5CT and/or ergotamine for that site should be the same in the four tissues studied. As shown in the table, the affinities of 5CT and ergotamine for the specific binding of ^{3}H-5HT, with $5HT_{1A}$, $5HT_{1B}$, and $5HT_{1C}$ receptors pharmacologically blocked (with pindolol and mesulergine) are different in each of the four tissues studied. Further data to be presented support the argument that there are additional "5-HT_1-like" binding sites in mammalian brain tissue and these sites may be heretofore uncharacterized, functional 5HT receptors.

DRUG	Rat cortex	Rat striatum	Calf cortex	Calf striatum
5CT	101±22 nM	82±5 nM	14±4 nM	0.8±0.1 nM
ergotamine	130±30 nM	51±5 nM	7±0.5 nM	5±0.8 nM

467.12

5-HT_1 RECEPTOR-MEDIATED INHIBITION OF PORCINE PIAL VENOUS TONE. M. Ueno, T.J-F. Lee. Dept. Pharmacol., Southern IL Univ. Sch. Med., P.O. Box 19230, Springfield, IL 62794-9230

We have demonstrated that 5-HT inhibits, via 5-HT_1-like receptors, the spontaneous rhythmic constriction (SRC) and KCL-or U46619-induced sustained active muscle tone (SAT) of porcine pial veins. In this study, we further characterized the 5-HT receptors mediating inhibition of SRC and KCL-or U46619-induced SAT of porcine pial vein, using an *in vitro* tissue bath technique. Changes in isometric vessel wall tension were measured. The results indicated that α-Me-5-HT_1 a 5-HT_2 agonist, induced a small enhancement of SRC and SAT. 2-Me-5-HT, a 5-HT_3 agonist, did not affect SRC and SAT. 8-OH-DPAT (a 5-HT_{1A} agonist), TFMPP (a 5-HT_{1B} agonist) and 5-methoxytryptamine (5-MT, a 5-HT_{1C} agonist) inhibited SRC and SAT in a dose-dependent manner. Based on the ED_{50} values, 5-MT is the most potent preferential 5-HT_1 agonist in inhibiting SRC and SAT. On the other hand, GR43175 (a 5-HT_{1D} agonist) did not affect SRC or SAT. Ketanserin, a 5-HT_2 antagonist, did not significantly affect the 5-MT inhibition of the SRC and SAT. Methysergide, a 5-$HT_{1\text{ and }2}$ antagonist, however, significantly blocked 5-MT induced inhibition of SRC and SAT. Results of this study indicate that multiple 5-HT_1 receptor subtypes are involved in 5-HT inhibition of SRC and SAT in porcine pial veins, although 5-HT_{1C} agonist appears to be most potent in inhibiting these contractions. (Supported by NIH HL27763, AHA/IHA and SIU-CRC)

467.13

CISAPRIDE EXCITES HIPPOCAMPAL PYRAMIDAL CELLS THROUGH 5-HT4-LIKE RECEPTORS. S.M.Roychowdhury* and E.G.Anderson. Dept. of Pharmacology, Univ. of Illinois at Chicago, Chicago, IL 60612.

5-HT typically inhibits then facilitates hippocampal pyramidal cell firing. The inhibition involves 5-HT1A receptors. The mechanism of facilitation remains uncertain. We examined the effects of 5-HT on hippocampal CA1 pyramidal neurons in slices incubated in a low calcium (0.1 mM)-high magnesium buffer (2.5 mM) buffer that blocked synaptic transmission and induced a slow, rhythmic bursting discharge (0.1-3 Hz), reflecting cell excitability. 5-HT commonly inhibited cell discharge, followed by off-stimulation upon washout. The inhibition was mimicked by 5-carboxamidotryptamine or 8-OH-dipropylaminotetralin and blocked by spiroxatrine, suggesting mediation by 5-HT1A receptors. The off-stimulation was blocked by 1-10μM of ICS 205-930. Alpha-methyl 5-HT, like 5-HT, produced inhibition followed by off-stimulation. The off-stimulation was blocked by 5 μM ICS 905-230. Cisapride, a 5-HT3 antagonist/5-HT4 agonist, stimulated pyramidal cell burst firing in a dose-dependent manner (10-100μM) which was blocked by 1-10 μM of ICS 205-930 but not by MDL 72222. The 5-HT3 agonist 2-methyl 5-HT had no effect on cell discharge. The phosphodiesterase inhibitor isobutyl methyl xanthine (IBMX) increased the cell firing rate at 10μM or more. Co-administration of subthreshold doses of IBMX (1μM) and cisapride (1μM) increased the excitation produced by 1 μM cisapride. We conclude that the 5-HT induced inhibition is mediated by 5-HT1A receptors while the off-stimulation appears to be mediated by 5-HT4 receptors.

467.14

AUTORADIOGRAPHIC LOCALIZATION OF SEROTONIN RECEPTORS IN THE RAT SPINAL CORD. K.B. Thor, S. Nickolaus*, M. Seggel and C. Helke. Lilly Research Laboratories, Indianapolis, IN 46285; USUHS and NIH, NINDS, LNP, Bethesda, MD.

Serotonin (5HT) is present in the spinal cord, where it exerts multiple effects. The present study describes the distribution of 5HT1A, 5HT1B, and 5HT1C/2 receptor subtypes using 2nM ^{3}H-8-OHDPAT or 100 pM ^{125}I-8-MeODPAT, 50 pM ^{125}I-cyanopindolol with 30 uM isoproterenol, and 200 pM ^{125}I-DOI, respectively, as ligands. 5HT1A and 5HT1B specific binding was determined by blockade with 1 μM 5HT, while 5HT1C/2 specific binding was determined by blockade with 1μM ketanserin. 5HT1A sites were located in laminae (L) I, II, and III, around the central canal (CC) and dorsal commissure gray (DCG), in sympathetic (SYM) and parasympathetic (PSYM) nuclei, and in the lateral subdivision of Onuf's nucleus. 5HT1B sites were preferentially located in L I and III (obviously less dense in L II), around the CC and DCG, in SYM and PSYM nuclei, and in the corticospinal tract. 5HT1C/2 sites were located throughout the gray matter, but were significantly more dense in the lateral motor nuclei and the intermediolateral portion of the SYM nuclei with greater densities at rostral thoracic levels than at lower thoracic and lumbar levels. PSYM nuclei showed no preferential localization of 5HT1C/2 sites. Differentiation of 5HT1C from 5HT2 sites is underway.

467.15

SEGMENTAL AND LAMINAR PATTERNS OF SEROTONIN BINDING IN CAT SPINAL CORD. L.M. Pubols, N.A. Bernau, L.A. Kane* and A.L. Burleigh. R.S. Dow Neurol. Sci. Inst., Good Samaritan Hosp. and Med. Ctr., Portland, OR 97209 and Dept. Physiol, OR Health Sci. Univ., Portland, OR.

Serotonin (5HT) binding sites in spinal cord segments C7-8, T8-9, L2, L6,and S1-2 were labelled for autoradiography with 2 nM [^{3}H]5HT, without and with 10 μM 5HT for total and nonspecific binding, respectively. Grain density was quantified using the video counting mode of a Bioquant image analysis system. Tritium standards were used to estimate the amount of binding. The level of specific binding in each region was classed as low (<46 fmol/mg), moderate (46-90 fmol/mg), or high (>90 fmol/mg).

All segments examined showed significant amounts of serotonin binding. In general, laminae V-VII showed low levels of specific binding, laminae IV, VIII, IX and X moderate levels, and laminae I-III high levels. As in the rat (Seybold and Elde, 1984), the thoracic intermediolateral cell column had a high level of specific binding. Clarke's nucleus in the thoracic and lumbar cord had moderate levels of binding. Overall, the segmental and laminar pattern of serotonin binding we observed in the cat is similar to the segmental and laminar pattern of serotonin immunoreactivity seen in rat spinal cord (Steinbusch, 1981).

(Support: NIH, NS19523)

467.16

REGULATION OF THE 5HT$_{1B}$ RECEPTOR IN OPOSSUM KIDNEY CELLS BY SEROTONIN. R.C. Pleus and D.B. Bylund. Dept. of Pharmacology, Univ. of Nebraska Med. Ctr., Omaha, NE 68198-6260.

The polyclonal cell line of the Opossum kidney (OK) is unique in that the only serotonin (5HT) receptor subtype it expresses is the 5HT$_{1B}$. In a time course experiment, OK cells were plated, 6 x 10^{6} cells per 150 mm culture disk and grown in serum substitute for 7 d. We observe a maximal decrease of ~39%, for 18-40 h of exposure, in 5HT$_{1B}$ receptors when cells are treated with 10 μM 5HT using [^{125}I]cynapindolol (10 to 1000 pM) as the radioligand. At 3 h of 5HT exposure, 5HT$_{1B}$ number decreased by 15%, whereas there was no down-regulation after 1 h of exposure to the same concentration. We tested the effect of 5HT exposure to the signal transduction system. The 5HT$_{1B}$ receptor is negatively coupled to adenylate cyclase through G$_{i}$ protein. We pretreated OK cells (plated at 6 x 10^{5}/35 mm culture dish, in serum substitute for 4 d) with 1 μM 5HT and [^{3}H]adenine followed by 3 m of 30 μM forskolin for cAMP stimulation. Data was expressed as % conversion of [^{3}H]cAMP/[^{3}H]ATP+[^{3}H]cAMP. The results demonstrate that 5HT for 1 h causes desensitization measured as a 15% decrease in efficacy at saturating concentrations of 5HT. Therefore, it appears that agonist exposure to 5HT$_{1B}$ receptor causes both down-regulation and desensitization. (NIH grant MH47354).

467.17

REGULATION OF 5-HT$_{1B}$ RECEPTORS IN OPOSSUM KIDNEY (OK) EPITHELIAL CELLS. C. D. Unsworth* and P. B. Molinoff. Dept. of Pharmacology, Univ. of Pennsylvania School of Medicine, Philadelphia, PA 19104.

Serotonin receptors present on membranes prepared from OK cells maintained in culture demonstrate pharmacological properties consistent with the 5-HT$_{1B}$ subtype, with high affinities for 5-HT and cyanopindolol and low affinities for other serotonergic compounds including 8-OH-DPAT, quipazine, and ketanserin. The density of receptors measured with ^{125}I-ICYP binding is approximately 40 fmoles/mg protein. Serotonin-mediated inhibition of forskolin-stimulated cAMP formation can be demonstrated in these cells with an IC$_{50}$ value of 3 nM and a maximal inhibition of 70-80%. Exposure of cells to 5-HT (0.1-10 μM) for a period of 16 hr led to a concentration-dependent decrease in receptor density, with a maximum decrease of 60-65%. Characterization of 5-HT-mediated inhibition of forskolin-stimulated adenylyl cyclase following exposure to 5-HT is complicated by a time- and concentration-dependent sensitization of the adenylyl cyclase system to forskolin. Exposure to 100 nM 5-HT for 16 hr did not result in a significant reduction of 5-HT$_{1B}$ receptor-mediated inhibition of cAMP formation despite a 40% decrease in receptor density. Exposure of cells to higher concentrations of 5-HT (1-10 μM) resulted in a progressive increase in the IC$_{50}$ for inhibition of cAMP formation together with a dose-dependent decrease in receptor density. A loss of receptors with no concomitant desensitization could be explained by the presence of "spare" receptors on these cells. This cell line represents a valuable model system in which the cellular mechanisms involved in the regulation of 5-HT$_{1B}$ receptor density and its coupling to adenylyl cyclase may be characterized. (Supported by USPHS grant MH 48125).

467.18

EFFECTS OF IBOTENATE, AMPA & 5,7-DHT LESIONS ON 5HT TRANSPORTER SITES IN RAT CORTEX AND HIPPOCAMPUS. I.A. Lawrence*, K. Shirakawa*, H.J. Olverman*, J.S. Kelly and S.P. Butcher*, University Department of Pharmacology, 1 George Square, Edinburgh, EH8 9JZ, Scotland.

Cholinergic neurones in the substantia innominata were destroyed by direct injection of either ibotenate or AMPA. After 7 days brain tissue was prepared for measurement of ChAT activity and ligand binding studies. [^{3}H]Citalopram was used to label the 5HT transporter sites. Similarly, serotonergic neurones were destroyed by intraventricular injection of 5,7-DHT. 14 days later, brain 5HT levels were measured and binding studies carried out.

ChAT activity in the cortex was reduced by 49.8% and 67.6% for ibotenate and AMPA lesions respectively but no significant changes were detected in density or affinity for the transporter site. While the destruction of serotonergic fibres reduced 5HT levels by a similar amount in cortex (77.8%) and hippocampus (83.4%) the reduction in the transporter site density in cortex (55.1%) and the hippocampus (> 95%) was significantly different ($P < 0.001$). These results support previous accounts of the localisation of 5HT transporter sites on serotonergic terminals, however, they raise the question of the heterogeneity of the 5HT innervation of the cortex as opposed to the hippocampus.

468.1

ACTIONS OF BUFOTENINE ON THE STORAGE AND RELEASE OF SEROTONIN (5HT) FROM RAT SPINAL CORD SYNAPTOSOMES. P.J. Monroe, D.L. Smith*, G.M. Williams, and D.J. Smith. Dept. of Anesthesiology, West Virginia University Health Sciences Center, Morgantown, WV 26506

The 5HT3 receptor agonist bufotenine was found to evoke a dose-dependant (100 nM - 10 μM) increase in [3H]-efflux from rat spinal cord synaptosomes which had been preloaded with [3H]-5HT. The effect of bufotenine on [3H]-efflux was not altered by the inclusion of ICS205-930 (a 5HT3 recetor antagonist) in, nor the removal of Ca^{++} from, the superfusion buffer. Furthermore, *in vivo* reserpine pretreatment did not alter the ability of bufotenine to evoke increases in [3H]-efflux until the tissue was almost totally depleted of 5HT stores. The ability of bufotenine to promote release was restored, however, with the addition of an inhibitor of monoamine oxidase.

Inhibition of 5HT uptake with fluoxetine blocked the bufotenine-induced increase in [3H]-5HT efflux, while having no effect on the increased [3H]-5HIAA efflux. Furthermore, those concentrations of bufotenine which were effective in promoting release were found to coincide with those that inhibited [3H]-5HT uptake (IC50 = 300 nM), and inhibited radioligand binding to the uptake carrier (Ki vs. [3H]-citalopram = 408 nM). Nevertheless, an interaction with the uptake carrier can not entirely explain the releasing action of bufotenine since other 5HT uptake inhibitors, including fluoxetine, do not promote release.

These data suggest that bufotenine is capable of releasing 5HT from a reserpine-resistant (presumably cytoplasmic) store. Furthermore, bufotenine apparently does not require the uptake carrier to enter the synaptosome since increases in [3H]-5HIAA efflux are observed in the presence of uptake inhibition. On the other hand, the sensitivity of bufotenine-induced 5HT efflux to uptake inhibiton suggests the carrier may be involved in the outward transport of the amine. It is somewhat paradoxical that bufotenine may use the uptake carrier as a mechanism to promote release, while also equipotently inhibit carrier-mediated transport into the synaptosome, however these actions are consistent with several other 5HT releasing agents, including parachloroamphetamine (Wong, Horng, and Fuller, Biochem. Pharmacol. 22:311-322,1972).

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468.2

BIOCHEMICAL EFFECTS OF EXPOSURE TO NITROUS OXIDE (N_2O) ON RAT BRAIN MONOAMINES. D. Emmanouil*, Z. Papadopoulou-Daifoti*, I. Kotsi* and R.M. Quock. Univ. Athens, Greece and Univ. Illinois Col. Med. at Rockford, IL 61107, U.S.A.

N_2O is reported to influence brain monoaminergic transmission (Passino et al., Soc. Neurosci. Abst. 13:913, 1987). To further explore possible effects of N_2O upon brain monoamines, male Sprague Dawley rats, 200 ± 20 g, were individually exposed to either 70% N_2O or room air for 15 min then sacrificed. The frontal cortex, striatum, hippocampus and hypothalamus were dissected for measurement of the monoamines dopamine and 5-hydroxytryptamine (5HT) and metabolites DOPAC, HVA and 5HIAA using HPLC with electrochemical detection. Our results show that N_2O exposure significantly raised levels of 5HIAA and increased the 5HIAA/5HT ratio in the hypothalamus but lowered the 5HIAA/5HT ratio in the frontal cortex due to elevated levels of 5HT. Recent work implicating an action of N_2O on benzodiazepine and perhaps GABA mechanisms (Emmanouil and Quock, IBRO 3rd World Congr. Abst., in press) suggests that the decreased 5HT turnover rate in the frontal cortex might be due to stimulation of GABA receptors that modulate 5HT release. The opposite effects of N_2O on hypothalamic 5HT transmission may also provide a basis for a distinct GABAergic or other system regulating 5HT turnover in that region. In conclusion, our data suggest adaptive changes of 5HT systems after N_2O exposure. (Supported by N.I.H. Grants DE-06894 and DE-09378.)

468.3

EARLY INDUCTION OF RAT BRAIN TRYPTOPHAN HYDROXYLASE (TPH) mRNA FOLLOWING PARACHLOROPHENYLALANINE (PCPA) TREATMENT. D.H. Park, D.M. Stone, H. Baker, K.S. Kim and T.H. Joh. Lab. Mol. Neurobiol., Cornell Univ. Med. Coll., Burke Med. Res. Inst., White Plains, NY 10605.

PCPA is a potent and selective inhibitor of TPH, the first and rate-limiting enzyme in the biosynthesis of serotonin. Thus, it might be a useful tool for studying the regulation of TPH gene expression. In the present study we assessed the time course and magnitude of the changes in TPH activity and mRNA as well as that of its product, serotonin (5-HT) in three brain regions, the dorsal raphe nucleus (DRN), hypothalamus (H) and caudal brainstem (CB). PCPA (300 mg/kg) was injected subcutaneously and rats were killed at 12 hrs, 1, 2, 4, 7, and 14 days post-treatment. TPH mRNA in the DRN, demonstrated by *in situ* hybridization, increased almost 2-fold by 1 day post-PCPA and returned to control levels by 4 days. In contrast, TPH activity in the DRN, H and CB was reduced to about 10% of control levels 1-2 days post-treatment returning to control levels by 2 weeks. The decrease and return of 5-HT immunostaining in fibers followed the same time-course as TPH activity. No effect was observed on aromatic-L-amino acid decarboxylase activity, immunoreactivity or mRNA levels. These data suggest that inhibition of TPH by PCPA-treatment results in the specific up-regulation of TPH mRNA expression and new enzyme synthesis.

Supported by MH44043.

468.4

PROTEIN KINASE A PHOSPHORYLATES BUT DOES NOT ACTIVATE RAT TRYPTOPHAN HYDROXYLASE. P.A. Johansen, M. Zhu*, K. Sankaran*, R.G.H. Cotton[1], and D.M. Kuhn. Lafayette Clinic & CCN Program, Wayne State Univ. Sch. of Med., Detroit, MI, and [1] The Murdoch Institute, Royal Children's Hosp., Melbourne, Australia.

The activity of tryptophan hydroxylase (TPH), the initial and rate-limiting enzyme in the biosynthesis of serotonin, is increased by Ca^{2+}/calmodulin-dependent protein kinase, presumably as a result of phosphorylation of TPH. Several studies suggest that cAMP also activates TPH, but a role for protein kinase A (PKA) in this process has not been established. Concentrations of cAMP, dibutyryl-cAMP and 8-thiomethyl-cAMP which activate endogenous PKA did not alter TPH activity in tegmentum, striatum or pineal gland, even when purified activator protein (protein 14-3-3) was added to the assay. Similarly, the catalytic subunit of PKA failed to activate TPH. To determine whether TPH is phosphorylated by PKA, crude extracts from rat striatum, tegmentum, and pineal were incubated with ^{32}P-ATP (2 μCi/tube). Proteins were subjected to SDS-PAGE and Western blotting, and autoradiograms were produced from the blots. TPH, identified on blots with a monoclonal antibody (PH8), was excised and it's ^{32}P content was determined by scintillation counting. The results clearly show that 20 μM cAMP or 1 μg purified PKA catalytic subunit increase the phosphorylation of TPH in each brain area. The cAMP-mediated phosphorylation of TPH was inhibited by 50 μM of the PKA inhibitor H-7. These results indicate that PKA phosphorylates TPH, however this phosphorylation does not appear to produce an increase in catalytic activity.

468.5

ORIGIN OF TRYPTOPHAN HYDROXYLASE-IMMUNOREACTIVE (TPOH-I) NERVE FIBERS IN RAT CEREBRAL BLOOD VESSELS. Z. Cohen[1,*], G. Bonvento[1,2*], P. Lacombe[1,2*], J. Seylaz[2*], E.T. MacKenzie[3] and E. Hamel[1]. [1]Montreal Neurological Institute, Montréal, H3A 2B4, Canada, [2]CNRS UA 641 75010 Paris, and [3]Cyceron, 14021 Caen, France.

Fibers immunoreactive for TPOH, the synthesizing enzyme for serotonin (5-HT), have been visualized around cerebral blood vessels (Neurosci. Lett., 1990, 116:269-274). In order to determine their origin(s), rats were subjected to bilateral removal of the superior cervical ganglion or to a neurotoxic lesion of brain 5-HT pathways (stereotaxic injection of 5,7-dihydroxytryptamine, J Neurochem, 1991, 56:681-689). Seven days later, major cerebral and small pial arteries were dissected free and processed for TPOH-immunoreactivity. Destruction of brain 5-HT ascending pathways did not significantly alter the distribution of TPOH-I fibers in major and small pial arteries as compared to controls (though efficacy of the lesion was confirmed by a considerable decrease in cortical TPOH-I terminals). Bilateral ganglionectomy markedly reduced TPOH-I fibers in all segments of the cerebrovascular bed; suggesting that cerebrovascular TPOH-I fibers are closely related to the sympathetic system. However, no TPOH-I cells could be clearly evidenced in rat superior cervical ganglion though in adjacent sections, cells were immunoreactive for dopamine ß-hydroxylase, the synthesizing enzyme for NA. Our failure to identify TPOH-I cells might suggest that a minute population of these cells exists in the ganglion and has eluded our detection, that the levels of TPOH within the cells are too low to be immuno-detected or, alternatively, that the cells of origin are located outside the core of the ganglion itself. Supported by MRC, QHSF and a France-Québec Scientific Collaboration.

468.6

MEASUREMENT OF EXTRACELLULAR SEROTONIN DURING SUCKLING-INDUCED PROLACTIN RELEASE J.J. Rutter*, M.J. Baumann, S.B. Auerbach, and J. Rabii. Dept. Biol. Sci., Rutgers Univ., Piscataway, NJ 08855.

A role for serotonin (5-HT) in CNS modulation of prolactin (PRL) surges is supported by physiological and pharmacological experiments. We have used microdialysis and peripheral blood sampling concurrently in unrestrained lactating rats to reexamine the possibility that 5-HT exerts a PRL releasing effect.

Dialysis probes were implanted in the anterior preoptic area (APOA) of lactating female rats. A 90 minute suckling bout, following 4 hours of pup separation, significantly increased PRL levels at 30 minutes to 215 ± 26 ng/ml (mean ± s.e.m., n=5) from a baseline of 6 ± 1 ng/ml, and levels remained elevated while the pups were present. In contrast, extracellular 5-HT release in the APOA was unchanged during suckling.

The APOA had been implicated as a PRL modulating site in earlier 5-HT turnover studies, so we verified probe placement in the hypothalamus by locally infusing the 5-HT releasing agent, fenfluramine (30 ug over 30 minutes). This resulted in a large increase in 5-HT (5337 ± 698 % of baseline) and PRL (118 ± 25 ng/ml from a baseline of 9 ± 1 ng/ml) at 30 min.

468.7

EXCITABILITY IS DECREASED DURING THE SEROTONIN-INDUCED SLOW DEPOLARIZATION OF MUDPUPPY CARDIAC NEURONS. R.L. Parsons, L.M. Konopka and J.C. Hardwick. Department of Anatomy and Neurobiology, University of Vermont College of Medicine, Burlington, VT 05405.

Many small intrinsic neurons in the mudpuppy cardiac ganglion exhibit 5-HT immunoreactivity. Local application of 5-HT produced a biphasic depolarization in approximately 50% of the mudpuppy parasympathetic postganglionic neurons responding to 5-HT. This biphasic 5-HT-induced depolarization was comprised of a fast, brief component followed by a slower, longer lasting period of depolarization. The fast depolarization was consistently accompanied by a decrease in membrane resistance (R_m). The slow 5-HT-induced depolarization was accompanied by an increase in R_m in 14 cells and a decrease in R_m in 12 cells. During the 5-HT-induced fast depolarization, action potentials often were initiated. In contrast, during the slow 5-HT-induced depolarization, no spiking occurred regardless of whether R_m decreased or increased. Application of long duration suprathreshold depolarizing current pulses to the parasympathetic neurons initiates multiple action potential activity. During the 5-HT-induced slow depolarization, fewer spikes were produced by the application of identical depolarizing current pulses. These results show that during the 5-HT-induced slow depolarization membrane excitability was depressed. Supported by NIH Grants NS-23978 and NS-25973.

468.8

INTRACEREBROVENTRICULAR 5,7-DIHYDROXYTRYPTAMINE INCREASES GFAP mRNA IN THE HIPPOCAMPUS BUT NOT IN THE STRIATUM OF RAT BRAIN:COMPARISON WITH d-FENFLURAMINE. C.Bendotti,S. Baldessari*,R. Rivolta*,R.Samanin*. Istituto"Mario Negri", Milan,Italy.

GFAP mRNA levels were measured by Northern blot analysis in different regions of rat brain to get information on potential neurotoxicity of drugs on central serotonin(5-HT) containing neurons. Intracerebroventricular administration of 5,7-dihydroxytryptamine(150µg/20µl)30 min after 25mg/kg intraperitoneally of desipramine,to protect noradrenergic nerve terminals,caused a significant increase of GFAP mRNA levels in the hippocampus but not in the striatum,5 days later. Intraperitoneal administration of d-fenfluramine (10 mg/kg b.i.d. for 4 days), a potential neurotoxic agent for 5-HT neurons, caused similar decreases of 5-HT in hippocampus (about 80%) and striatum (about 60%) but did not change GFAP mRNA levels in either region. Since 5-HT has been found to exert an inhibitory influence on GFAP synthesis in brainstem astrocytes culture (Le Prince et al.Dev. Brain Res. 1990,51,295), it is not clear whether the changes of GFAP mRNA levels were secondary to modifications in the serotonergic control of GFAP synthesis,to astrocytes hyperplasia or both.

468.9

D-LYSERGIC ACID DIETHYLAMIDE (LSD) POTENTLY ENHANCES A HYPERPOLARIZATION-ACTIVATED CATION CURRENT (I_h) IN FACIAL MOTONEURONS (FMNs).
J.C. Garratt[2] and G.K. Aghajanian[1,2]. Depts. of Pharmacology.[1] and Psychiatry[2], Yale University. New Haven CT. 06508.

The facial motor nucleus has a high density of binding sites and mRNA for 5-hydroxytryptamine$_2$ (5-HT$_2$) receptors. 5-HT has been shown to induce a slow depolarization/inward current in FMNs via 5-HT$_2$ receptors. In the present study, the effects of LSD on two components of the 5-HT response, a slow depolarization and an increase in a hyperpolarization-activated cation current (I_h), were examined in an *in vitro* slice preparation of rat facial motor nucleus by current clamp and voltage clamp methods. 5-HT increased the electrical excitability of FMNs and induced a slow depolarization. LSD (10-100nM) produced a slowly developing, sustained increase in the excitability of FMNs but, in contrast, produced little or no inward current and progressively blocked the depolarization produced by 5-HT.

Since LSD produced an increase in excitability without an inward current this led us to examine the effects of LSD as well as 5-HT on voltage-dependent currents. 5-HT was found to produce a small enhancement of I_h current in FMNs. Interestingly LSD (100nM) was more efficacious than 5-HT at increasing this current. The effects of LSD on I_h persisted over 1hr, with maximal effect seen after 20 mins. The reversal potential for I_h was -30mV, thus having the characteristics of a mixed Na^+/K^+ channel. The 5-HT$_2$ agonists, 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM) and (2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) also enhanced I_h to a degree intermediate between 5-HT and LSD.

Our findings suggest that LSD has dual actions in FMNs: (1) as a full agonist on an I_h current (2) as a low efficacy partial agonist on the 5-HT induced inward current.

468.10

SEROTONIN (5HT) EXCITES HYPOGLOSSAL MOTONEURONS IN DECEREBRATE CATS. H. Tojima*, L. Kubin, A.I. Pack* and R.O.Davies. Department of Animal Biology and Center for Sleep and Respiratory Neurobiology, University of Pennsylvania, Philadelphia, PA.19104.

There has been increased interest in the central mechanisms controlling the activity of hypoglossal (XII) motoneurons due to their importance in maintaining upper airway patency during sleep. A recent report showed that 5HT tonically depressed XII nerve activity in an isolated brainstem-spinal cord preparation from a newborn rat (Monteau et al. Neurosci.Lett. 111:127,1990). In contrast, it increased excitability of adult rat facial motoneurons both in vivo and in vitro (Rasmussen and Aghajanian, Synapse 5:324, 1990). The 5HT receptors that mediate these excitatory effects are present in both facial and XII motor nuclei (Pazos et al. Brain Res. 346:231, 1985). We assessed the effects mediated by 5HT receptors localized within the XII motor nucleus on XII motoneurons in decerebrate, paralyzed, vagotomized, artificially ventilated cats. 5HT or its antagonists were injected into one XII motor nucleus while monitoring the activity of both genioglossal branches of the XII nerve. The antagonists were: methysergide, ketanserin and LY53857 (1-2.5 mM in saline, 80-250 nl). All depressed XII nerve activity (20-90% of control). Injections of 5HT (10 mM, 100-300 nl) were excitatory. The 5HT-induced excitation was strong enough to overcome the XII motoneuron depression induced by injections of carbachol into the pons, a depression functionally similar to that occurring during the atonia of REM sleep (Kimura et al. J. Appl. Physiol. 60:2280, 1990). Thus, in the decerebrate cat, XII motoneurons receive a tonic excitatory drive mediated by serotonergic receptors located within the XII nucleus. These receptors are likely of type 1C or 2. Withdrawal of the serotonergic excitatory drive may underlie, at least in part, the depression of XII motoneuron activity during REM sleep. (Supported by HL42236.)

468.11

ELECTROPHYSIOLOGICAL AND BIOCHEMICAL STUDIES ON THE MECHANISM OF ACTION OF THE 5- HT$_2$ ANTAGONIST MDL 28,133 ON MDMA-INDUCED SLOWING OF A9 DOPAMINE (DA) NEURONS. Stephen M. Sorensen, Teresa M. Humphreys*, Vicki L. Taylor*, and Christopher J. Schmidt , Marion Merrell Dow Research Institute, Cincinnati, OH 45215

Recent clinical reports of the therapeutic efficacy of 5-HT$_2$ receptor antagonists in schizophrenia have renewed interest in the mechanism by which serotonin (5-HT) modulates dopaminergic neurotransmission.. Here we discuss electrophysiological and biochemical experiments using the 5-HT$_2$ receptor antagonist MDL 28,133 (1-4-fluorophenyl)-2[4-[(4-methanesulfonamido-phenyl)carbonyl]-1-piperidinyl]-ethanone) and the DA/5-HT releasing drug, methylenedioxymethamphetamine (MDMA) to further characterize the modulation of DA metabolism produced by 5-HT in A9 neurons. Extracellular recording was used to record from A9 DA neurons in chloral hydrate anesthetized rats. Following systemic MDMA (15 mg/kg i.v.) there was a marked slowing (80%) in the majority of DA neurons recorded. Pretreatment with MDL 28,133 (0.2 mg/kg i.v) blocked this slowing . Coadministration of L-DOPA (100 mg/kg i.v.) eliminated the blockade of MDMA-induced slowing by the 5-HT$_2$ antagonist so that MDMA again significantly slowed the firing rate of the A9 neurons. In biochemical studies, MDL 28,133 blocked the long-term serotonergic deficits produced by the administration of a high dose of MDMA. The ability of MDL 28,133 to prevent MDMA-induced neurotoxicity was lost in animals pretreated with L-DOPA. These results support the hypothesis that 5-HT acting through 5-HT$_2$ receptors may be permissive for the DA synthesis produced by DA releasing agents such as amphetamine and MDMA.

468.12

INVESTIGATIONS INTO THE SEROTONERGIC-DOPAMINERGIC INTERACTIONS MEDIATED BY 3,4-METHYLENEDIOXYMETHAMPHETAMINE (MDMA). C.J. Schmidt, G.M. Abbate*, C.K. Black* and V.L. Taylor*. Marion Merrell Dow Research Institute, Cincinnati, OH 45215.

We have examined several potential mechanisms through which 5-HT$_2$ receptors could regulate the effects of MDMA on the dopaminergic system. Administration of the selective 5-HT$_2$ receptor antagonist, MDL 28,133, had no effect on the accumulation of striatal dopamine (DA) produced acutely by gamma-butyrolactone (GBL), but did block the same effect when produced by MDMA. In animals treated with α-methyl-p-tyrosine, neither MDMA nor MDMA plus MDL 28,133 altered the rate of DA depletion. Pretreatment with MDL 28,133 was also without effect on the ability of apomorphine to reduce the accumulation of DOPA in GBL-treated rats. These results suggest that 1) the stimulation of DA synthesis produced by MDMA is not due to blockade of a tonically active, inhibitory 5-HT input, and 2) the effect of MDMA on DA cell firing is not mediated by 5-HT$_2$ receptor activation directly but requires release from the newly synthesized pool of DA. Furthermore, the sensitivity of the DA autoreceptors controlling striatal synthesis does not appear to be modified by MDL 28,133. We conclude that 5-HT$_2$ receptors play a direct role in the effects of MDMA on the dopaminergic system at the level of the MDMA releasable pool of DA.

468.13

EFFECTS OF NEONATAL REMOVAL OF SUPERIOR CERVICAL GANGLION ON 5-HT AND TRH IMMUNOREACTIVITY IN THE INTERMEDIOLATERAL CELL COLUMN OF THE RAT SPINAL CORD. *P. Poulat[1], L. Marlier[1], N. Rajaofetra[1], C. Oliver[2], A. Privat[1] and N. König[1].* [1] U.336 INSERM, USTL, pl. E. Bataillon, 34095 MONTPELLIER. [2]U.297 INSERM, Blvd P. Dramart, 13000 MARSEILLE, FRANCE.

The intermediolateral cell column (IML) contains serotonin (5-HT) and thyrotropin-releasing hormone (TRH) expressing fibers. Pups (1-3 days) were anaesthetized and the right SCG was removed. After various delays, the rats were sacrificed by intracardiac perfusion with 5% glutaraldehyde. Sections from C8-T4 levels of the spinal cord were processed for 5-HT and TRH immunocytochemistry using the PAP procedure. Image analysis revealed a significant 60% loss of 5-HT-immunoreactivity (IR) at C8-T1 levels on the lesioned side at all stages. At T2-T4 levels, this loss was 15% at 1 month and 30% at 3 months. For TRH-IR, there was a significant loss of 20% at 2 weeks, and 60% at 1 and 3 months, at C8-T1 levels on the lesioned side. At T2-T4 levels, a loss of 30% of TRH-IR appeared at 3 months. These results bring further evidence for 5-HT and TRH colocalisation in the same fibers in the IML. Neonatal removal of SCG presumably induces degeneration of a subset of sympathetic preganglionic neurons which in turn induces severe loss of 5-HT immunoreactive fibers innervating them. (supported by IRME, FRMF & HEUMANN).

468.14

SEROTONIN INHIBITION OF GLUTAMATE-EVOKED FIRING IS ATTENUATED IN THE NUCLEUS ACCUMBENS OF RATS FOLLOWING PRETREATMENT WITH P-CHLOROAMPHETAMINE. J. Smith, S.R. White and K. Paros*. Dept. of VCAPP, Washington State Univ., Pullman, WA 99164.

The amphetamine derivative, p-chloroamphetamine (PCA) is neurotoxic to fine caliber serotonin-containing axons that arise from the dorsal raphe nucleus (Mamounas and Molliver, *Exp. Neurol.* 1988, 102:23). The nucleus accumbens (NA), a major component of the brain reward pathway, receives part of its serotonin (5HT) innervation from the dorsal raphe nucleus. This study examined whether responses of nucleus accumbens cells to locally applied serotonin would be altered in rats that had been pretreated with neurotoxic doses of PCA. Male Sprague-Dawley rats received PCA (10 mg/kg, ip) on two successive days. Two weeks later the rats were anesthetized with urethane and prepared for single cell recording. 5HT applied by microiontophoresis (20-60 nA for 60 s) produced a significant decrease in glutamate-evoked firing of NA cells within 30 s of 5HT ejection onset in the control animals. This inhibition by 5HT was significantly depressed in PCA-pretreated rats compared to controls for all doses of 5HT that were tested. Thus, partial 5HT denervation by neurotoxic amphetamine derivatives alters responses of NA cells to subsequently applied 5HT, and presumably to 5HT that may be released from spared terminals.

468.15

RAPHE GRAFTS IN THE ENTORHINAL CORTEX OR IN THE HIPPOCAMPUS: EFFECTS ON HIPPOCAMPAL ELECTROPHYSIOLOGY AND BEHAVIOUR. G. Richter-Levin and M. Segal, The Weizmann Inst., Center for Neuroscience, Rehovot 76100, Israel.

The dentate gyrus (DG) and the entorhinal cortex (EC) are major targets of the midbrain raphe serotonergic fibers. Depletion of forebrain serotonin (5-HT) (by 5,7-DHT, icv) increased the responsiveness of the DG to perforant path (PP) stimulation and reduced its responsiveness to the serotonin releasing drug - fenfluramine (FFA). We compared 5-HT - depleted rats, grafted with embryonic raphe tissue into either the DG (DGG) or the EC (ECG). DGG rats responded to both PP stimulation and FFA application like controls, whereas ECG rats had a significantly different responsiveness profile. The differences between the two grafted groups are not due to the inability of raphe grafts to survive in the EC, since in all of the rats, examined with ^{3}H-imipramine binding, we detected grafted serotonergic terminals. When the septo-hippocampal connection is disrupted, depletion of forebrain 5-HT adversly affects the ability of rats to perform a spatial memory water-maze task. In rats that were injected with colchicine into the septum (1.5 ug/0.5 ul x2) together with 5,7-DHT, DGG but not ECG rats performed the task significantly better than double-lesioned rats that were not grafted. These results suggest that the serotonergic modulation of the DG responsiveness to afferent stimulation has a role in memory, the importance of which is revealed when other hippocampal modulatory systems are disrupted. *Supported by a grant from MINERVA foundation, Munich, Germany.

468.16

SEROTONERGIC DRUGS INCREASE MHPG-SO_4 IN RAT HYPOTHALAMUS. K. W. Perry* and R. W. Fuller, Lilly Research Laboratories, Eli Lilly & Co. Lilly Corporate Center, Indianapolis, IN 46285.

Previous neuroanatomic and functional evidence suggests that serotonergic input can modify noradrenergic neuron function. We have used concentrations of MHPG-SO_4 (3-methoxy-4-hydroxy-phenylglycol sulfate) as an index of norepinephrine release and metabolism in brain after administration of serotonergic drugs. Quipazine, a direct acting serotonin agonist, caused ~80% increase in MHPG-SO_4 in rat hypothalamus at 1 hour after a 2.5 mg/kg s.c. dose. 8-Hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), a selective 5-HT_{1A} agonist, increased MHPG-SO_4 ~50% 1 hr after a 0.3 mg/kg s.c. dose. The increases were dose-related for both drugs and occurred over the same dose range which caused a decrease in 5-HIAA (5-hydroxyindoleacetic acid), the metabolite of serotonin which decreases in response to direct or indirect acting serotonin agonists. The quipazine-induced increase in MHPG-SO_4 was antagonized by metergoline, and the 8-OH-DPAT-induced increase was antagonized by pindolol. The serotonin uptake inhibitor fluoxetine increased MHPG-SO_4 by 50-60% at 1-2 hrs after a 10 mg/kg i.p. dose. MHPG-SO_4 levels returned to normal within 6 hrs after fluoxetine although 5-HIAA levels remained decreased for 24 hrs. These findings extend earlier literature suggesting that modification of serotonergic input to noradrenergic neurons alters norepinephrine release.

468.17

REDUCTION BY SEROTONIN OF GABAERGIC SYNAPTIC TRANSMISSION IN HIPPOCAMPAL CA3 PYRAMIDAL CELLS IN VITRO. S. Oleskevich and J.-C. Lacaille. Centre de recherche en sciences neurologiques, Département de physiologie, Université de Montréal, Montréal (Qué.), Canada H3C 3J7.

The adult rat hippocampus receives a relatively dense and heterogeneous serotonin (5-HT) innervation. We have characterized electrophysiologically the effects of 5-HT on CA3 pyramidal cells and on GABAergic synaptic transmission in rat hippocampal slices. A microdrop of serotonin (500μM) was applied locally in stratum lucidum during intracellular recording from CA3 pyramidal cells. Several direct effects of 5-HT on pyramidal cells were obtained such as a membrane hyperpolarization, an increase in membrane conductance and a decrease in the slow afterhyperpolarization (sAHP) that follows a burst of action potentials (n=7 cells). These effects were similar to those previously shown for CA1 pyramidal cells. Inhibitory postsynaptic potentials (IPSPs) were elicited orthodromically in CA3 pyramidal cells by electrical stimulation of mossy fibers. These IPSPs were biphasic, consisting of an early $GABA_A$, Cl^--mediated component and a late $GABA_B$, K^+-mediated component. Both the early and late IPSPs were attenuated by serotonin (n=7 cells). Monosynaptic IPSPs were elicited by direct electrical stimulation of interneurons during bath application of the excitatory amino acid antagonists CNQX (20μM) and AP-5 (40μM). 5-HT caused a decrease in the amplitude of the early and late monosynaptic IPSPs (n=7 cells). The possiblity of a presynaptic effect of 5-HT on the glutaminergic afferents onto interneurons thus appears unlikely. Overall, the results suggest that 5-HT reduces GABAergic synaptic transmission in CA3 pyramidal cells. Studies of 5-HT actions on direct postsynaptic responses mediated by $GABA_A$ and $GABA_B$ receptors will be necessary to discriminate between pre- and postsynaptic sites of action of serotonin in the CA3 hippocampal region. (Supported by FRSQ and MRC).

468.18

AGING EFFECTS ON GABAERGIC AND SEROTONINERGIC SYSTEMS OF THE RAT SUPERIOR COLLICULUS. C. De la Roza[1*], V. Steffen[2*], A. Machado[2*], I. de Andres[1] and J. Cano[2*]. 1. Dep. Morfología, Fac. Medicina, Univ. Autónoma Madrid. 28029. 2. Dep. Bioquímica, Bromatología y Toxicología, Fac. Farmacia, Univ. Sevilla. 41012 Spain.

Biochemical (HPLC-UV detector) and immunocytochemical (HRP) techniques were used to study the GABAergic and serotoninergic neurotransmitter systems in the superior colliculus (SC) of adult and aged rats. GABA concentration was determined according to the slightly modified method of Maldonado et al. (1989) using a precolumn derivatization technique with Phenylisothyocyanate as the tagging reagent using Pico-tag and UV detection. To determine the cellular distribution of the neurotransmitters, 70 μm serial sections of brain tissue -fixed with a mixture of aldehydes- were processed for GABA or serotonin immunohistochemistry and were analized with light microscopy. GABA concentration did not show statistically significant changes in aging. However, since there was a significant decrease of the weight of the SC in aged rats, there is an age-related loss of GABA in the SC. This finding is consistent with a conspicuous decrease in the number of GABA immunoreactive neurons in aged rats. Likewise, serotonin immunoreactive fibers showed networks of remarkably swollen varicosities in aged rats, suggesting a possible axonal degeneration.

Supported by grants DGICYT PB 88/169 and PB 87/0929.

468.19

INTERACTIONS BETWEEN SEROTONIN (5HT) AND TRH ON SLOW K^+ CURRENTS OF HIPPOCAMPAL CA1 NEURONES. L. Ballerini*, R. Corradetti, A. Nistri[1] and A.M. Pugliese*. Pharmacol. Depts., Univ. Florence, Italy and [1] Queen Mary and Westfield College, London, U.K.

At spinal synapses 5HT and TRH act as co-transmitters by blocking K^+ currents. In rat hippocampal slices the effects of 5HT and TRH were studied using single electrode clamping of CA1 cells. At -60 mV holding potential (in the presence of 1 μM tetrodotoxin) 30 μM 5HT elicited an outward current (0.15 $\pm$ 0.03 nA) associated, in 50% of neurones, with a 27 $\pm$ 3% fall in input resistance. Depolarizing steps activated slow K^+ currents which, after leak subtraction, were found to be depressed (-35% at -20 mV) by 5HT. TRH (10 μM) had little effect on steady current or input resistance, but it reduced by 18% (at -20 mV) the slow K^+ current. Co-application of 5HT and TRH gave smaller (40%) reductions in this current than those predicted by the sum of the effects of the two drugs. The slow K^+ current remaining in Ca^{2+} free solution was identified as the delayed rectifier (I_K) which was attenuated by 9% and 26% in the presence of 5HT or TRH, respectively. Co-application of these agents did not change I_K. We suggest that since 5HT diminished the total slow K^+ current (mainly by blocking its Ca^{2+}-sensitive component) this action would reduce the efficacy of inhibitory K^+ conductance systems. TRH appeared to occlude the effect of 5HT on the slow K^+ current, suggesting a modulatory role of this peptide in some excitatory actions of 5HT present at depolarized membrane potentials.

468.20

SELECTIVE INCREASE IN EXTRACELLULAR SEROTONIN IN RAT MOTOR N. V DURING ORO-BUCCAL ACTIVITY. S.B. Auerbach, E. Auerbach* and B.L. Jacobs. Biol. Sciences, Rutgers Univ., New Brunswick, NJ 08855,

The activity of some mesencephalic serotonin (5-HT) neurons is selectively increased during jaw and tongue movements (Neuropsychopharm. 3:473-479, 1990). We have used in vivo microdialysis to determine if extracellular 5-HT in the rat CNS is also increased during feeding or grooming. Measurements were made in motor n. V (MoV) and striatum before and after grooming, or feeding and drinking. In the first sample collected after the end of a 24 hr fast, extracellular 5-HT in MoV was increased 47 $\pm$ 11% (n=5). Similarly, when grooming was induced by application of moistened soil to the body, 5-HT in MoV was increased 78 $\pm$ 37% (n=7). In contrast, striatal 5-HT was not consistently changed during periods of either feeding or grooming. Together with electrophysiological data, these results suggest that 5-HT in brainstem motor nuclei may be involved in facilitating repetitive movements. (Supported by NSF; SBA, and by AFOSR and NIMH; BLJ)

468.21

INCREASED 5-HT AND DOPAMINE RELEASE IN RAT LATERAL HYPOTHALAMUS AND N. ACCUMBENS FOLLOWING SYSTEMIC ETHANOL INJECTION. D. Benjamin, D.J. Knapp, E.R. Grant*, and L.A. Pohorecky. Rutgers University, Center of Alcohol Studies, Piscataway, NJ 08855-0969.

Ethanol (ET) has previously been shown to increase extracellular levels of serotonin (5-HT) and dopamine (DA) in the nucleus accumbens (NA). To determine if this effect is regionally specific, levels of these neurotransmitters and their metabolites were measured in the lateral hypothalamus (LH) and NA concurrently using intracerebral microdialysis followed by HPLC-EC, prior to and following intraperitoneal injection of ET. Male Wistar rats were prescreened for ET preference, then implanted with guide cannulae that terminated 0.5 mm above the NA and the LH. Following recovery from surgery, 2 mm dialysis probes were implanted and testing was initiated on the following day. Probes were perfused with pH 7.6 Ringer's solution at a rate of 1 uL/min, and dialysate samples were taken every 20 min. At least 3 baseline samples were taken prior to injection with vehicle or ET (0.5 or 1.0 g/kg). While vehicle injection had no substantial effect on the concentration of 5-HT or DA in either area, injection of ET resulted in dose-related increases in the concentration of 5-HT, DA, and 3,4-dihydroxybenzeneacetic acid (DOPAC) in dialysate. DOPAC and 5-HT increased before DA in both regions. Tenfold increases in 5-HT and DA were observed in both areas within 1 h of ET injection, and increases in the NA were preceded by increases in LH. These results demonstrate that ET injection increases 5-HT and DA release in LH as well as NA, and suggests that the primary site of action for ET effects on brain monoamines remains to be determined. (Supported by USPHS AA05306, AA08499, AA08206, and a Smithers Foundation Grant)

468.22

ELECTROCONVULSIVE SHOCK MODIFIES THE INHIBITORY EFFECT OF SELECTIVE μ and δ OPIOID AGONISTS ON [^{3}H]5HT RELEASE IN RAT HIPPOCAMPAL SLICES. F. Orzi,G. Lapucci and F.Passarelli - Neurosci.Dept.,University "La Sapienza" III Neuro-V.le dell'Universita' 30 00185 Rome (Italy)

It has been shown an involvement of opiate-receptors in the mechanism of action of electroconvulsive shock (ECS) (Belenky G.L. and Holaday J.W., 1979). We previously reported the inhibition of [^{3}H]5HT-release by a delta-selective agonist [D-Pen^2,D-Pen^5]enkephalin (DPDPE) and μ-selective agonist [D-Ala^2,N-methyl-Phe^4,Gly^5-ol]enkephalin (DAGO)(Passarelli and Costa, 1989). The aim of present study is to determine whether repeated ECS affects the modulation of 5HT release by both selective μ and δ opioid agonists in hippocampal slices. Male Sprague-Dawley rats submitted to repeated ECS, once a day for 7 days, were killed 24 hrs after the last of the 7 ECS sessions and the brain quickly dissected. Hippocampal slices (0.4 mm thickness) were incubated for 30 min at 37°C in Krebs' medium containing 0.1 μM [3H]5HT creatinin sulphate (s.a. 12.3 Ci/mmol) and bubbled with a mixture of 95% O2 and 5% CO2. The slices were then transferred to superfusion chambers and superfused with Krebs'medium. Each slice was stimulated twice at 60 min (S1) and 104 min (S2) after the onset of superfusion by exposure to a medium containing 30 mM of KCl for 4 min. The fractional release was expressed as the ratio S2/S1. The S2/S1 value was 1.00 ± 0.12 (n=5)in control animals, 0.94 ± 0.2 (n=4) in repeated ECS-treated animals and 0.94 ± 0.2 (n=3) in single ECS-treated animals respectively. After the addiction of DPDPE (1μM) and DAGO (0.1 μM) 20 min before the second stimulation, a clear reduction of K^+-evoked release of 5-HT was observed showing S2/S1 values of 0.45 ± 0.03 (n=3) and 0.44 ± 0.05 (n=5) respectively.ECS-treatment, by itself, did not alter the K^+-evoked release of 5-HT as reported before.In contrast repeated ECS treatment did reverse the inhibitory effect of both DPDPE and DAGO.The S2/S1 values in repeated ECS-treated animals were 1.08 ± 0.2 (n=3) in presence of DPDPE and 0.95 ± 0.16 (n=6) in presence of DAGO. The results suggest that repeated ECS treatment is
followed by opioid receptors desensitization.

SEROTONIN II

469.1

REGULATION OF 5-HT TRANSPORTER ACTIVITY BY PROTEIN KINASES. B. J. Hoffman, Laboratory of Cell Biology, NIMH, Bethesda, MD 20892.

Protein kinases are regulated by receptor activation via second messenger cascades. Phosphorylation by protein kinase C (PKC) and cAMP-dependent protein kinases have been shown either to enhance or to inhibit function of receptors and ion channels. The effects of signal transduction pathways on 5-HT transporter activity was investigated by analyzing ^{3}H-5-HT uptake into rat basophilic leukemia cells (RBL). Uptake was determined by incubation for 20 min in 0.2 uM ^{3}H-5-HT with or without 10 uM paroxetine. RBLs cultured in the presence of 10 uM cAMP analogues, 8-(4-chlorophenylthio)-cAMP or dibutyrylcAMP, for 16 hrs. exhibited a 225 +/- 11% increase in uptake compared to control. Under identical conditions, treatment of RBLs with 100 nM 12-myristate, 13-acetate phorbol (TPA) inhibited uptake by 53 +/- 3% relative to control. These data indicate that PKC and cAMP-dependent protein kinases have opposing effects on the 5-HT transporter. These results suggest that neurotransmitters and hormones may influence synaptic 5-HT levels not only by regulating release but also by modulating re-uptake.

469.2

Essential Sulfhydryl Groups in Neuronal Serotonin Transporter Function: Evidence for Differential Mechanisms in the Binding of Cocaine and Amphetamines to the Serotonin Transporter. W.A. Wolf* and D.M. Kuhn. Cellular and Clinical Neurobiology Program, Dept. of Psychiatry, Wayne State Univ., and Lab of Neurochemistry, Lafayette Clinic, Detroit, MI 48207.

The neuronal serotonin (5-HT) transporter, as studied by ^{3}H-paroxetine binding, was slowly inactivated by the sulfhydryl-selective alkylating agent, N-ethylmaleimide (NEM), in brain membrane preparations ($T_{1/2}$>120 min at 25°C using 3 mM NEM). ^{3}H-Paroxetine binding to digitonin-solubilised 5-HT transporter was more potently and more rapidly inactivated by NEM ($T_{1/2}$=12 min at 25°C using 3 mM NEM). Some ligands (5-HT, fluoxetine, citalopram, cocaine) which bind to the neuronal 5-HT transporter could protect against the inactivation of ^{3}H-paroxetine binding caused by NEM in digitonin-solubilised preparations while others (p-chloroamphetamine) could not. Hydrophobic sulfhydryl reagents (p-OHmercuribenzoate, dithiobis-[2-nitrobenzoic acid], N-phenylmaleimide, methyl methanethiosulfonate) were more potent than NEM at inactivating ^{3}H-paroxetine binding while the hydrophilic sulfhydryl reagents, iodoacetate and iodoacetamide, were completely ineffective in this regard. Finally, a distinct class of reduced sulfhydryls unassociated with the ligand binding site was found to be required for 5-HT translocation in nerve terminal membrane vesicles. ^{3}H-5-HT uptake in nerve terminal membrane vesicles (Km = 50 nM, Vmax = 0.14 pmoles/mg prot/sec) was rapidly inactivated by NEM (approximately 50% inhibition within 15 min incubation at 25°C with 1 mM NEM), although ^{3}H-paroxetine binding in these NEM-treated preparations was unaltered (98 $\pm$ 4% of control). Neither 5-HT (100 uM) nor cocaine (10 uM) could protect against the transport-inactivating effects of NEM.

469.3

AUTORADIOGRAPHIC COMPARISON OF IMIPRAMINE AND CITALOPRAM BINDING IN THE HUMAN HYPOTHALAMUS.

K.Y. Little, G.E. Duncan, Depts. of Psychiatry and Cell Biology and Anatomy, University of North Carolina, Chapel Hill, NC 27599.

^{3}H–imipramine binding in post mortem brain tissue has been considered a measure of serotonin uptake sites, and altered binding in suicides suggested as evidence of a serotonergic abnormality. In an initial evaluation of these assumptions, we compared the autoradiographic topography of ^{3}H–imipramine binding (5nM) in hypothalamus to that of ^{3}H–citalopram binding (2nM), a highly selective ligand for the serotonin transporter. Hypothalamic tissue was obtained at autopsy from acute trauma victims within 24 hours of death, as authorized by the Chief Medical Examiner. ^{3}H–imipramine binding in the hypothalamus was dense in the supraoptic and paraventricular nuclei, but was relatively low and diffuse elsewhere. In contrast, high densities of ^{3}H–citalopram binding sites were distinctly concentrated in the ventromedial, arcuate, and lateral nucleii. At these concentrations, highly selective hypothalamic binding of ^{3}H–imipramine and ^{3}H–citalopram occurs in distinct nucleii, demonstrating that ^{3}H–imipramine binding cannot be considered a specific label for serotonin terminals in human hypothalamus. Further, an autoradiographic study in rats found different binding patterns in the hypothalamus for both ligands compared to those in humans, suggesting that rat modeling of human serotonergic function may have limited validity.

469.4

PRESENCE OF ^{3}H-PAROXETINE BINDING SITES IN THE HYBRIDOMA CELL LINE NG 108-15. X. Zhang, J.E. Rubinstein and P.M. Whitaker-Azmitia. Dept. of Psychiatry, State Univeristy of New York, Stony Brook, New York, 11794.

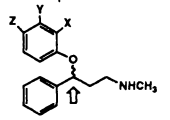
NG 108-15 cells are a hybridoma cell line of mixed glial-neuronal origin, which we are currently studying as a model for effects of serotonin receptors on the development of serotonin neurons. In the course of these studies, we tested for the presence of the serotonin transporter by using the radiolabelled specific uptake inhibitor ^{3}H-paroxetine.

Cells were grown in complete media in 25 cm^2 flasks for homogenate binding assays, or on two-well slides for autoradiography. In both systems, non-specific binding was determined by 100 nM fluoxetine. Saturation analysis (using 0.1 to 6.0 nM ^{3}H-paroxetine) indicated a single population of sites with a B_{max} of 85 fmoles/mg and a K_d of 1.2 nM. Preliminary autoradiographic analysis showed that the density of label was higher in a subpopulation of the cells.

469.5

IODINATED LIGANDS FOR SEROTONIN (5-HT) REUPTAKE SITES. H. Kung, S. Chumpradit, M.P. Kung, J. Billings, S.M. Tejani-Butt, A. Frazer. Dept's of Radiology & Psychiatry, U. of Pennsylvania, Phila., PA 19104.

Pharmacological actions of many antidepressants are based on blockage of presynaptic reuptake sites for 5-HT or norepinephrine (NE). In order to develop radioactive ligands for 5-HT reuptake sites, a series of iodinated ligands with high selectivity and affinity was developed.



	X	Y	Z
Tomoxetine(TMT)	CH_3	H	H
Nisoxetine	OCH_3	H	H
Fluoxetine	H	H	CF_3
4-Iodo-TMT	CH_3	H	I
3-Iodo-2-Demethyl-TMT	H	I	H

Optical isomers (R- and S-) of iodinated tomoxetine and its related derivatives were prepared. The affinity and selectivity of the synthesized compounds for 5-HT and NE uptake sites were determined in *in vitro* binding studies with [^{3}H]paroxetine (for 5-HT uptake sites) or [^{3}H]nisoxetine (for NE uptake sites]. S-3-iodo-2-demethyl-tomoxetine and R-4-iodo-tomoxetine display the highest affinity to the 5-HT uptake sites (Ki = 0.8 nM for both compounds for 5-HT sites versus Ki = 2 and 134 nM, respectively, for NE sites). Other pairs of isomers (R-3-iodo-2-demethyl-tomoxetine and S-4-iodo-tomoxetine) show lower affinity to both 5-HT and NE reuptake sites. The ^{125}I-labeled derivatives of these compounds have been prepared from the corresponding tributyl-tin derivatives. These ligands may be useful as potential tools for mapping serotonin neuron projections *in vivo* and *in vitro*. (USPHS grant support NS-24538 & MH 48125)

469.6

GINKGO BILOBA EXTRACT INCREASES AND PROLONGS THE SYNAPTOSOMAL ^{3}H SEROTONIN UPTAKE ACTIVITY.

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A Ginkgo biloba extract (EGb 761, 4 to 16 µg/ml) increased ^{3}H serotonin (^{3}H 5HT) uptake (≈ +25%) by synaptosomes incubated in pH 7.4 Krebs-Ringer buffer previously oxygenated (95% O_2, 5% CO_2). Such an increase was also obtained when mice were treated with EGb 761 (100 mg/kg, 16h and 2h) before sacrifice. This increase in ^{3}H 5HT uptake was not observed in the presence clomipramine 10^{-6} M (which abolished the 5HT uptake). EGb 761 (4 to 16 µg/ml) did not increase the ^{3}H dopamine uptake by synaptosomes prepared from mouse striatum. Synaptosomes lost their ^{3}H 5HT uptake ability after about 1h at 37°C. This decrease in uptake activity was prevented by EGb 761 (from 4 to 16 µg/ml). The prevention also concerned the ^{3}H dopamine uptake. The decrease in amine uptake induced by 1h exposition at 37°C was not reversed by EGb 761. We suggest that the oxygenated buffer could induce a lipoperoxydation of the synaptoplasmic membrane and modify the amine uptake system. EGb 761 could protect synaptosomes from alteration during the exposition at 37°C by its free radicals scavenger properties.

469.7

BEADED SEROTONERGIC AXONS ARE NOT TYPICALLY ASSOCIATED WITH CALBINDIN- OR PARVALBUMIN-POSITIVE NEURONS IN RAT BRAIN. K.J. Axt and M.E. Molliver. Dept. Neuroscience, The Johns Hopkins Univ. Schl. Med., Baltimore, MD 21205.

In cat and marmoset neocortex, and rat hippocampus, serotonergic (5-HT) axons have been shown to form basket-like terminals upon a subset of GABA-ergic neurons which express calbindin D_{28k} (CaBP). 5-HT axons do not appear to be associated with GABA-ergic neurons which express parvalbumin (PV). The 5-HT axons which surround CaBP-positive neurons have distinctive large, spherical varicosities (beaded axons). Two morphologic subtypes of 5-HT axons (fine and beaded) can be differentiated pharmacologically with the 5-HT neurotoxin p-chloroamphetamine (pCA), which selectively ablates fine 5-HT axons, while sparing beaded axons. In this study, the pCA-lesion model is used to reveal whether beaded 5-HT axons, which have a specific, limited distribution, are typically associated with CaBP- or PV-immunoreactive neurons (CaBP and PV antibodies were gifts of M.R. Celio and P. Emson).

We examined areas which are densely innervated by beaded 5-HT axons: cingulate cortex, lateral entorhinal area (LEA), septum, olfactory bulb, and hippocampus. Generally, in these areas no association is found between beaded 5-HT axons and CaBP- or PV-positive cell bodies. For example, in LEA, beaded 5-HT axons densely innervate layer III, whereas CaBP-positive cell bodies are located predominantly in layer II. In septum, 5-HT axons form baskets around neurons which are immunonegative for CaBP or PV. Occasionally, we observed beaded 5-HT axons around CaBP-positive cells in CA3 of hippocampus. In summary, in the rat, most CaBP- and PV-positive neurons are not innervated by beaded 5-HT axons, and most 5-HT beaded axons are not associated with CaBP- or PV-positive cell bodies; these 5-HT axons are likely to be associated with other types of neurons. [Support: NS 15199 and PMAF]

469.8

DUAL SEROTONERGIC PROJECTIONS TO RAT OLFACTORY BULB: p-CHLOROAMPHETAMINE (PCA) SELECTIVELY DAMAGES DORSAL RAPHE AXONS WHILE SPARING MEDIAN RAPHE AXONS.

M.E. Molliver and L.A. Mamounas. Dept. of Neuroscience, The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

The serotonergic (5-HT) innervation of the main olfactory bulb (MOB) in the rat arises from the dorsal (DR) and median raphe (MR) nuclei of the midbrain (McLean and Shipley, 1987). As in cerebral cortex, the MOB is innervated by two morphologically dissimilar types of 5-HT axons: fine axons with minute varicosities found primarily in the infraglomerular layers of the MOB and beaded axons with large, spherical varicosities located in the glomerular layer. In neocortex, fine 5-HT axons arising from the DR are selectively damaged by the amphetamine neurotoxin PCA, while beaded axons from the MR are spared (Mamounas and Molliver, 1988). The present study evaluates whether PCA is selectively toxic to DR axons in the MOB, while sparing MR axons in this area. We compared retrograde axonal transport of the fluorescent tracer Fluoro-Gold from the MOB to the raphe nuclei in PCA-treated (10 mg/kg) and control rats; 5-HT axons in the MOB that are ablated by PCA should not transport the tracer to their cell bodies of origin in the raphe nuclei, thus allowing us to specify the brainstem origins of the damaged axons. PCA caused extensive loss of fine 5-HT axons in the MOB, while beaded 5-HT axons were spared. Concomitant with the loss of fine axons, there was a large reduction (79%) in the number of retrogradely-labeled 5-HT neurons in the DR after treatment with PCA; in contrast, there was no reduction in the number of retrogradely-labeled 5-HT neurons in the MR. These results indicate that the DR projection to the olfactory bulb is selectively damaged by PCA, while the MR projection is preferentially spared. Moreover, the results suggest that fine 5-HT axons located in the infraglomerular layers of the MOB arise from the DR, while beaded axons in the glomerular layer arise from the MR. Support: NS-15199; NIDA 271-90-7408.

469.9

ABERRANT REINNERVATION OF RAT CEREBRAL CORTEX BY SEROTONERGIC AXONS AFTER DENERVATION BY p-CHLORO-AMPHETAMINE (PCA). L.A. Mamounas and M.E. Molliver. Dept. of Neuroscience, The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

The neurotoxic amphetamine PCA causes extensive degeneration of fine serotonergic (5-HT) axons in cerebral cortex, yet spares beaded 5-HT axons and most 5-HT cell bodies in the raphe nuclei. The present study employs 5-HT immunocytochemistry to evaluate regrowth of 5-HT axons into denervated regions at longer survival times after PCA administration. From one to six months after PCA administration, normal-appearing fine 5-HT axons slowly reinnervate cerebral cortex; beaded 5-HT axons, which survive drug treatment, do not sprout into zones denervated of fine axons. An increase in 5-HT axon density is first detected in frontal cortex at 1-2 months after PCA, and reinnervation extends to posterior regions of neocortex at 4-6 months; however, the density of fine 5-HT axons never reaches control levels in caudal areas of cortex. At survival times of 6-12 months after PCA, there is a subsequent decrease in the density of normal-appearing fine 5-HT axons in cortex concomitant with the emergence of structurally abnormal 5-HT axons which are unusually tortuous, thickened, and form numerous tangles. One year after PCA, these aberrant axon tangles form a large proportion of the 5-HT axon plexus in cortex. In age-matched control rats, aberrant axons of this type are not seen in young adults, yet they appear in increasing frequency, thickness, and tortuosity in aged rats (1-2 years of age); nonetheless, the overall 5-HT innervation density is not diminished in aged rats. These findings suggest that neurotoxic amphetamines administered to young rats may lead to initial regenerative sprouting followed by accelerated aging of 5-HT projections later in life. Thus, despite the early regenerative response of 5-HT neurons after PCA-induced injury, the normal 5-HT innervation pattern is not re-established. Support: NIDA DA-04431 & 271-90-7408.

469.10

FLUOXETINE ATTENUATES FENFLURAMINE INDUCED INCREASES IN EXTRACELLULAR SEROTONIN *IN VIVO* K.E. Sabol, I.B. Richards, L.S. Seiden. University of Chicago, Dept of Pharm./Phys. Sci.,Chicago, Il 60637

Fenfluramine is an amphetamine derivative that causes acute increases in extracellular serotonin *in vivo*, and long term decreases in serotonin tissue concentrations. Fluoxetine, a serotonin uptake inhibitor, protects against the long term decreases in serotonin concentrations caused by fenfluramine. The purpose of the present experiment was to determine whether fluoxetine prevents the acute increase in extracellular serotonin induced by fenfluramine. Rats were implanted with BAS 4 mm dialysis probes in the right hippocampus. Fourteen to eighteen hrs later, baseline extracellular serotonin was measured. Perfusion flow rate was 0.65 ul/min; samples were analyzed by HPLC-EC. After a minimum of six 20-min samples were collected, rats were given one of the following treatments: 12.5 mg/kg fenfluramine (FEN, N=4); 10.0 mg/kg fluoxetine (FLUOX, N=4); or 10.0 mg/kg fluoxetine followed by 12.5 mg/kg fenfluramine 20 mins later (FLUOX+FEN, N=4). Pre-drug baseline serotonin was 0.4±0.09 pg/10 ul for the FEN group; 0.4±0.08 pg/10 ul for the FLUOX group; and 0.3±0.08 pg/10 ul for the FLUOX+FEN group. Forty mins post injection, extracellular serotonin was 37.7±3.41 pg/10 ul for the FEN group; 1.9±0.51 pg/10 ul for the FLUOX group; and 6.5±1.18 pg/10 ul for the FLUOX+FEN group. These results demonstrate that fluoxetine substantially attenuates the increase in extracellular serotonin induced by fenfluramine. They are consistent with the view that the serotonergic effects of fenfluramine and fluoxetine are both mediated through the serotonin uptake carrier mechanism. (Supported by: NIDA DA-00085 and RSA-10562 L.Seiden.)

469.11

EFFECTS OF (+) MDMA ON MAO-B ACTIVITY IN RAT BRAIN STEM AND HIPPOCAMPUS. E.T. KOKOTOS LEONARDI AND E.C. AZMITIA. New York University, Dept. of Biology, NY,NY 10003

We have previously shown an inhibition of MAO-A activity with MDMA at an IC-50 of $5x10^{-5}$M in rat brain stem and hippocampus in both mature and immature rats (Kokotos and Azmitia, 1990). We also observed an increase in MAO-A activity at lower concentrations of the drug in two month old rat brain stem and hippocampus. As the primary monoamine oxidase present in serotonergic terminals is MAO-B, we sought to assay the effects of MDMA on MAO-B actvity in immature (one month old) rat brain stem and hippocampus as well as mature (two month old) rat brain stem and hippocampus. In brief, rat brain stems and hippocampi were dissected from one month- and two month old rats and homogenized 10x v/w in ice cold 0.01M PB for 10 strokes. The homogenates were centrifuged to obtain a mitochondrial-rich fraction. MDMA was added to homogenates at concentrations ranging from 10^{-4}M to 10^{-9}M. MAO-B activity was assayed by measuring 3H-DA degradation product by scintillation counting. An inhibition of MAO-B activity was observed in both age groups with an IC 50 of $5x10^{-5}$M. A stimulation of MAO-B activity was observed in one month old hippocampus in nanomolar concentrations ($p<0.001$). These results may explain some of the actions of MDMA on brain serotonergic function. Supported by NIDA contract 271-87-8144.

469.12

5-HT AND 5-HIAA LEVELS ARE INCREASED FOLLOWING MDMA IN RAT FETAL CULTURE MEDIA. X. F. Gu, AND E. C. Azmitia. Dept. of Biology, New York University, New York, NY 10003.

A method was developed to determine the levels of serotonin (5-HT) and its metabolite 5-hydroxyindoleacetic acid (5HIAA) in medium conditioned by cultured rat (Sprague-Dawley) fetal serotonergic neurons.

A raphe slice from 14-day-old fetal brain was dissected and dissociated. The cells were plated onto 96-well plates with an initial plating density of 0.9 x 10^6 cells/cm^2 in a volume of 200µl per well. Since there is a significant level of 5-HT in serum contained in complete neuron medium (CNM) which is necessary for the growth of fetal cells, a serum-free medium was required to detect the absolute level of 5-HT. Cells were cultured at 37°C for 1 day with CNM and then for 2 days with serum-free CNM. Medium (160µl) was then collected from each well and analyzed by reversed-phase high-performance liquid chromato-graphy with electrochemical detection. The mobile phase containing 60% acetonitrile was delivered at a flow rate of 1.0 ml/min onto a 10 cm x 3.2 mm column with ODS 3µm packing size. Samples (20µl) were automatically injected and analyzed by using a dual potentiostat electrochemical detector. The peak sizes were integrated using BASELINE 810 software. The lower limit of peak detection in this study was 0.2pmol for 5-HT and .1pmol for 5-HIAA per 20µl injection. This method was used to study the effects of 3,4-methylenedioxymethamphetamine (MDMA) on levels of 5-HT and 5-HIAA of fetal serotonergic neurons. There is no detectable level of 5-HT and 10^{-9}moles of 5-HIAA per well of control. After MDMA 10^{-5}M application, there is 10^{-7} moles of 5-HT and $5x10^{-9}$ moles of 5-HIAA. These results demonstrate that MDMA induces release of endogenous 5-HT from fetal serotonergic neurons. (NIDA contract, 271-87-8144)

469.13

SUBSTITUTED AMPHETAMINES CAUSE SEROTONIN RELEASE *IN VITRO* VIA A COMMON MECHANISM. U.V. Berger, X.F. Gu and E.C. Azmitia. Dept. of Biology, NYU, New York, NY 10003.

The neurotoxic amphetamine derivatives p-chloroamphetamine (PCA), 3,4-methylenedioxymethamphetamine (MDMA), methamphetamine (METH) and fenfluramine (FEN) cause release of serotonin (5-HT) from presynaptic nerve terminals. This release is primarily Ca-independent and blocked by 5-HT uptake inhibitors, suggesting that it proceeds via the uptake carrier. Yet, it is unknown whether this uptake carrier-mediated release is the same for all of these compounds. In this study, we tested the hypothesis that if these drugs had different mechanisms of causing release, then their effects would be additive when used in combination. Thus, we determined whether a combination of two drugs releases more 5-HT than either drug alone at an equivalent concentration. Drug-induced 5-HT release was studied using crude rat brain synaptosomes preloaded with ^{3}H-5-HT (100 nM). First, dose-response curves were determined, then two drugs were combined at their approximate EC_{50} concentration. EC_{50}'s for the different drugs ranged from 3 µM to 11 µm with PCA > FEN > MDMA > METH. The release caused by all four drugs was significantly attenuated by the 5-HT uptake blocker fluoxetine (80 nM). In no case did the combinations of two drugs at the EC_{50} exceed the releasing effect of the single drugs at the equivalent (i.e. $2xEC_{50}$) concentration. This lack of a synergistic effect of the drug combinations suggests that all of these amphetamine compounds compete for the same site(s) when causing release, implying that they act via a common mechanism. Currently, we are using the same experimental approach to determine whether these drugs share a common mechanism when causing toxicity to primary cultures of serotonergic neurons. Supported by NIDA contract 271-90-7403 and the Swiss National Foundation.

469.14

METHYLENEDIOXYAMPHETAMINE (MDA) DESTROYS 5-HT AXONS AND TERMINALS IN BRAINSTEM NUCLEI OF RAT. J.A. Harvey, P. Iannuzzelli, A.G. Romano and S.E. McMaster. Depts. Anatomy & Pharmacology, Medical College of Pennsylvania, Philadelphia, PA 19129.

Although MDA is well known to selectively destroy 5-HT axons and terminals in forebrain, its possible actions on brainstem have not been examined. Therefore, we have compared the effects of MDA on serotonergic projections in both forebrain and brainstem. Animals were injected twice a day, for four consecutive days, with MDA (40 mg/kg per day, s.c.) or saline. Brains were obtained two weeks after the last injection and processed for 5-HT immunocyto-chemistry. MDA produced a severe loss of 5-HT immunoreactive fibers and terminals throughout the trigeminal sensory nucleus, vestibular nucleus, and superior olivary nucleus of the brain stem which was comparable to effects in forebrain structures such as neocortex and hippocampus. Fluoxetine (10 mg/kg, injected i.p., 20 min prior to MDA) blocked all evidence of neurotoxicity in forebrain and brainstem. Our results indicate that the ablation of 5-HT axons and terminals produced by MDA is more extensive than previously reported. (Supported by NIDA Grant DA04944-04).

469.15

CELL BODY LOSS UNDERLIES PERSISTENT SEROTONERGIC DEFICITS INDUCED BY (±)3,4-METHYLENEDIOXYMETHAMPHETAMINE (MDMA) IN PRIMATES. G. A. Ricaurte, J. L. Katz, and G. Hatzidimitriou*. Department of Neurology, Francis Scott Key Med. Ctr., Johns Hopkins School of Medicine and NIDA Addiction Research Center, Baltimore, MD. 21224

This study examined the basis for the lasting serotonin (5-HT)-depleting effects of (±)3,4-methylenedioxymethamphetamine (MDMA) in the primate. Six adult squirrel monkeys (*Saimiri sciureus*) were used. Three animals were administered MDMA (5 mg/kg, s.c., twice daily for 4 days); three saline-treated animals served as controls. Eighteen months later, immunocytochemical studies were performed using an antibody directed at 5-HT. Sections through the forebrain of MDMA-treated animals showed a marked reduction in the number of 5-HT axons in cortical and subcortical regions. Counts of neurons in the dorsal, median and B9 raphe cell groups revealed a concomitant loss of 5-HT-immunoreactive cell bodies. Each of the raphe nuclei examined was comparably affected. These findings indicate that the toxic effects of MDMA in the primate are not limited to axons but involve neuronal perikarya as well. Further, they suggest that cell body loss forms the basis for the persistent 5-HT deficits induced by MDMA in nonhuman primates. Possible permanent effects in humans using MDMA ("Ecstasy") recreationally need to be considered. [USPHS DA05707]

469.16

3,4-METHYLENEDIOXYMETHAMPHETAMINE (MDMA) DOES NOT ALTER RAT PINEAL SEROTONIN CONTENT. T.H. Champney and R.T. Matthews. Dept. of Anatomy, College of Medicine, Texas A&M University, College Station, Texas 77843-1114.

The illicit drug, MDMA (ecstasy), produces a sustained depression in serotonin (5HT) content in many brain regions of rats. This depression is presumably produced by a neurotoxic effect of MDMA at the nerve terminal utilizing a presynaptic 5HT reuptake system. The pineal gland contains large quantities of intracellular cytosolic 5HT without nerve terminals or well characterized 5HT reuptake systems. By determining MDMA's effects on pineal 5HT content, the mechanism of MDMA's neurotoxicity can be investigated. Male albino rats have been injected with MDMA acutely (ip) using several dose and time response protocols or have received MDMA subchronically (sc) to determine if MDMA alters pineal 5HT content. A significant dose dependent decline ($p<0.05$) in 5HT content 2 h after MDMA administration (10 and 30 mg/kg) is observed in the frontal cortex, the hypothalamus and the striatum, but not in the brain stem or pineal gland. Likewise, the decline in 5HT content after MDMA (10 mg/kg) is maximal 2 h after injection in the frontal cortex (73%), the hypothalamus (48%) and the striatum (53%), but is unaffected in the brain stem or the pineal. Two weeks after 8 MDMA injections (12 h apart, 10 mg/kg/injection) or a single para-chloroamphetamine (pCA, 10 mg/kg, ip) injection, 5HT levels are significantly reduced ($p<0.05$) in the frontal cortex (38% and 88%), the hypothalamus (21% and 41%) and the striatum (22% and 84%). Brain stem 5HT content falls 60% after pCA, but is unaffected by MDMA. Pineal 5HT content is unaltered by MDMA or pCA administration. These results suggest that the pineal is resistant to the neurotoxic effects of MDMA or pCA. This resistance may be due to different membrane properties of pinealocytes when compared to serotonergic neurons or to the intracellular localization of 5HT in pinealocytes.

469.17

PRENATAL COCAINE AND FENFLURAMINE ADMINISTRATION INHIBIT SEROTONIN AND DOPAMINE FIBER OUTGROWTH.

H.M. Akbari, H.K. Kramer, and E.C. Azmitia.

Dept. of Biology, New York Univ., Washington Sq. East, NY, NY 10003.

Prenatal cocaine exposure has been found to disrupt the development of the serotonin (5-HT) and catecholamine systems as assessed by immunocytochemical methods (Akbari et al., 1990). In this study, we examined the effects of prenatal cocaine and fenfluramine exposure on the development of cortical 5-HT and dopamine (DA) innervation.

Cocaine (40mg/kg s.c.), fenfluramine (5mg/kg s.c.) or vehicle was administered to pregnant Sprague-Dawley rats from gestational day 13 to partuition. Cortical 5-HT and DA fiber densities were assessed on postnatal days (PND) 1, 7, and 28 using high affinity ^{3}H-5-HT and ^{3}H-DA uptake. Prenatal cocaine exposure resulted in a decrease in the 5-HT and DA high affinity uptake on PND 1 and PND 7. On PND 1, forebrain 5-HT uptake levels were decreased to 84% of control and DA levels to 75% of control. On PND 7, ^{3}H-5-HT cortical uptake was reduced to 64% and ^{3}H-DA to 51% of control. By PND 28, ^{3}H-5-HT uptake had returned to control levels. ^{3}H-DA uptake was increased to 135% of control in cocaine-treated animals by PND 28. A similar pattern was observed following gestational fenfluramine exposure. On PND 1 and 7, ^{3}H-5-HT uptake was reduced to 68% and 58% of control and ^{3}H-DA uptake to 73% and 50% of control. By PND 28, 5-HT and DA uptake had returned to control levels in the fenfluramine-treated group.

Our results indicate that both prenatal cocaine and prenatal fenfluramine disrupt the normal development of cortical 5-HT and DA fiber systems. Although the decreases are observed only in the early postnatal period, they may cause behavioral deficits in the adult by disrupting the normal "chemical imprinting" process. Supported by NIDA contract # 271-A7-A144.

469.18

EARLY POSTNATAL COCAINE EXPOSURE FAILS TO MODIFY CORTICAL SEROTONIN AND DOPAMINE INNERVATION.

H. K. Kramer, E. C. Azmitia, and H. M. Akbari.

Dept. of Biology, New York University, NY, NY 10003.

We have previously shown (Akbari et al., 1991) that prenatal exposure to cocaine decreases serotonin (5-HT) fiber density postnatally. These changes were attenuated by continued cocaine administration through the early postnatal period. This study examines the effects of early postnatal exposure to cocaine on the 5-HT and dopamine (DA) systems.

Newborn Sprague-Dawley pups were injected with either cocaine (10mg/kg s.c.) or vehicle on postnatal days (PND) 1-5. Cortical 5-HT and DA fiber densities were quantified on PND 7 and PND 28 with high affinity ^{3}H-5HT and ^{3}H-DA uptake. No significant differences were observed in DA uptake on either PND 7 or PND 28. No differences in 5-HT uptake were seen on PND 7; however, on PND 28 a 23% increase in ^{3}H-5-HT uptake was seen in the cocaine group. Although this result was not statistically significant, it raises the possibility that early postnatal cocaine treatment may stimulate 5-HT fiber outgrowth.

Our results indicate that postnatal cocaine administration in the rat does not cause developmental changes in the 5-HT and DA systems comparable to those seen following prenatal cocaine exposure. As with the adult rat brain, early postnatal cocaine exposure does not seem to significantly alter fiber density in these two systems. We are currently studying the effects of higher doses of cocaine (20 and 30mg/kg), and the noradrenergic system's contribution to this developmental question. Supported by NIDA contract # 271-A7-A144.

NEUROTRANSMITTERS: MOLECULAR NEUROBIOLOGY

470.1

NEURONAL GABA TRANSPORTER: IN VITRO PHARMACOLOGY AND DISTRIBUTION OF GENE EXPRESSION IN RAT AND HUMAN BRAIN. Y. Xia, C. Whitty, M. Poosch, G. Kapatos, M. Bannon. Dept. of Psychiatry, Wayne State University, Detroit, MI 48201.

The γ-aminobutyric acid (GABA) transporter is important in the regulation of GABAergic synaptic transmission. A rat neuronal GABA transporter clone (GAT-1; obtained from J. Guastella and colleagues) was calcium phosphate transfected into COS-7 cells. After 48 hrs, ^{3}H-GABA uptake was analyzed. The effects of various GABA transporter inhibitors were assessed. Nipecotic acid, a nonselective but potent inhibitor of GABA transporter and 2,4-diaminobutyric acid, which selectively inhibites the neuronal type GABA transporter, exhibited IC_{50}s of 10 μM and 300 μM, respectively. β-alanine and 4,5,6,7-tetrahydroisoxazolo[4,5-c]pyridin-3-ol, which are selective for the glial type of GABA transporter, inhibited GABA uptake by 10-20% at 1 mM. These results agree with the initial observations on this clone (Science 249:1303-1306, 1990) when expressed in Xenopus laevis oocytes. A BstXI-derived subclone corresponding to the 5' 333 bp of GAT-1 is being used for quantitation of GAT mRNA by nuclease protection assay. The GAT gene mRNA expressed in rat cortex, cerebellum, hippocampus, striatum, hypothalamus, and olfactory bulb are in the range of several pg/ng total RNA. Studies of the developmental profile of GAT gene expressed in these tissues are currently underway. A 276 bp human GAT-1 subclone corresponding to the 3rd transmembrane region and the 2nd external loop of the full length clone was obtained by PCR amplification using human putamen cDNA. This subclone is currently being used to quantitate the regional distribution of the GABA transporter in various human brain regions. Supported by: NIDA 06470.

470.2

DIVERSITY OF NEUROTRANSMITTER TRANSPORTER cDNAs IN BRAIN LIBRARIES. S. Shimada, S. Kitayama*, C.-Liang Lin*, A. Patel#, M. Kuhar#, P. Gregor and G.R. Uhl. Lab. Mol. Neurobiol., #Lab. Mol. Pharm., NIDA/ARC, Dept. of Neurol., Nsci., JHU Sch Med, Box 5180, Balto., MD 21224.

Recent cloning of cDNAs encoding GABA and norepinephrine transporters (GABAT and NET) reveals areas of high sequence homology. This family may also include the dopamine transporter that is a key molecular target for cocaine and MPTP, as well as the glutamate and serotonin transporters. We have used oligo-nucleotides corresponding to the 1st, 2nd, and 6th putative GABAT/NET transmembrane regions to clone PCR product cDNAs from brain libraries.

PCR product TM111 displays 52% amino acid identity with rat GABAT and 54% with NET; this also contrasts with a partial mouse GABAT cDNA which shows 95% identity with the rat sequence. Northern analyses show a 4 kb major band that hybridizes with TM111 and is present in mRNA from several brain regions but not from liver, pancreas or eye.

Screening a ventral midbrain library with oligo-nucleotide sequences conserved in GABAT, NET and TM111 reveals 0.005% positives; TM111 sequences are present in 0.001% of plaques from a whole brain library. Neuro-transmitter transporter cDNAs are thus individually rare, but members of a family of related genes with sequence conservation in putative transmembrane regions.

470.3

A cDNA ENCODING A NOVEL NEUROTRANSMITTER TRANSPORTER IS SELECTIVELY EXPRESSED IN SUBSTANTIA NIGRA. J. Kilty and S.G. Amara Section of Molecular Neurobiology, Howard Hughes Medical Institute, Yale University School of Medicine, New Haven , CT 06510

Degenerate oligonucleotides based on regions of amino acid sequence similarity between the cloned norepinephrine (NE) and GABA transporters were used in a polymerase chain reaction (PCR) with rat midbrain cDNA. The sequence of one of the cDNAs amplified in this reaction diverges significantly from the NE and GABA transporter cDNAs, but predicts a protein with extensive amino acid identity with the NE transporter. When used as a hybridization probe this 684 basepair PCR product detects a single 4.4 kilobase mRNA on Northern blots. On blots containing RNA isolated from a variety of specific brain regions, the intensity of this band was strongest in the substantia nigra, with faint signals in the brainstem and olfactory bulb. No hybridizing band could be seen in RNA from cortex, cerebellum, basal ganglia, kidney, lung, adrenal, or PC12 cells. The predicted sequence and tissue distribution of this mRNA identifies this clone as a likely candidate for a dopamine transporter.

470.4

ISOLATION OF NEW MEMBERS OF A TRANSPORTER GENE FAMILY BY SEQUENCE HOMOLOGY BASED PCR. J. A. Clark, A. A. Fluet, and S. G. Amara. Department of Molecular Neurobiology, HHMI, Yale University School of Medicine, New Haven, CT 06510.

Comparison of the amino acid sequences of clones encoding a GABA transporter from rat brain and a norepinephrine transporter from human neuroblastoma cells has led to the identification of regions of sequence with significant homology. Degenerate oligonucleotides were designed based on three of these conserved regions. Using the polymerase chain reaction along with these oligos we have isolated several novel DNA fragments from midbrain, spinal cord, C6 neuroblastoma cell, and retinoblastoma Y79 cell cDNA's. Sequencing these cloned PCR fragments has revealed a family of genes which are related to both the GABA and norepinephrine clones, but are clearly new members of this sodium-dependent transport family. Northern analysis has shown that each of these potential transporters has its own unique distribution throughout the rat CNS and peripheral tissues. Several of these fragments are presently being cloned in order to obtain full length sequences and allow complete characterization of these transport proteins.

470.5

UPTAKE, TRANSPORT AND TRANSLATION OF EXOGENOUS VASOPRESSIN (AVP) mRNA IN BRATTLEBORO RAT HYPOTHALAMO-NEUROHYPOPHYSIAL SYSTEM

G. F. Jirikowski, P. P. Sanna, D. Maciejewski-Lenoir* and F. E. Bloom. Dept. of Neuropharmacology, Scripps Clinic and Res. Fdn., La Jolla, CA 92037.

Following our recent detection of oxytocin mRNA in hypothalamic axons by in situ hybridization (Jirikowski et al, 1990) we have extended our studies to AVP mRNA, and have employed Brattleboro rats, genetically incapable of AVP expression, as a means to detect the metabolic fate of exogenous AVP mRNA injected intracerebrally. RNA extracted from hypothalami of osmotically challenged rats as well as synthetic riboprobes coding for the Arg-vasopressin (AVP) precursor were injected into the hypothalamo-neurohypophysial tract of homozygous Brattleboro rats. A radiolabelled riboprobe coding for AVP was found to be selectively accumulated and transported in axons to magnocellular perikarya within 2 h, while the antisense sequence failed to show cellular accumulation. Brattleboro rats injected with either the natural RNA preparations or with the synthetic sense probe showed immunoreactive AVP in magnocellular neurons and their distal axons by 18h and a temporary (1-5 days) significant increase in urine osmolarity. Our results suggest that magnocellular hypothalamic neurons are capable of uptake, axonal transport and translation of certain natural and synthetic mRNA sequences. This may reflect a novel means of interneuronal communication generally. Supported by grants AA06420 and NS22347. GFJ is a Heisenberg Fellow.

470.6

IMPORTANCE OF POLY-A TAILS FOR EXPRESSION OF EXOGENOUS RNA IN BRATTLEBORO RAT HYPOTHALAMO-NEUROHYPOPHYSIAL SYSTEM

D. Maciejewski-Lenoir*, P.-P. Sanna, G. F. Jirikowski, F.E. Bloom
Dept Neuropharm, Scripps Clinic & Research Fdn., La Jolla, CA 92037

Poly A(+) and poly A(-)RNA fractions, obtained from the hypothalami of osmotically challenged Lewis rats as well as cRNA coding for Arg-Vasopressin (AVP) were injected unilaterally into the hypothalamo-neurohypophysial tract of young homozygous Brattleboro rats. After 2 hours survival, the magnocellular hypothalamic nuclei of animals injected with 1 µg poly A(-) RNA contained Arg-Vasopressin immunostaining and immunoreactive peptide, ipsilateral to the injection site. After 18 hours, the Brattleboro rats injected with poly A(-) contained immunoreactive AVP in the median eminence and in the posterior pituitary lobe. Plasma AVP levels and urine osmolarity of animals injected with poly A(-) and synthetic AVP RNA were significantly increased within 120 minutes and remained elevated for up to 6 days after the injection. Similar effects could be observed with 50 ng of AVP-cRNA. The injection of 1µg poly A(+) RNA proved to be less effective, despite the presence of much more mRNA coding for AVP. To assess further the importance of the poly A tail in this process, we annealed the poly A(+) fraction with synthetic poly T tails and then treated with RNAse H in order to remove partially the poly A tails. The detailed poly A(+) fraction also significantly increased the urine osmolarity of injected Brattleboro rats, suggesting that the absence of poly A tail is of functional importance for the uptake, transport and translation of RNA by magnocellular hypothalamic neurons. Supported by grants AA06420 and NS22347.

470.7

REGULATION OF GAD PROTEIN LEVELS AND GENE EXPRESSION IN RAT CORTEX IN VITRO AND IN VIVO. K. Rimvall and D.L. Martin. Wadsworth Center for Labs. and Res., NY State Dept. Health, Albany, NY 12201.

We have previously shown that the two GAD proteins, GAD65 and GAD67, are present in cultures of prenatal rat cortex enriched in GABAergic neurons. Total GAD activity was reduced by 60% in cultures and 30% in vivo when intracellular GABA levels were increased >300% by chronic treatment with the GABA-transaminase inhibitor γ-vinylGABA (GVG). Levels of other amino acids (glutamate, glutamine, aspartate and alanine) changed only slightly. In cultures, the effects of GVG were mimicked by adding GABA to the medium, indicating a negative feedback mechanism. Immunoblotting experiments showed that the level of GAD67 protein was reduced by 76% in cultures and 79% in vivo, but the level of GAD65 protein was unchanged by GVG treatment. The possibility that the decrease in GAD67 is due to selective changes in gene expression was investigated. Total RNA and mRNA were extracted from rat brains and cultured neurons. Northern blotting with ^{32}P-labeled cDNA's from the genes coding for GAD65 and GAD67 (Erlander et al., Neuron, in press), showed that the cerebellum and cortex of adult rats and cultured GABAergic cells contain a 3.7 kb GAD67-mRNA. The effect of manipulating GABA levels on the levels and stability of GAD mRNA in cultures and in vivo are under study. Supported by Grants MH35664 and NS853102 from USPHS-DHH.

471.1

S-ANTIGEN (ARRESTIN) FAMILY: EXPRESSION AND IDENTIFICATION OF MULTIPLE FORMS IN RAT AND BOVINE TISSUES WITH SYMPATHETIC INNERVATION. C.M. Craft, D.H. Whitmore*#, R. Gonzalez*, T.H. Thai*. Lab. Mol. Neurogen., Dept. of Psychiatry, UT Southwestern Med. Sch., and VA Med. Ctr., Dallas. TX, 75235 and #Dept. of Biology, Univ. Texas, Arlington, TX.

Specific desensitization of activated receptors is mediated through kinases (K) and other cofactors to impair the capacity to activate guanine nucleotide binding proteins (Gs, Gi, Go) in the signal transduction cascade. In the retina, S-antigen (SAG) enhances inhibition of light-activated rhodopsin (R*) after phosphorylation by RK. In neuronal tissues with adrenergic receptors, an analogous protein, beta-arrestin (BAR), participates with BARK in homologous desensitization of B-adrenergic receptors (Lohse et al., 1991, Science 248:1547). Expression of mRNAs and proteins encoding SAG and BAR are detectable in mammalian retina and pineal gland (Craft et al., 1991, J. Neurochem. 55:1461). Antigenic epitopes are shared and highly conserved between members of the arrestin family; however, multiple genes encode the different members. With labeled polymerase chain reaction (PCR) and specific primers designed for regions for either SAG or BAR, we examined mRNA distribution. Total RNA was isolated from rat or bovine tissues and first strand cDNA synthesized with reverse transcriptase. We sequenced gel-purified PCR fragments with either the primers used for amplification or internal, shared primers. The labeled PCR products were further analyzed with restriction endonucleases that cut either SAG (Sst 1/ Rsa1) or BAR (Sau1/ Hpa II). SAG was detected in rat pineal, retina, Hardarian gland, and ciliary processes but none in heart, cerebrum, cerebellum or adrenal. BAR was expressed in all of these. Several PCR products analyzed from the pineal, cerebellum and adrenal were neither SAG or BAR; however, each had conserved arrestin-like domains, suggesting alternative arrestins for specific G-protein coupled receptors. Grant support: NINDS/NIH and VA Med.

471.2

MECHANISM OF RECEPTOR DYSFUNCTIONS IN HYPERREACTIVE AIRWAYS

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Hyperreactive airways (Asthma) have been proposed to be a disease of hypermuscarinic and hypo-ß-adrenergic functions. In order to study its mechanism, we employed an animal model of guinea pigs sensitized with ovalbumin and subsequently challenged by a single and multiple exposures to the antigen. When their tracheal responses to acetylcholine and isoproterenol were measured in vitro, the tracheas isolated after multiple challenges were more sensitive to acetylcholine but less sensitive to isoproterenol. However, the binding studies with [^{3}H]QNB and [^{125}I]CYI showed no changes in the number and affinity of M_3-muscarinic and β_2-adrenergic receptors. These observations suggest that inflammatory reactions occurring in the lungs modify the postreceptor signal transduction steps, possibly G proteins.

471.3

AUTORADIOGRAPHIC LOCALIZATION OF ALPHA 1 ADRENOCEPTORS ALONG THE RAT SPINAL CORD AS REVEALED BY [^{3}H]PRAZOSIN : EFFECT OF NORADRENERGIC DENERVATION. C. Roudet, M. Savasta, and C. Feuerstein. INSERM-LAPSEN U.318, Pavillon de Neurologie, CHU de Grenoble, BP 217, 38043 GRENOBLE cedex 9, France.

The distribution of α_1 adrenoceptors along the different segments of spinal cord (cervical, thoracic, lumbar and sacral) of normal rats has been studied by quantitative autoradiography using the specific α_1-antagonist [^{3}H]Prazosin as a ligand. The same study was performed with rats bearing specific lesion of NA spinal cord system carried out by intracisternal (IC) injection of 6-hydroxy-dopamine (6-OHDA) and after transsection of spinal cord at level T8-T9. The binding of [^{3}H]Prazosin to spinal cord sections from the different segment of spinal cord was saturable, reversible and of high affinity (Kd ≈ 0.14nM). It occurred at a single population of sites and possessed the pharmacological features of the α_1-adrenoceptors. The results can be summarized as follows : 1) In control rats, the quantitative analysis of α_1 adrenoceptor densities revealed an homogeneous distribution of these receptors along all segments of the spinal cord with a similar pattern in the various subregions of the grey matter at all levels. However, the central area of the grey matter and the region where the motoneurons are localized presented the highest densities of α_1 adrenoceptors, while in the dorsal horn (proximal part) densities were the lowest. This distribution of α_1 adrenoceptors only partly corresponded to the distribution of NA nerve terminals revealed by immunohistochemistry. 2) After specific lesion of NA system by 6-OHDA IC an increase of α_1 adrenoceptor densities (13-43%) was observed at all levels of the spinal cord. 3) Transsection of spinal cord at T8-T9 level induced a similar increase of α_1 adrenoceptor densities (22 - 42%) in downstream segments (lumbar and sacral) as compared to that observed after 6-OHDA lesion. No significant effect has been observed in upstream segments.

These results showed that specific lesioning (6-OHDA) or non specific destruction (transsection) of NA spinal cord system caused a supersensitivity of α_1 adrenoceptors localized on target cells of this system.

471.4

IMPLANTED OSMOTIC MINI-PUMPS UP-REGULATE AND IMIPRAMINE DOWN-REGULATES BETA-ADRENOCEPTOR ACTIVITY IN RAT CA1 NEURONS; ADINAZOLAM INDUCES ONLY MARGINAL CHANGES. R.S. Neuman, Fac. of Medicine., Memorial University., St. John's, Nfld. Canada A1B 3V6

There are numerous reports that chronically administered antidepressants down-regulate beta-adrenoceptor function. We have now compared the triazolobenzodiazepine antidepressant, adinazolam (ADZ), with imipramine (IMI) in this regard. Rats were untreated or received ADZ (0.64 mg/kg/day; 2 or 15 days), IMI (10 mg/kg/day; 2 or 15 days) or saline (15 days). Drugs and saline were administered by osmotic mini-pumps implanted s.c. Hippocampal slices (500 um) were obtained on the appropriate treatment day for intracellular recording from CA1 neurons. Current injection (0.7-0.9 nA, 100 ms) through the microelectrode elicited 6 to 11 spikes followed by a slow afterhyperpolarization (AHP). In untreated rats, isoproterenol (ISO; 0.5 nM-100 uM) dose-dependently reduced the AHP by 56% with an EC50 of 80 nM. In contrast, the maximum depression was 70% with an EC50 of 20 nM in saline treated rats. Thus, the presence of a mini-pump, perhaps by increasing the level of stress, shifted the ISO dose response curve to the left. In comparison to the saline control, IMI shifted the curve to the right (2 and 15 days) and reduced the maximum inhibition (32%; 15 days). Dose response curves for ISO, in ADZ treated rats (2 and 15 days), were similar to the saline control and each other except for the maximum reduction of the AHP (70% and 58% respectively). In conclusion, implanted mini-pumps up-regulate and IMI down-regulates functional beta-adrenoceptor activity. In contrast, ADZ only marginally effects this activity.

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471.5

Cytoskeletal Aspects of the Regulation of β-Adrenergic Receptor mRNA. Christopher Hough and De-Maw Chuang. Biological Psychiatry Branch,, NIMH, Bethesda, MD 20892.

The β_1- and β_2-adrenergic receptor mRNA levels in C_6 glioma cells are differentially regulated in response to isoproterenol. The nature of the changes in β-AR mRNA levels varied with different cell culture conditions. For example, at confluency, β_2-AR mRNA was down-regulated in response to isoproterenol treatment, but at low cell density β_2-AR mRNA could be transiently up-regulated. Because the cytoskeleton determines the morphology of cells in culture, we investigated the effect of microtubule-altering agents on β-AR mRNA regulation. The microtubule disrupter, colchicine, at 10 μM, induced a time dependent up-regulation of β_2-AR mRNA and down-regulation of β_1-AR mRNA. Simultaneously, the cells withdrew their processes, leaving large holes in otherwise confluent sheets of cells. Colchicine did not interfer with isoproterenol induced down-regulation of β_2-AR mRNA, but appeared to partially attenuate the down-regulation of the β_1-AR mRNA species. Taxol, a microtubule bundle promoter, produced effects opposite to those of colchicine: taxol at 10 μM induced the down-regulation of β_2-AR mRNA and early up-regulation of the β_1 mRNA species. Taxol had little or no effect on isoproterenol induced down-regulation of either receptor mRNA species. These results suggest that aspects of the cytoskeleton, play a role in the regulation of β-AR mRNA.

471.6

EFFECT OF CHRONIC TRICYCLIC ANTIDEPRESSANT TREATMENT ON β1-ADRENERGIC RECEPTOR (β1-AR) mRNA IN RAT FRONTAL CORTEX AND CULTURED GLIOMA C6 CELLS. K. Hosoda, P. H. Fishman and R. S. Duman. Lab. of Molecular Psychiatry, Depts of Pharmacology and Psychiatry, Yale Univ. School of Medicine, New Haven, CT 06508. Lab. of Molecular and Cellular Neurobiology, National Institute of Neurological Disorders and Stroke, NIH, Bethesda, MD 20892.

One of the most consistent effects of nearly all classes of antidepressant treatment is down-regulation of β1-AR binding sites in rat cerebral cortex. Incubation of C6 glioma cells with a tricyclic antidepressant also decreases β1-AR binding, indicating an effect of the drug treatment directly on receptor coupled signal transduction pathways. The present study examines levels of β1-AR mRNA in rat brain and C6 cells to elucidate the molecular mechanisms mediating the regulation of β1-AR by antidpressant treatments. Absolute levels of β1-AR mRNA were determined by RNA-excess solution hybridization. Riboprobes corresponding to the sense and antisense DNA strand of β1-AR were synthesized with T7 & T3 RNA polymerase. Unlabelled cRNA was used to generate the standard curve. Chronic imipramine administration (21 d, 15 mg/kg, i. p.) significantly decreased levels of β1-AR mRNA in rat frontal cortex. This effect was not observed after only 3 or 7 d of treatment. Treatment of C6 glioma cells with desipramine (5 d, 10 μM) also significantly decreased levels of β1-AR mRNA. These results suggest that long-term changes in levels of β1-AR in response to antidpressant treatment may occur through regulation of levels of mRNA and gene expression.

471.7

THE INFLUENCE OF CHRONIC ANTIDEPRESSANT TREATMENTS ON THE EXPRESSION OF 5-HT2 RECEPTOR mRNA IN RAT FRONTAL CORTEX. M.O. Butler and R.S. Duman Laboratory of Molecular Psychiatry, Department of Psychiatry, Yale University School of Medicine, New Haven, CT 06508.

Earlier studies have shown that chronic administration of most classes of antidepressant drugs decreases the level of 5-HT2 receptor binding in brain. Electroconvulsive shock (ECS), however, has been shown to increase levels of 5-HT2 receptor binding in rat brain. This raises some doubt concerning the role of the 5-HT2 receptor in antidepressant action since electroconvulsive therapy is considered to be extremely efficacious in the treatment of major depression.

The present study examines the influence of chronic antidepressant treatments on levels of 5-HT2 receptor mRNA in an attempt to address this discrepancy and to elucidate the molecular mechanisms underlying regulation of 5-HT2 receptors. RNA samples obtained from frontal cortex of control and treated rats were analyzed by Northern blot using a nick translated 5-HT2 receptor cDNA clone. Chronic ECS (10 daily treatments) increased levels of 5-HT2 receptor mRNA by about 60% ($p<.05$). Additionally, we have begun to study regulation of 5-HT2 mRNA by antidepressant drug treatments. Preliminary studies have indicated a similar increase of 5-HT2 receptor message in frontal cortex of chronic imipramine treated rats (18 days, 15 mg/kg i.p.) Therefore, although ECS and antidepressant drugs change 5-HT2 binding in opposite directions, these treatments appear to regulate levels of receptor mRNA and possibly gene expression in a similar up-regulated manner.

471.8

DEAFFERENTATION INDUCES A DIFFERENTIAL PATTERN OF EXPRESSION OF THE VARIOUS GUANINE NUCLEOTIDE BINDING PROTEIN mRNAs IN RAT STRIATUM M. Memo, A. Valerio *,M. Ribola *,M. Buonamici, P. Sarmientos * and P.F. Spano Section of Pharmacology, Dept. of Biomed. Sci. and Biotech., School of Medicine, University of Brescia, and Research and Development, Farmitalia-Carlo Erba, Erbamont Groups, Nerviano-Milano, Italy

This study was designed to assess the relative abundance of the mRNAs encoding various forms of Gs (long and short), Gi (1, 2, and 3) and Go α subunits in the striata of rats unilaterally injected with 6-hydroxy (6-OH) dopamine in the substantia nigra.A PCR-derived method was used to identify and quantitate the relative abundance of the different mRNAs (Memo et al.,Mol Pharmacol 1991 in press). Chemical deafferentation induced a differential pattern of expression of the mRNAs encoding the various α subunits of G proteins. In the striatum of rats lesioned with 6-OH dopamine in the substantia nigra, the mRNA levels of the Gαo and Gαi-1 subunit were increased by about 2-3 times, those of Gαi-3 decreased by 60 % and those of Gαi-2 and Gαs were unchanged. The changes in the pattern of expression of various G proteins are correlated with the development of functionally supersensitive striatal dopamine D-2 receptors.

471.9

EFFECTS OF REPEATED ADMINISTRATION OF DESIPRAMINE (DMI) OR ELECTROCONVULSIVE SHOCK (ECS) ON NOREPINEPHRINE (NE) UPTAKE SITES MEASURED BY 3H-NISOXETINE (3H-NIS) AUTORADIOGRAPHY. M.E. Bauer,* K.J. Kellar,+ and S.M. Tejani-Butt, Dept. Psych., Univ. of Pa Sch. of Med. & Dept. Vet. Affairs Med. Ctr., Phila., PA 19104 & +Dept. Pharmacol., Georgetown Univ. Sch. of Med. Wash., D.C. 20007.

The development of radioligands for monoamine "transporters" allows one to determine if these sites undergo regulation analogous to monoamine receptors. To determine if repeated administration of DMI or ECS regulate NE uptake sites in rat brain, the binding of 3H-NIS was measured using quantitative autoradiography. We have recently shown that 3H-NIS binds specifically and with high affinity to a single population of NE uptake sites (Eur. J. Pharmacol. 191:239, 1990) One group of rats received DMI (a NE uptake blocker) (10 mg/kg, daily) i.p. for 21 days and was killed 48 hr after the last injection. Another group of rats received ECS (increases release of NE) daily for 12 days (150 mA, 60Hz, 300 msec. duration) and was killed 24 hr after the last shock. DMI caused modest (15-30%) but statistically significant decreases in the binding of 3H-NIS in 5 of 17 areas measured. By contrast, except for the paraventricular nucleus of the thalamus where ECS caused a modest (20%) but statistically significant increase in binding, no other brain region was affected by ECS. In general, then, ECS does not seem to exert wide-spread effects on NE uptake sites in brain. It remains to be determined if the DMI-induced loss of binding of 3H-NIS in specific areas of brain represents a functional regulation of the NE uptake sites. (Research funds from the Dept. of Vet. Affairs and USPHS grants MH 45472 and MH 41819).

471.10

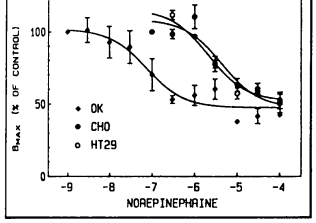
SEMICHRONIC ADMINISTRATION EFFECTS OF ADRENERGIC DRUGS ON ADRENOCEPTORS DENSITY IN DIFFERENT BRAIN AREAS. V. Alemán, M.E. Alvarez and *G.P. Jiménez*. Dept. Physiology, CINVES-TAV, IPN, Mexico, D. F., Mexico.

We wished to determine how both α_1 and α_2 adrenoceptors density is regulated by adrenergic drugs injected semichronically. Ninety six ninety-day-old rats were divided in three control and three drug treated groups. Rats were intradermically administered, 0.75 mg/kg of amphetamine every 12 hr during ten days, Prazosin (i.p.)1.0 mg/kg and Atipamezole (i.p.)3.5 mg/kg. Two hr after the last injection, cerebral areas were dissected and membrane fractions obtained. (^{3}H)-Prazosin and (^{3}H)-RX781094 were used as ligand for α_1 and α_2 receptors respectively. Amphetamine decreased α_1 receptors density in temporo-parietal cortex (T-PC) and in caudate (CN), but it increased in amygdala (Am). Amphetamine produced changes in α_2 receptors density, thus in frontal cortex (FC) and T-PC it was decreased significantly but it increased in hippocampus (Hp) and Am. Prazosin produced important decrements in FC, Hp and in CN α_1 receptors but it produced increments of α_2 in T-PC and Hp.Finally atipamezole increased α_1 receptors density in FC, T-PC, Am and CN. Receptors α_2 decrements were observed in FC, T-PC and Am. This type of regulation is in accord with either the administration of antagonists or agonists, with the presence of both hetero and autoreceptors and furthermore it depends on the brain area analyzed. But some unexplained changes were present too.

471.11

AGONIST INDUCED DOWN-REGULATION OF THE α_2-ADRENERGIC RECEPTOR SUBTYPES HAVE MARKEDLY DIFFERENT CHARACTERISTICS. P.E. Shreve, M.L. Toews and D.B. Bylund, Dept. of Pharmacology, Univ. of Nebraska Med. Ctr., Omaha, NE 68198-6260.

The purpose of the present study was to determine the effect of agonist pretreatment on α_2 adrenergic receptor (A2AR) number in cell lines expressing different A2AR subtypes. The cell lines used were Chinese hamster ovary (CHO) cells which have been stably transfected with the gene coding for the A2AR, human colonic adenocarcinoma (HT29) cells, and opossum kidney (OK) cells which have been pharmacologically characterized to express α_{2A}, α_{2A}, and α_{2C} subtypes of the A2AR, respectively. Cells were pretreated t norepinephrine (NE) at increasing concentrations for 24 h before harvesting. Binding studies with [^{3}H] rauwolscine showed that NE produced a concentration dependent down-regulation of the A2AR in all 3 cell lines (figure). The EC50 of NE was 0.073, 4, and 2 μM in OK, CHO, and HT29 cells, respectively. Thus NE was 27- to 55-fold more potent at down-regulating the A2AR in OK cells as compared to CHO and HT29 cells, respectively. These data suggest that differences in agonist induced down-regulation of the A2AR appear to be receptor subtype selective. This research was supported by NIH grant GM37664.



471.12

EFFECTS OF ANTIMYCIN A ON AGONIST BINDING TO ADRENERGIC RECEPTORS OF INTACT CELLS. S.J. Zhu*, P.E. Shreve and M.L. Toews. Dept. Pharmacology, Univ. Nebraska Med. Ctr., Omaha, NE 68198-6260.

For beta adrenergic, alpha-1 adrenergic, and alpha-2 adrenergic receptors and for muscarinic acetylcholine receptors, agonists have been shown to exhibit markedly lower apparent affinities in competition binding assays performed on intact cells than in assays with isolated membrane preparations. We have postulated that agonists induce receptor internalization during the course of the assay and that the apparent low affinity results from inaccessibility of hydrophilic agonists to internalized receptors. Since depletion of intracellular ATP with antimycin A has been shown to prevent both internalization and low affinity binding in the case of muscarinic receptors, the effects of antimycin A on low affinity binding to beta, alpha-1 and alpha-2 adrenergic receptors were studied. In DDT$_1$ MF-2 smooth muscle cells, antimycin A pretreatment increased affinity of the agonist epinephrine for beta receptors whereas binding of the antagonist metoprolol was not changed. However antimycin A did not increase epinephrine affinity for alpha-1 receptors in these cells. In Chinese hamster ovary cells expressing beta receptors, antimycin A increased affinity of the agonist isoproterenol but not the antagonist metoprolol. Similarly, in Chinese hamster ovary cells expressing alpha-2 receptors, antimycin A increased affinity of the agonist norepinephrine but not the antagonist prazosin. These results are consistent with involvement of receptor internalization (or another ATP-dependent mechanism) in low affinity binding of agonists to intact cell beta and alpha-2 receptors. They extend previous results suggesting that different mechanisms are involved in the case of alpha-1 receptors. (Supported by GM34500).

471.13

UPREGULATION OF β ADRENERGIC RECEPTORS AND β_2 m RNA BY CARBAMAZEPINE IN VITRO. G.Chen*, C.Hough, H.Manji, D.M.Chuang,I.N.Mefford and W.Z.Potter*, Exp Ther Branch and Biol.Psych. Branch, NIMH,Bethesda, MD 20982

Despite the widespread clinical use of carbamazepine (CARB), the molecular mechanisms underlying both its anticonvulsant and mood-stabilizing effects remain unknown. We have recently demonstrated that chronic in vitro exposure of C6 glioma cells to desipramine (DMI) results in a significant downregulation of β adrenergic receptors (βARs). Given the close structural similarity between DMI and CARB, and their opposite effects on seizure threshold, we examined CARB's effects on βARs. In contrast to DMI, incubation of C6 glioma cells with 40 uM CARB in vitro resulted in a significant increase in βARs. The upregulation of βARs was evident within 3 days of incubation, and exhibited a dose-response profile (10-100 uM). Moreover, the upregulation of beta receptors was accompanied by a marked increase in β_2 mRNA. CARB's effects on β_2 mRNA were also time and dose-dependent (and occured within the drugs therapeutic range). The mechanism(s) by which CARB and DMI produce opposite effects on βARs is currently under investigation.

NEUROENDOCRINE REGULATION II

472.1

EFFECT OF AGING ON INDUCTION OF FOS IN THE SUPRAOPTIC AND PARAVENTRICULAR NUCLEI IN RESPONSE TO CHRONIC DEHYDRATION. L. Trojanczyk, J.A. Olschowka and C. D. Sladek, Univ. of Rochester Sch. of Med., Rochester, N.Y. 14642.

In previous studies, this laboratory demonstrated that the vasopressin (VP) response to 72 hours of water deprivation is attenuated in aged rats (Neurobiol. Aging 1:293, 1981). Aged rats have a decreased concentration of VP in the neural lobe, and VP synthesis is decreased (Exp Gerontol. 22:113-125, 1987). These observations led to the hypothesis that defects in the mechanisms that regulate VP synthesis and release in response to stimuli may precipitate the observed aging associated deficits in VP release. Fos is a candidate for a signal involved in stimulus-transcription coupling in VP neurons, because it was recently demonstrated that the *c-fos* gene is induced in supraoptic and paraventricular neurons following dehydration. In this study we evaluated the effect of 72 hours of water deprivation on fos induction in the SON and PVN nuclei in 4, 14, and 30 month old Fischer 344 rats. Animals were processed for c-fos immunocytochemistry using an antisera against the M-peptide region of the c-fos protein. This antisera cross reacts with fos-B and fos-related-antigen 1, and therefore provides an index of activation of the Fos family of antigens. Densitometric analysis of the SON and PVN demonstrated c-fos induction in all water deprived rats regardless of age. The density of staining (analyzed from the integrated optical density) was increased in both nuclei following dehydration (SON: F=12.05, p=0.002; PVN: F=21.68, p=0.0001). There was also a significant increase in the number of cells expressing fos in both nuclei in the dehydrated animals (SON: F=11.64; p=0.002; PVN: F=9.11, p=.0056). There was no significant effect of age on the density of fos staining nor any significant interaction effect in either nuclei. These results suggest that a deficit in the induction of the fos or fos related proteins in response to dehydration is not responsible for the attenuation of VP release observed in aged rats, however further studies are warranted to evaluate the effect on c-fos specifically. Supported by R35-AG09016.

472.2

C-FOS PROTO-ONCOGENE EXPRESSION IN HYPOTHALAMUS AND VASOPRESSIN REGULATION AFTER DEHYDRATION. J. M. Ding and J. Buggy, Dept. of Physiology, University of South Carolina School of Medicine, Columbia, S.C. 29208

The proto-oncogene *c-fos* codes for the nuclear protein Fos which acts as a transcriptional regulator linking short-term stimuli to long-term responses by altering secondary target gene expression. This study in rats examined *c-fos* and vasopressin gene expression in brain areas associated with body fluid regulation in response to various dehydration stimuli. *Fos* is induced by acute intracellular dehydration or cerebroventricular injection (ICVT) of carbachol (but not by hypovolemia or ICVT angiotensin) in the hypothalamic magnocellular nuclei of paraventricular (PVN), supraoptic (SON), and nucleus circularis as well as in the dehydration-transducing structures of lamina terminalis: AV3V nucleus medianus, OVLT, and SFO. The induction of *fos* in magnocellular nuclei requires stimulus transduction since AV3V lesion attenuated *fos* induction by intracellular dehydration or carbachol in the PVN and SON. Dual-label immunocytochemical co-localization of *fos* and vasopressin in the magnocellular neurons of PVN and SON suggests that *fos* may regulate vasopressin. *In situ* hybridization analysis after acute dehydration revealed a rapid and transient *fos* induction followed by a slower-onset increase in vasopressin mRNA. The increase in vasopressin mRNA persisted for up to 2 days even after rehydration. Furthermore, prevention of fos translation by cycloheximide attenuated the dehydration-induced increase in vasopressin mRNA. These results support a role for *fos* in vasopressin regulation following intracellular dehydration.

472.3

DIFFERENTIAL RESPONSE OF ARGININE VASOPRESSIN (AVP) mRNA IN THE HYPOTHALAMIC PARAVENTRICULAR AND SUPRAOPTIC NUCLEI TO ANTIGLUCOCORTICOID TREATMENT IN LEAN AND OBESE ZUCKER RATS. U. Pesonen, M. Koulu and R. Huupponen', Dept. of Pharmacology, Univ. of Turku, SF-20520 Turku, Finland.

Arginine vasopressin (AVP) is a physiological regulator of ACTH secretion. It acts synergistically with corticotropin releasing factor (CRF) potentiating its action on pituitary corticotrophes. In the present study we have investigated the effects of antiglucocorticoid treatment on AVP mRNA expression in the hypothalamus of genetically obese Zucker rats. These rats are hyperphagic, hyperinsulinemic and there is also evidence of abnormal regulation of the hypothalamus-pituitary-adrenal -axis in obese Zucker rats.

Six obese and six lean controls were administered with mifepristone (RU486), a glucocorticoid receptor antagonist, 10 mg/kg p.o. twice daily for four days. Control animals were treated similarly with vehicle. The expression of AVP mRNA in the hypothalamic paraventricular (PVN) and supraoptic (SON) nuclei was quantitated using *in situ* hybridization assay with ^{35}S-labelled oligonucleotide probe.

Our results show that obese Zucker rats have significantly ($p<0.05$) lower basal expression of AVP mRNA in the PVN, but not in the SON compared to lean controls. Mifepristone treatment significantly ($p<0.01$) increased AVP mRNA levels in the PVN of obese animals, but did not modify AVP mRNA levels in the PVN in lean animals. In contrast, mifepristone lowered ($p<0.05$) AVP mRNA content in the SON in lean animals.

The results suggest that the regulation of AVP neurons in the PVN is altered under basal conditions in obese Zucker rats. Opposite responses to mifepristone treatment propose that glucocorticoids elicit different feedback control on AVP mRNA in the hypothalamus of lean and obese Zucker rats.

472.4.

MODULATION OF SUPRAOPTIC AND PARAVENTRICULAR PEPTIDE mRNA LEVELS BY THE POSTERIOR ARCUATE / PREMAMMILLARY REGION. S.E. Bachus, M. Palkovits*, and W.S. Young,III, Lab. Cell Biology, NIMH, Bethesda, MD 20892.

We previously demonstrated that hemisections anterior to the mammillary bodies dramatically elevated expression of galanin in the supraoptic (SON) and paraventricular (PVN) nuclei of the hypothalamus (Neurosci. 1:115). In order to determine the origin of fibers mediating this effect, we made electrolytic lesions (Physiol Behav. 23:421) of various posterior hypothalamic sites. We used hybridization histochemistry with ^{35}S-oligonucleotide probes against galanin, as well as cholecystokinin, and angiotensin mRNAs. Other rats' SONs were implanted with micropipettes (Brain Res. 235:174) containing the retrograde fluorescent tracer, Fluorogold. These rats were examined 5 days later for the presence of retrogradely labeled cells in the posterior hypothalamus.

Electrolytic lesions in the region of the premammillary nuclei and mammillary and premammillary portions of the arcuate nucleus elevated levels of galanin transcripts in the PVN and SON. Lesions of the mammillary and supramammillary nuclei were ineffective. The effective lesions also elevated levels of cholecystokinin mRNAs in the PVN and SON, whereas those for angiotensin were reduced in SON. Fluorogold-retrogradely labelled cells were present in the arcuate and premammillary nuclei after injections that included the SON, although potential contributions from extra-SON uptake are still being analyzed. We are also attempting more specific lesions with ibotenic acid and neonatal monosodium glutamate, but our results presented here suggest that projections from the posterior arcuate/premammillary region modulate SON and PVN gene expression.

472.5

THE GABA AGONIST MUSCIMOL REDUCES VASOPRESSIN SYNTHESIS AND RELEASE. Michelle M. Roberts and Alan G. Robinson, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

GABA is a major inhibitory transmitter of the neurohypophysis and GABAergic mechanisms are believed to modify both basal and stimulated vasopressin (AVP) release. We studied the effects of the GABA agonist muscimol on the synthesis and release of AVP and oxytocin (OT) in the rat. Acute intracerebroventricular (ICV) injection of muscimol (160 ng in 1 μL artificial CSF [aCSF]) inhibited the AVP and OT response to hemorrhage and hypertonic saline infusion. Following a 5 ml hemorrhage, plasma AVP levels were reduced to 26%, 46%, 27% and 59% at 5, 10, 15 and 25 min in muscimol treated rats relative to aCSF. Plasma OT levels were reduced to 18%, 15%, 14% and 27% of controls. Following infusion of 2 M saline (10 ml/kg over 60 min), plasma AVP was suppressed to 35%, 37% and 16% at 30, 60 and 90 min in muscimol-treated rats relative to aCSF. OT was dramatically suppressed to levels 5%, 16% and 33% of control responses. We next demonstrated that chronic infusion of muscimol into the lateral ventricle reduced basal synthesis rates of both AVP and OT. Following a 3 day infusion of 50 ng muscimol per hour, AVP synthesis rates were reduced from a baseline value of 1.5 ng/h to undetectable levels. OT synthesis decreased from a basal rate of 4.1 ng/h to 0.5 ng/h. AVP mRNA levels in muscimol treated rats were reduced to 53% of control. Plasma sodium and hydration were normal in all groups of rats. We conclude that acute ICV injection of muscimol inhibits AVP and OT release in response to both hemorrhagic and osmotic stimuli. Chronic infusion of muscimol inhibits basal AVP and OT synthesis and reduces AVP mRNA levels. These studies indicate that GABA is a major inhibitor of physiologic release and synthesis of AVP.

472.6

STIMULATION OF NEUROPHYSIN EXPRESSION IN CULTURED NEURONS BY CYCLIC AMP IS REVERSIBLE. C.D. Sladek and M.J. Gallagher.* Univ. Rochester Sch. Med., Rochester, N.Y. 14642

Previous studies have demonstrated that the number of vasopressin (VP) neurons present in primary diencephalic cultures can be markedly augmented by treatment with drugs that elevate intracellular cyclic AMP (cAMP; Oeding,et al, J. Neuroendo., 1990). In order to evaluate the effect of this drug treatment on VP secretion by hypothalamic cultures, we have exposed primary dispersed hypothalamic cultures derived from 14 day old fetal Sprague-Dawley rats to forskolin (25μM) and the phosphodiesterase inhibitor, IBMX (500μM) either continually or intermittently for up to 32 days. Culture medium was collected throughout the culture period for VP radioimmunoassay. At the end of the experiment, cultures were stained immunocytochemically for neurophysin (NP). As reported by previous investigators, exposure to the drugs for 11 days resulted in a 10X increase in the number of NP positive neurons. This increase in the cell number was sustained during longer periods of exposure. During this period the cells continued to increase in size and develop extensive neuritic processes. This increase in cell size and complexity was reflected in an increase in the concentration of VP in the culture medium from 1.4±0.15 pg/ml at 11 days to 8.4±0.6pg/ml after 32 days of drug treatment. The VP concentration remained undetectable (<1.25pg/ml) in non-treated cultures throughout this period. The effect on NP expression required the continuous presence of the drugs. Removal of the drugs from day 11-18 of culture resulted in an almost complete loss of NP positive cells, however re-exposure to the drugs reinstated the NP expression in a time dependent fashion. These results demonstrate that VP secretion and maturation of NP neurons in dispersed hypothalamic cultures can be markedly stimulated by chronic treatment with cAMP elevating drugs, but continuous exposure to the drugs is necessary to sustain the effect. Supported by RO1-DK19761 and PHS S7RRO5403-29.

472.7

ALTERED CNS EXPRESSION OF CYTOPLASMIC AND HETERONUCLEAR VP RNA IN THE BRATTLEBORO RAT. P. Szot, M.D. Brot and D.M. Dorsa. GRECC, Seattle VAMC, WA 98108 and Dept. of Pharmacology, Univ. Washington, Seattle,WA 98195.

The HOM-BB rat lacks the ability to synthesize VP and its neurophysin due to a single base pair deletion in the VP gene. This mutation in the gene results in a reading frame shift and inefficient translation of the mRNA encoding the peptide. The level of cytoplasmic VP mRNA was quantified autoradiographically by in situ hybridization in the paraventricular (PVN), supraoptic (SON) and suprachiasmatic (SCN) nuclei, as well as the extrahypothalamic nuclei of the bed nucleus of the stria terminalis (BNST) and medial amygdala (MA). The HOM-BB rat showed a significant reduction in the amount of VP mRNA expressed per cell compared to LE or HET-BB rats, while the number of hybridization positive cells was equivalent. In contrast, the SCN, BNST and MA of the HOM-BB rat showed a significant reduction in the number of VP mRNA positive cells and amount of VP mRNA being expressed than the LE or HET-BB rat. The number of VP mRNA expressing cells in the BNST and MA of the HOM-BB rat was not enhanced by elevating testosterone levels as in the normal LE rat. An oligonucleotide to intron 2 of the VP gene was used to detect heteronuclear VP RNA. Significant increases in the number of cells expressing the nuclear VP RNA were observed in the PVN (58%) and SON (62%), but not in the SCN of the HOM-BB rat when compared to the LE or HET-BB rat. These data suggest that the trans-cription rate of the VP gene in the magnocelluar nuclei of the HOM-BB rat may be elevated, though this increase does not appear to result in higher levels of mutant cytoplasmic VP mRNA.

472.8

COLD EXERTS OPPOSITE EFFECTS ON TRH mRNA LEVELS IN NEURONS OF THE PARAVENTRICULAR NUCLEUS (PVN) AND PREOPTIC AREA (POA), INDEPENDENT OF CHANGES IN BARORECEPTOR ACTIVITY. R.T. Zoeller, D. Dolan*, M.F. Nichols* and J. C. Schadt. Dept. Anat/Neurobiol. and Dalton Research Center, Univ. Missouri Sch. Med., Columbia, MO 65212.

TRH neurons of the POA have been implicated in the control of body temperature (T_b); their activity is believed to contribute to maintenance of T_b during cold exposure. Because TRH mRNA levels in other hypothalamic nuclei are linked to TRH release (Zoeller et al., *Endocrinology* 127:2955, 1991), we hypothesized that TRH mRNA levels in POA neurons would be elevated by cold exposure. Using *in situ* hybridization and single-cell analysis, we found that TRH mRNA levels in neurons of the POA were significantly lower ($p<0.01$) in animals exposed to 6h 5°C (n=8) compared to 25°C (n=11). Cold exposure produces changes in cardiovascular function which may account for some effects of cold. We manipulated blood pressure with 60 min infusions of Na nitroprusside (SNP, n=8), phenylephrine (PE, n=11), or saline (n=14) to initially investigate possible involvement of these TRH neurons in regulating cardiovascular function. Neither drug significantly affected TRH mRNA levels in PVN; nor did SNP affect TRH mRNA levels in POA. However, we did find that SNP significantly elevated TRH mRNA levels in specific raphe nuclei ($p<0.01$). These data suggest that separate populations of TRH neurons may be involved in different aspects of T_b regulation and cardiovascular function. [Supported by: AHA, MO Affiliate (RTZ) and HL31218 (JCS).

ENDOCRINE REGULATION II

473.1

INTERACTION OF F8Fa WITH DYNORPHIN IN MODULATING NEUROPEPTIDE SECRETION FROM ISOLATED NERVE ENDINGS OF THE RAT NEURAL LOBE. K. PAYZA. Laboratory of Biochemical Genetics, NIMH Neuroscience Center, WAW-113, St. Elizabeth's Hospital, Washington, D.C. 20032.

The neuropeptide FLFQPQRFamide (F8Fa) was tested for modulatory effect on secretion of the hormones oxytocin (OT) and vasopressin (VP) in isolated nerve endings of rat neural lobe, where F8Fa has been localized. The endings were loaded onto 0.45 μm filters, perfused with Ringer containing physiological salts and protease inhibitors, and stimulated by perfusion with 35 mM $[K^+]_o$. The OT and VP secreted into the eluate were measured by RIA. Dynorphin A 1-8 (1 μM) inhibited secretion of both OT and VP. F8Fa (2 μM) blocked the inhibition of OT and VP secretion induced by dynorphin A 1-8. These effects of F8Fa were similar to naloxone (10 μM), which also blocked or attenuated the effect of dynorphin on OT and VP secretion. These results suggest a local modulatory role for F8Fa in the rat neural lobe.

473.2

OPIOIDS INHIBIT SYNAPTIC INPUTS FROM THE REGION OF THE ORGANUM VASCULOSUM OF THE LAMINA TERMINALIS (OVLT) TO THE SUPRAOPTIC NUCLEUS (SON) IN VITRO. S.J.A. MacMillan, *Centre for research in Neuroscience, Montreal General Hospital and McGill Univ., Montreal, H3G 1A4.*

The activity of SON neurones is thought to be regulated by inputs from the region anterior and ventral to the third ventricle (AV3V). This input which is thought to be important, in part, in osmotic control of neurohypophysial hormone release is believed to be opioid sensitive. Using a superfused hypothalamic explant preparation and intracellular recording techniques we have examined the effects of opioids on this input to the SON. Morphine applied by addition to the superfussion line (1-10μM) inhibited the spontaneous firing of MNCs (n=21) but this was associated with only small membrane hyperpolarisations. However morphine (1μM) profoundly inhibited (n=5) spontaneous presynaptic potentials (PSPs). Electrical stimulation of the OVLT region produced short latency short duration mixed excitatory and inhibitory PSPs followed by longer latency excitations in 17 MNCs. Morphine (1μM) inhibited both early and late components of this response (n=11). These inhibitions peaked within 2-5 minutes of morphine reaching the tissue and recovered within 10 minutes of return to control solution and were prevented (n=4) by the opioid antagonist naloxone (1μM). We conclude that opioids exert a presynaptic inhibitory action on inputs from the OVLT region to the SON. *(Supported by the MRC)*

473.3

OSMOTIC STIMULATION OF THE ORGANUM VASCULOSUM OF THE LAMINA TERMINALIS (OVLT) ACTIVATES RAT MAGNOCELLULAR NEUROSECRETORY CELLS (MNCs) IN VITRO. D. Richard and C.W. Bourque. Centre for Research in Neuroscience, McGill University, Montreal, Canada.

The OVLT has been implicated as a central osmoreceptor involved in the regulation of neurohypophysial hormone release. Possible functional interactions were examined during intracellular recordings obtained in superfused explants of rat hypothalamus. Brief (5-10 sec) hypertonic stimulation (20-50 mOsm) of the OVLT area induced depolarization and increased firing in each of five local neurons tested. Selective osmotic stimulation of the OVLT also caused increases in the frequency of spontaneous postsynaptic potentials and accelerated the firing rate in each of 14 MNCs tested. These effects were dose-dependent and persisted for 1-3 minutes before reversing to control rates. In contrast to the pronounced depolarizations (3-10 mV) and decreases in input resistance (10-25%) observed during direct osmotic stimulation of MNCs (n=8), increases in firing rate recorded from MNCs during OVLT stimulation were not accompanied by changes in impedance or membrane potential. These results suggest that neurons located in or near the OVLT convey information concerning fluid osmolarity to MNCs via synaptic input to the supraoptic nucleus. Supported by FCAR and the MRC.

473.4

EFFECTS OF NEUROTENSIN ON RAT MAGNOCELLULAR NEUROSECRETORY CELLS (MNCs) IN VITRO. K. Kirkpatrick and C.W. Bourque. Montreal General Hospital and McGill University, Montreal, Canada H3G 1A4

The tridecapeptide neurotensin (NT) has been shown to induce antidiuresis in dogs. In this study we have examined the actions of this peptide on hypothalamic MNCs. Intracellular recordings were obtained from supraoptic nucleus MNCs in superfused rat hypothalamic explants. Application of NT (.1-5 μM) depolarized and enhanced spike discharge in each of 23 cells tested. Eight of these cells were classified as being vasopressinergic on the basis of their ability to display phasic firing. These effects were mimicked by the NT 8-13 fragment (n=7) and persisted in Ca^{++}-free solutions containing Mn^{++} (2mM, n=3). In addition to these effects NT decreased the magnitude of the Ca^{++}-dependent afterhyperpolarization (AHP) that follows spike trains in MNCs (n=4), as well as the corresponding outward current measured under hybrid clamp (n=2). Intracellular injections of Cs^{+} sufficient to abolish the AHP (n=4) did not prevent NT-induced depolarizations. These results suggest that activation of postsynaptic NT receptors can contribute to the regulation of vasopressin release by exciting MNCs in two distinct manners. *Supported by FCAR and MRC.*

473.5

VASOPRESSIN RELEASE BY CENTRAL ANGIOTENSIN II IS MEDIATED THROUGH AN ANGIOTENSIN TYPE-1 RECEPTOR AND THE DRINKING RESPONSE IS MEDIATED BY BOTH AT-1 AND AT-2 RECEPTORS. David C. Hogarty* and M. Ian Phillips. Dept. of Physiology, College of Medicine, University of Florida, Gainesville, FL 32610.

Angiotensin II, given centrally, releases vasopressin, increases blood pressure and stimulates drinking. With the advent of new receptor subtype antagonists, we are able to investigate whether the effects are mediated by AT-1 receptors or AT-2 receptors in the brain. Adult male Sprague Dawley rats were cannulated and 5 days later catheterized in the common carotid artery for blood pressure measurements. All experiments were carried out in chronic rats. Three treatments were given i.v.t. in 2 ul ACSF at 30 min intervals. 1) 50 ng Ang II, 2) AT-1 antagonist 0.7 μg DuP or AT-2 antagonist 7.0 μg EXP, + 50 ng Ang II, 3) 50 ng Ang II to test for recovery. Controls to test repeated doses were also used. Blood pressure and drinking responses were recorded and an arterial blood sample drawn for radioimmunoassay of arginine vasopressin. The results showed that plasma AVP release in response to Ang II, is reduced 70% by AT-1 antagonism but not altered by AT-2 antagonist. EXP had no significant effect on the pressor response, but DuP significantly blocked the pressor response ($p<0.01$). Drinking was also antagonized by DuP (64%), and partially by EXP (50%). The results show that the central vasopressin and blood pressure responses to Ang II i.v.t. are mediated by AT-1 receptors and the drinking response is mediated by AT-1 and partly by AT-2 receptors.

473.6

A D1-DOPAMINE RECEPTOR AGONIST AND ANGIOTENSIN II STIMULATE OXYTOCIN RELEASE IN THE LACTATING RAT VIA ACTIONS IN MAGNOCELLULAR HYPOTHALAMIC REGIONS. S.L. Parker and W.R. Crowley. Dept. of Pharmacology, University of Tennessee-Memphis, Memphis, TN 38163.

Dopamine activates OT release in the lactating rat, and our recent results suggest that the D1 receptor is preferentially involved (Neuroendocrinology 53: 493, 1991). The objective of the present studies was to test whether D1 stimulation of OT release occurs via an action in the hypothalamic magnocellular nuclei. Lactating Holtzman rats received cannula implants in the third ventricle (3v) or unilaterally above the paraventricular nucleus (PVN) or supraoptic nucleus (SON) at 4-7 days postpartum. Seven days later the D1-specific dopamine agonist SKF 38393 (SKF) was injected into the 3v at 12.5 or 50 μg/5 μl or into the PVN or SON regions at 8 μg/1 μl. Controls received an equal volume of vehicle. All sites were subsequently treated with angiotensin II (AII; 95 pmoles) as positive control. SKF dose-dependenly elevated plasma OT at 5-60 min following injections into the 3v. Highly significant stimulation was also seen following injection into the SON, and a lesser, but significant, stimulation was apparent after treatment of the PVN. The increase in plasma OT after 50 μg SKF in the 3v was attenuated by prior iv injection of the D1 antagonist SCH 23390 (750 μg/kg). AII significantly stimulated OT release from all three injection sites, but the same dose was ineffective after iv administration. Plasma OT levels were unaffected by vehicle injection at any site. SKF in the 3v also dose-dependently increased plasma concentrations of prolactin (PRL) and produced a strong priming of PRL release to subsequent 3v injection of AII.

473.7

OSMOSENSITIVITY OF MAGNOCELLULAR NEUROSECRETORY CELLS (MNCs) ISOLATED FROM THE SUPRAOPTIC NUCLEUS OF THE ADULT RAT. Stephane H.R. Oliet and Charles W. Bourque. *Centre for Research in Neuroscience. Montreal General Hospital and Mc Gill University, Montreal, PQ.*

Small (1-5%) increases in plasma osmolarity induce the release of oxytocin and vasopressin from the axon terminals of MNCs in the neurohypophysis. While a number of other osmoresponsive inputs may be involved, an endogenous osmosensitivity of MNCs is thought to be important for the in vivo response. We have examined the effects of small hyperosmotic stimuli on MNCs and non-MNCs acutely dissociated from the adult rat. Immunostaining revealed that 96% of the neurons with a cross-sectional area (CSA) > 160 μm^2 are oxytocin or vasopressin-positive. In response to a 3-10% increase in osmolarity (+ Mannitol), all cells (MNCs and non-MNCs) showed small reductions in CSA (< 5%). Whole-cell current clamp recordings from MNCs revealed reproductible and reversible depolarization and increase in firing rate (n=10). Non-MNCs (CSA < 50 μm^2) failed to show either response (n=6). Under voltage-clamp the response of MNCs was associated with the appearance of an ionic current reversing around -20 mV (n=3). These results confirm the intrinsic osmosensitivity of MNCs . The persistence of this response in isolated MNCs in the whole-cell configuration opens the way to an analysis of signal transduction in a physiological osmoreceptor. Supported by FCAR, FRSQ and MRC.

473.8

DISTRIBUTION AND ORIGINS OF GABAergic PROJECTIONS TO THE PARAVENTRICULAR NUCLEUS. B.L. Roland, M.R. Brown and P.E. Sawchenko, The Salk Institute, La Jolla, CA 92037 and UCSD Medical Center, San Diego, CA 92103.

Axonal transport and immunohistochemical methods were used to characterize the organization of glutamic acid decarboxylase-immunoreactive (GAD-IR) projections to the paraventricular nucleus of the hypothalamus (PVH) in the rat. In line with prior reports, GAD-IR varicosities were found to be densely and quite uniformly distributed throughout the hypothalamus, including the PVH and the supraoptic nucleus (SO). Small crystalline implants of the retrograde tracer, true blue, into the PVH labeled GAD-IR cells in the anterior periformical region, portions of the anterior hypothalamic area immediately ventral to the PVH and just doral to the optic tract at the level of the SO, and within the PVH, itself. Because possible uptake of retrograde tracer by local dendritic processes might have yielded false positive results, a combined anterograde transport (PHA-L)-immunohistochemical approach was used in an attempt to confirm some putative local sources of GAD-IR inputs. Tracer injections in the region immediately ventral to the PVH, or within the anterior third of the nucleus, labeled moderate axonal projections to the PVH; a variable, and generally small, proportion of anterogradely labeled axons and terminals in the PVH also displayed GAD-IR. Some PHA-L injections also labeled GAD-IR projections to the SO. These results suggest that GABAergic projections to the various visceromotor cell types in the PVH and SO arise at least principally from several diffusely distributed local sources. Peri- and intra-nuclear GABAergic neurons could provide an intermediary by which documented (and generally inhibitory) limbic system influences on neuroendocrine function are exerted.

473.9

ELECTROPHYSIOLOGICAL DIFFERENCES BETWEEN IDENTIFIED OXYTOCIN AND VASOPRESSIN NEURONS RECORDED INTRACELLULARLY FROM RAT SUPRAOPTIC NUCLEUS *IN VITRO*.

W.E. Armstrong, B.N. Smith & M. Tian. Dept. of Anat. Neurobiol., Univ. Tenn., Memphis, Memphis, TN 38163.

Oxytocin (OT)- and vasopressin (VP) -secreting neurons exhibit different patterns of spontaneous and evoked activity *in vivo*. To determine whether differences exist in the membrane properties of these two cell types, supraoptic neurons were recorded intracellularly *in vitro*, injected with biocytin or Neurobiotin, and immunocytochemically identified as either OT or VP-containing.

OT and VP neurons exhibited similar I/V relationships, input resistances, and membrane time constants. In response to positive pulses eliciting brief spike trains, both types exhibited spike frequency adaptation, although the amount was variable across neurons. Both types exhibited a similar long lasting afterhyperpolarization following a spike train which was reduced by apamin (up to 100 nM), known to block a Ca^{++}- dependent K^+ current in these neurons. The great majority of VP neurons exhibited a depolarizing afterpotential (DAP) following brief spike trains. While most OT neurons did not exhibit a DAP, a minority did. Continuous current injection which altered the membrane potential failed to force OT neurons to exhibit phasic bursting activity, including those few with a DAP. Many VP neurons adopted or spontaneously exhibited the phasic pattern, but several did not, and instead fired continuously, not unlike OT neurons.

The results on phasic bursting are consistent with those from several other labs, i.e., neurons exhibiting this pattern contain VP. However, the lack of this activity, or the expression of continuous firing, is clearly not a unique signature for OT neurons. Similarly, while the voltage-sensitive DAP is undoubtedly related to the phasic, bursting pattern, its presence, while characteristic of, is not unique to VP neurons. Supported by NIH #NS23941(WEA) and NIMH #MHO9933 (BNS).

473.10

THE EFFECTS OF HISTAMINE ON IMMUNOCYTOCHEMICALLY IDENTIFIED VASOPRESSIN NEURONS IN RAT SUPRAOPTIC NEURONS RECORDED INTRACELLULARLY *IN VITRO*.

B.N. Smith and W.E. Armstrong Dept. of Anat. Neurobiol., Univ. Tenn., Memphis, Memphis, TN 38163.

The effects of histamine on immunocytochemically identified vasopressin neurons in the rat supraoptic nucleus were investigated using intracellular recording techniques from the hypothalamo-neurohypophysial explant. Exogenous application of histamine (100 nM-100 μM) to vasopressinergic neurons results in a 3-10 mV membrane depolarization, accompanied by an increase in the amplitude of the depolarizing afterpotential and a coincident decrease in the afterhyperpolarizing potential following current-evoked trains of action potentials. The amplitude of the depolarizing afterpotential is also enhanced by histamine when the afterhyperpolarizing potential is blocked by 100-200 μM d-tubocurarine, as well as in the presence of 3 μM tetrodotoxin and 200 μM d-tubocurarine, indicating a post-synaptic action of histamine on the depolarizing afterpotential that is not simply a reflection of a decrease in the afterhyperpolarizing potential. In addition, the depolarizing afterpotential is enhanced by histamine in the presence of the histamine H_2-receptor antagonist, cimetidine (1 μM), or by the histamine H_1-receptor agonist 2-thiazolylethylamine (0.1-1 mM). Although preliminary, this result suggests that the effects of histamine on vasopressin neurons in the SON are mediated by the H_1-receptor.

The present findings indicate that histamine directly enhances the depolarizing afterpotential following current-evoked trains of action potentials observed in vasopressin neurons, possibly via histamine H_1-receptors.

Supported by NIMH Predoctoral Fellowship #MH09933 (B.N.S.) and NIH grant #NS23941 (W.E.A.).

473.11

IBOTENIC ACID LESIONS OF MEDIAN PREOPTIC NUCLEUS DO NOT INFLUENCE BARORECEPTOR INDUCED INHIBITION OF SUPRAOPTIC VASOPRESSIN-SECRETING NEURONS IN THE RAT.

R. Nissen, J.T. Cunningham, A.M. Allen & L.P. Renaud, Neuroscience Unit, Ottawa Civic Hospital, Ottawa, Ontario, Canada. K1Y 4E9.

Electrical stimulation of two forebrain structures, the diagonal band of Broca (DBB) and the median preoptic nucleus (MnPO), induces $GABA_A$ receptor mediated synaptic inhibition of hypothalamic supraoptic vasopressin (VP) secreting neurons. The DBB relays inhibitory baroreceptor information associated with increases in blood pressure to VP-secreting neurons. The purpose of this study was to determine whether MnPO neurons are similarly involved in baroreceptor induced suppression of supraoptic VP neurons. Male Long-Evans rats were anesthetized with pentobarbital and stereotaxically injected with 0.25μl of ibotenic acid (5μg/μl) into the DBB or MnPO. Following a minimum of 3 days recovery, animals were reanesthetized with pentobarbital and prepared for extracellular recording from identified SON neurons using a transpharyngeal approach. The spontaneous activity of the VP neurons tested in control, DBB lesioned or MnPO lesioned rats were not different (8.5±0.7, 7.0±0.6, 9.9±0.8 spikes/sec respectively). While acute increases in blood pressure, achieved by intravenous administration of metaraminol (10μg/10μl), reliably arrested the activity of 18/18 neurons in the control group, ibotenate lesions in DBB significantly reduced the number of VP neurons responding to similar blood pressure elevations (8/21). In contrast, MnPO lesions had no effect on the sensitivity of vasopressin neurons to baroreceptor induced cessation in activity (24/29). Thus, MnPO neurons, which are involved in body fluid homeostasis, are not necessary for the transmission of inhibitory baroreceptor information to the supraoptic VP-secreting neurons. (Supported by FRSQ, Ontario Heart & Stroke, MRC & NHMRC of Australia)

473.12

INJECTIONS OF NOREPINEPHRINE (NE) IN THE DIAGONAL BAND OF BROCA (DBB) ATTENUATE THE ACTIVITY OF RAT SUPRAOPTIC (SON) VASOPRESSIN NEURONS.

J.T. Cunningham, R. Nissen, & L.P. Renaud, Neuroscience Unit, Ottawa Civic Hospital, Ottawa, Ontario Canada K1Y 4E9.

In the rat, electrophysiological studies have demonstrated that transient drug-induced elevations in arterial blood pressure, which are sufficient to activate peripheral baroreceptors, produce a brief cessation in the spontaneous activity of SON magnocellular neurosecretory cells. This response is selective for vasopressin-secreting neurons and appears to be mediated by the noradrenergic innervation of the DBB. The present study evaluated whether or not direct NE injections into the DBB could similarly arrest the spontaneous activity of SON vasopressin secreting neurons. Extracellular recordings were obtained from antidromically identified SON neurons in pentobarbital anesthetized male, Long-Evans rats using a transpharyngeal approach. A triple barrel injection pipette containing NE (10 uM), pontamine sky blue, and the artificial CSF vehicle (with 0.1 % ascorbic acid) was placed in the DBB. Neurosecretory neurons were characterized as either vasopressin- or oxytocin-secreting based on their responses to increases in blood pressure produced by intravenous injections of metaraminol (10 ug/10 ul). Injections of 200 nl NE into the DBB region arrested the spontaneous activity of 78% of the SON vasopressin-secreting neurons. In contrast, only 11% of the oxytocin secreting-neurons showed a comparable decrease in excitability to the same NE injections. Vehicle injections did not influence the activity of any of the neurons tested. No changes in blood pressure were associated with the NE injections in the DBB. These results are consistent with the hypothesis that the baroreceptor-sensitivity of vasopressin neurons is mediated by a NE mechanism in the DBB. (Supported by Heart & Stroke Fond. of Ont., FRSQ and the MRC)

473.13

SODIUM DEPRIVATION BLUNTS HYPOVOLEMIA-INDUCED NEUROHYPOPHYSEAL SECRETION OF OXYTOCIN (OT) AND VASOPRESSIN (AVP) IN RATS.

E.M. Stricker, J.G. Verbalis. Departments of Behavioral Neuroscience and Medicine, University of Pittsburgh, Pittsburgh, PA 15260.

The present studies determined the effects of dietary sodium intake on neurohypophyseal secretion of OT and AVP in response to plasma volume deficits in rats. Adult male rats were fed either standard sodium-rich diet or pelleted sodium-deficient (NaD) diet for 8 days, and then were injected sc with 5-10 ml of 30% polyethylene glycol (PEG) solution to induce hypovolemia. In rats maintained on either diet, secretion of OT did not begin until plasma volume deficits reached 25-30%, while AVP secretion increased gradually over this range. However, when hypovolemia was more pronounced, secretion of both hormones was significantly blunted in rats given NaD diet. These effects did not reflect a reduced responsiveness of the neurohypophyseal system because normal secretion of AVP and OT was observed after iv infusion of 2 M NaCl. Nor did they reflect insensitivity to hypovolemia because rats allowed access to water drank normally after 30% PEG treatment (13.9 ± 0.7 ml in 7 hr). Instead, the results likely represent a specific reduction of the stimulatory baroreceptor inputs into OT and AVP neurons during dietary sodium deprivation. In light of the pronounced salt appetite of rats maintained on NaD diet for 8 days, and the known co-activation of magnocellular and parvocellular OT neurons under some circumstances, the present observations are consistent with recent findings relating activity in central OT neurons with inhibition of salt appetite in rats.

473.14

CENTRAL SOMATOSTATIN INHIBITS CHOLECYSTOKININ-STIMULATED RELEASE OF MAGNOCELLULAR OXYTOCIN IN CONSCIOUS RATS.

R.E. Blackburn, E.M. Stricker, and J.G.Verbalis. Departments of Behavioral Neuroscience and Medicine, University of Pittsburgh, Pittsburgh, PA 15261.

Recent evidence has demonstrated a direct projection from the nucleus tractus solitarius (NTS) to oxytocin (OT) cells in the hypothalamic supraoptic nucleus (SON) that contains neurons immunoreactive for somatostatin (SS-28). Since NTS afferents are known to influence release of OT in response to peripheral cholecystokinin (CCK) and since SS-28 has been shown to suppress activity of SON cells in vitro, we studied the effect of central SS-28 administration on CCK-stimulated OT release. Conscious male rats were given either SS-28 (300 ng/rat in 5 μl) or vehicle (aCSF, 5 μl) icv. Blood samples for basal plasma OT levels were obtained 30 min later, or rats were injected with CCK (10 μg/kg or 100 μg/kg, ip) and then sampled 5 min later. In another group, plasma OT levels were measured 30 min after administration of hypertonic saline (2M NaCl, 2 ml, ip). Plasma OT levels (pg/ml) in each group are summarized below (** $p < 0.01$ compared to aCSF-treated control rats):

	aCSF (n)	SS-28 (n)
basal	4.2 ± 1.1 (5)	5.6 ± 1.5 (7)
CCK: 10 μg/kg	35.2 ± 2.4 (10)	7.8 ± 1.1 (12)**
100 μg/kg	42.2 ± 17.8 (7)	8.6 ± 2.4 (5)**
2M NaCl	28.1 ± 9.6 (7)	19.6 ± 4.9 (5)

These data indicate that central injection of SS-28 selectively inhibits CCK-induced OT release, while leaving osmotically-stimulated OT release intact. Our results therefore provide one of the first demonstrations of the importance of specific OT cell-innervating NTS pathways on neurohypophyseal secretion in conscious rats.

473.15

NALOXONE INHIBITS SODIUM APPETITE IN THE HYPOVOLEMIC RAT BY A MECHANISM INVOLVING CENTRAL OXYTOCIN. J.G. Verbalis, R.E. Blackburn and E.M. Stricker. Departments of Medicine and Behavioral Neuroscience, University of Pittsburgh, Pittsburgh, PA 15261.

Sodium appetite in the rat is commonly associated with suppressed plasma oxytocin (OT) levels and can be inhibited by a variety of treatments that lead to increased pituitary OT secretion. Systemic administration of OT does not, however, abolish saline ingestion, and this has led us to postulate that it is central rather than peripheral OT which mediates the sodium appetite. To test our hypothesis, we investigated the effect of peripheral administration of naloxone, a treatment known to stimulate both peripheral and central OT secretion, on saline ingestion in the polyethylene glycol (PEG)-induced hypovolemia model of sodium appetite. Male rats were treated with PEG (30% w/w, 5 ml, sc) and deprived of food and water for 24 h, after which they were injected with either naloxone (2.5 mg/kg in 2 ml, ip) or isotonic saline (2 ml, ip) 30 min prior to a 5-h drinking test period. Naloxone administration was associated with a marked increase in plasma OT levels (252.0 ± 45.4 pg/ml, n=9 compared to 56.9 ± 6.0 pg/ml in saline-treated PEG rats, n=6, $p<0.001$). In one-bottle (0.5 M NaCl) drinking tests naloxone completely abolished the sodium ingestion observed in control rats (5-h intake = 8.3 ± 0.9 ml). This inhibition of 0.5 M NaCl ingestion by naloxone could be reversed by prior icv administration of the OT-receptor antagonist [d(CH_2)$_5$,Tyr(Me)^2,Orn^8]vasotocin at doses of either 10 μg (5-h intake = 5.9 ± 1.4 ml) or 1 μg (5-h intake = 4.1 ± 0.6 ml). These data demonstrate that naloxone, like other stimuli for OT release, completely inhibits sodium appetite in the hypovolemic rat. Since this inhibition could be reversed by icv pretreatment with an OT-receptor antagonist, the results also provide evidence that brain OT participates in naloxone-mediated inhibition of hypovolemia-induced sodium appetite in rats.

473.16

CENTRAL ADMINISTRATION OF SEROTONIN INCREASES PLASMA VASOPRESSIN LEVELS BY ACTIVATION OF 5-HT$_{2/1C}$ RECEPTORS. R.H. Alper, P.E. Pérgola, A.F. Sved and J.L. Voogt. University of Kansas Medical Center, Kansas City, KS 66103 and University of Pittsburgh, Pittsburgh, PA 15260.

We have demonstrated that intraventricular (icv) serotonin (5-HT) increases blood pressure and decreases heart rate by activation of 5-HT$_{2/1C}$ receptors in conscious rats (Pérgola and Alper, *Am J Physiol*, in press, 1991). The bradycardia, but not the hypertension, is eliminated by iv pretreatment with a V_1-vasopressin antagonist. To determine if 5-HT acts at the 5-HT$_{2/1C}$ receptor to increase plasma vasopressin (AVP) levels, 5-HT (2.5 μg in 5 μl saline containing 0.1% ascorbic acid) was administered icv to conscious male Sprague-Dawley rats instrumented with venous and arterial catheters. Arterial blood samples (0.5 ml, replaced with sterile saline) were obtained prior to and 5, 15 and 30 minutes after the injection of 5-HT. In rats pretreated with saline iv, 5-HT increased plasma AVP at 5, but not 15 or 30 minutes; the icv injection of vehicle did not alter AVP. In rats pretreated with the selective 5-HT$_{2/1C}$ receptor antagonist LY 53857 (100 μg/kg, iv), 5-HT was no longer capable of increasing AVP. LY 53857 alone did not alter plasma AVP. In addition, prolactin and corticosterone were measured in these same rats. As anticipated, 5-HT increased plasma levels of both hormones. In contrast, plasma renin activity was not affected by 5-HT. The 5-HT-induced increase in prolactin and corticosterone was not altered by the 5-HT$_{2/1C}$ antagonist. These data suggest: 1) 5-HT increases AVP secretion by activation of 5-HT$_{2/1C}$ receptors following a time-course similar to that observed previously in our hemodynamic studies, and 2) although 5-HT was also capable of increasing the secretion of prolactin and corticosterone, these effects are not mediated exclusively by activation the same 5-HT receptor subtype.

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473.17

CENTRAL CATECHOLAMINERGIC PATHWAYS ARE REQUIRED FOR VASOPRESSIN RELEASE FOLLOWING SPLANCHNIC OSMORECEPTOR ACTIVATION. M.S. King and A.J. Baertschi, Neuroscience Program, University of Virginia, Charlottesville, VA 22908.

Previous studies in conscious rats demonstrated that peripheral osmoreceptors located at post-absorptive sites within the splanchnic mesentery are activated following hypertonic gastric infusions and project to the CNS through a spinal, and not a vagal, afferent pathway (Choi-Kwon and Baertschi, AJP, '90,'91). Electrolytic lesions indicated that, within the CNS, the osmotic pathway traverses the pontine reticular formation about 1mm below the locus coeruleus (LC; King and Baertschi, Br. Res., '91). In the current set of experiments, to determine the role of cell bodies and fibers of passage in this area, chemical lesions were induced one week prior to gastric infusions of 598 mOsm/kg (2ml/4min). Microinjection of 6-hydroxydopamine (6-OH-DA; 4ug/400nl; n=6) reduced the AVP response to hypertonic gastric infusions by 66.0% ($p<.05$; mean increase over baseline was 2.83 ± .49 pg/ml), indicating that a catecholaminergic component in this region is required for the AVP response. The effect of 6-OH-DA was reversed by desipramine pretreatment (25mg/kg; n=6; 5.78 ± .88 pg/ml). Ibotenic acid injections did not alter the AVP response (n=6; 4.83 ± 1.02 pg/ml), indicating that fibers of passage, and not cell bodies, in this area are part of the central osmotic pathway. Finally, microinjections of 5,7-dihydroxytryptamine (n=6; 5.36 ± 1.54 pg/ml) and vehicle (n=7; 7.58 ± 2.29 pg/ml) also did not alter the AVP response. These results demonstrate that a catecholaminergic pathway, which traverses the pons about 1mm below the LC, is a vital part of the central neural pathway which carries peripheral osmotic information to the hypothalamus. The origin of this pathway (i.e. NTS or VLM) and possible forebrain relays (i.e. preoptic area) are currently being investigated. [Supported by NSF BNS-8819877 and NIH RO1 NS27644].

473.18

DRINKING AND GLUCOSE UTILIZATION IN THE MAGNOCELLULAR SYSTEM (MCS) AFTER OSMOTIC AND Na STIMULATION. M. Kadekaro, M.L. Terrell, J.S. Harris*, S. Freeman*, J.Y. Summy-Long§, E.M. Koehler*§. Div. of Neurosurgery, UTMB, Galveston, TX; §Dept. of Pharmacology, Hershey Med. Ctr., Penn. State Univ., Hershey, PA.

We compared in male albino rats (n=71) the effect of jugular infusion (200 μl/min for 10 min) of 0.15 M NaCl, 0.85 M NaCl, 1.7 M mannitol or 1.7 M mannitol + 0.85 M NaCl on water intake and on the activity of the MCS, measured with the [^{14}C]deoxyglucose method. Plasma osmolality (mOsm/kg H_2O) increased after 0.85 M NaCl (Δ=14), 1.7 M mannitol (Δ=13) and mannitol + NaCl (Δ=26). Plasma [Na^+](mEq/l) either increased with NaCl (+8.8), decreased with mannitol (-7.7) or was unchanged after the combination of both. Drinking was similar in all groups but the activity of the MCS was stimulated to different degrees. Increases in glucose utilization in the supraoptic n. (SON, +18%) and neural lobe (NL, +52%) were observed with 0.85 M NaCl but only in the NL (+34%) with mannitol. With mannitol + NaCl glucose utilization increased in the SON (+58%), paraventricular n. (+28%) and NL (+152%). These increases were larger than the sum of changes induced by infusion of individual solutions.

Combined osmotic and hypertonic NaCl treatments, therefore, potentiate the activity of the MCS but not the drinking response. [Supported by NIH grants NIDS 2RO1 NS23055 (M.K.) and HD25498 (J.S-L.)].

473.19

FLUOXETINE INDUCES VASOPRESSIN ABNORMALITIES IN ACTIVITY-BASED ANOREXIA. P.F. Aravich, T.S. Rieg, I. Ahmed, S. Downing & T.J. Lauterio. Depts. Anatomy/Neurobiology & Internal Medicine, Eastern Virginia Medical School, Norfolk, VA 23501; V.A. Med. Ctr., Hampton, VA 23667.

Vasopressin (VP) and serotonin (5HT) abnormalities occur in anorexia nervosa (AN). It has been proposed that fluoxetine (FLX), an indirect acting 5HT agonist, may be useful in the treatment of AN. Because of the relationship between exercise and AN, we have been exploring activity-based anorexia (ABA) in the rat (restricted feeding 1.5 hrs/day; free running wheel access 22.5 hrs/day). This experiment determined the effects of FLX on VP in ABA. Suprachiasmatic (SCN), circulating and dorsal vagal complex (DVC) VP systems were evaluated. Adolescent male rats (N=83) were divided into ABA (following a 25% weight loss) and control groups, which included weight-matched (BWM), exercised (EXR) and non-exercised freely fed (AD LIB) rats. Subgroups were injected with FLX HCl (15 mg/kg, ip) or vehicle under resting, food-deprived conditions. The animals were killed by decapitation 0.5 hrs after injection. Fluoxetine induced ABA-specific VP abnormalities in the SCN but not the circulating or DVC VP systems. Specifically, FLX elevated SCN VP content in ABA rats but not controls. As a result, while basal SCN VP levels in ABA were not different from BWM and AD LIB controls, stimulated levels were reliably elevated compared to these groups. Fluoxetine caused an overall increase in plasma VP across groups. The BWM controls---but not the ABA and EXR rats---had elevated plasma VP levels under basal conditions. Finally, there was no effect of FLX (or behavioral treatment) on DVC VP content. Consequently, ABA is associated with specific 5HT abnormalities in the SCN VP system. Since disrupted circadian rhythms increase susceptibility to ABA (see ES Stanley et al., this meeting), these data raise concerns regarding the potential negative impact of FLX on circadian rhythms in anorexia nervosa. Support: V.A. Merit Award (PFA).

473.20

ORIGIN OF NEUROHYPOPHYSIAL MAMMALIAN FMRF-NH$_2$-LIKE PEPTIDE. E.A. Majane and H.-Y.T. Yang. Lab. of Biochem.Genetics, NIMH Neuroscience Ctr.@ St. Elizabeths, Washington, DC 20032.

The mammalian FMRF-NH2-like peptide, F-8-F-NH$_2$ (NPFF), is concentrated in nerve fibers and terminals of the neural lobe (NL)of rat pituitary where it can be regulated by osmotic stimuli. In this study, the source of pituitary NPFF was investigated by various lesions. Seven days following surgery, the content of NPFF in the NL was depleted by pituitary stalk transection implicating the hypothalamus as the source of pituitary NPFF. A group of NPFF positive cell bodies in a periventricular region of hypothalamus was revealed immunohistochemically however a lesion of this region failed to affect pituitary NPFF. Since the regulation of NL NPFF and AVP correlates very closely, we chose to lesion the AVP-containing magnocellular neurons of the supraoptic nuclei. Seven days following a bilateral lesion of the SON, the pituitary levels of both AVP and NPFF were reduced to 50% of control. These results strongly suggest that at least part of the NPFF in the NL arises from SON where it may be co-localized with AVP.

473.21

THE EFFECT OF SALT LOADING ON SEROTONERGIC ACTIVITY IN SPECIFIC BRAIN REGIONS OF THE RAT: J.A. Saydoff and M.S. Brownfield. Neurosci. Training Prog., Sch. Vet. Med., Univ. Wisconsin, Madison, WI 53706.

The aim of this study was to establish potential brain sites where serotonin (5-HT) influences osmoregulation of vasopressin. We previously demonstrated that depletion of brain 5-HT with 5,7-dihydroxytryptamine blocks the increase in plasma vasopressin due to i.p. hypertonic saline administration.

Groups of 10 male rats were given water (W) or 2% NaCl (SL) to drink for for 2 days and killed by decapitation. The midbrain (MB), hippocampus (HI), basal hypothalamus (BHT), dorsal hypothalamus (DHT), ventral forebrain (VF), septum (SE), and caudate nucleus (CN) were dissected. High pressure liquid chromotograhy with electrochemical detection was used to measure 5-HT, 5-hydroxyindoleacetic acid (5-HIAA), and dihydrophenylacetic acid (DOPAC). 5-HIAA and DOPAC are the major metabolites of 5-HT and dopamine respectively. The 5-HIAA/5-HT ratio was used as one of the indexes of serotonergic activity.

Analysis of covariance by brain region plotting 5-HIAA vs 5-HT revealed a similar pattern for the MB, BHT, and VF regions. This striking pattern in the MB, BHT, and VF indicated that salt loading increased both 5-HT and 5-HIAA. The SL group 5-HIAA/5-HT ratio was increased compared to the W group in the MB ($p < .01$) and CN ($p < .05$). The SL group 5-HIAA was increased compared to the W group in the BHT ($p < .01$) and HI ($p < .05$). Salt loading decreased 5-HT in the CN ($p < .05$). There were no significant changes in DOPAC concentration due to salt loading suggesting dopamine is not involved in the osmoregulatory response to salt loading.

These results demonstrate salt loading increases serotonergic activity in the brain regions that previous lesion studies have proven to be essential for osmoregulation of vasopressin. This study suggests a serotonergic pathway linking the MB to the BHT and VF is the circuit for osmoregulation of vasopressin by 5-HT. Supported by Univ. Wisc. Grad. Sch.

473.22

GLUTAMATE-INDUCED RELEASE OF VASOPRESSIN FROM THE ACUTE HYPOTHALAMO-NEUROHYPOPHYSIAL EXPLANT.

R.S. Greenwood, R.B. Meeker and J.N. Hayward. Dept. of Neurology and Pediatrics and Medicine and Neurobiology Curriculum, University of North Carolina, Chapel Hill, NC 27599

Recent studies (Meeker et al, 1989, 1991; Swanson et al, 1991; Van den Pol et al 1990) suggest that glutamate plays an important role in the excitatory control of vasopressinergic magnocellular neuroendocrine cells. To assess the influence of glutamate on vasopressin secretion we stimulated acute hypothalamo-neurohypophysial explants from male Sprague-Dawley rats in vitro with 10^{-5} M glutamate by perfusion or by direct microinjection into the supraoptic nucleus. Glutamate administered by microinjection or by perfusion induced a significant and rapid release of vasopressin not observed in hypothalamo-neurohypophysial explants from controls or in hypothalamo-neurohypophysial explants perfused or microinjected with other putative neurotransmitters. These results indicate that glutamate is a potent stimulus for vasopressin release even when locally applied to the supraoptic nucleus. The ablility of glutamate to induce vasopressin release from the hypothalamo-neurohypophysial explant is consistent with its proposed role as an important excitatory neurotransmitter in the vasopressinergic magnocellular neuroendocrine system.

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473.23

QUANTITATIVE MAPPING OF GLUTAMATE IMMUNOREACTIVE PRESYNAPTIC TERMINALS IN THE REGION OF THE SUPRAOPTIC NUCLEUS.

D.J. Swanson*, R.B. Meeker, R.S. Greenwood and J.N. Hayward, Department of Neurology and Neurobiology Curriculum, University of North Carolina, Chapel Hill, NC 27599.

To evaluate the relative contribution of putative glutamatergic synapses to the innervation of magnocellular neuroendocrine cells in the rat, thin sections were stained with colloidal gold using an anti-glutamate antibody. Electron micrographs taken from the main body of the supraoptic nucleus (SON), the ventral dendritic neuropil (VDN) of the SON and the perinuclear zone (PNZ) dorsal to the SON were evaluated for the presence of glutamate immunoreactivity in presynaptic endings. At least two populations of presynaptic endings were apparent based on relative densities of colloidal gold postembedding staining: 1) putative glutamate terminals with a broad distribution of colloidal gold densities (26 to 100 particles/um^2) and 2) non-glutamate terminals with low densities of colloidal gold (0-25 particles/um^2. As many as 45% of the total terminals were glutamate-immunoreactive in the SON and VDN although the absolute number of terminals in the VDN was more than 50% greater than in the SON. The PNZ received the greatest density of total presynaptic terminals of the three regions sampled. However, a lower percentage (33%) of the terminals in the PNZ were glutamate immunoreactive. These results indicate that a large portion of the synaptic input in the basolateral hypothalamus represents glutamate-containing terminals. We consider it likely that glutamate or a glutamate-like transmitter is used extensively at excitatory amino acid receptors in the neuroendocrine hypothalamus.

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ENDOCRINE REGULATION III

474.1

CONVERGENCE OF INNERVATION IN THE PITUITARY INTERMEDIATE LOBE.

S. Soinila, N. Bäck and G.J. Mpitsos. Dept. Anat., Univ. Helsinki, Finland and Oregon State Univ., Marine Sci. Ctr, Newport, OR 97365.

The intermediate lobe of the rat pituitary consists of a homogenous population of endocrine cells synthesizing α-MSH and β-endorphin. The intermediate lobe cells secrete these hormones under neural regulation from several loci. Several neurotransmitter-specific systems are involved in this regulation. However, it is not known whether each system affects a particular subpopulation of cells or whether all cells receive multiple innervation. We have used immunohistochemical techniques to visualize serotonin-, dopamine-, GABA-, enkephalin- and acetylcholine-containing systems, and computerized image analysis to study the distribution of these innervation systems within the lobe. Colocalization of these substances was also examined. Our results favor the notion that most if not all intermediate lobe cells receive innervation from several brain nuclei. This makes the intermediate lobe a useful model to study the mechanisms of multitransmitter regulation by converging innervation.

474.2

RELEASE OF cLHRH-I IN VITRO: EFFECTS OF CATECHOLAMINES AND AGING.

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The release of cLHRH-I was monitored in perfusion of microdissected slices of medial basal hypothalamus (MBH) in response to norepinephrine (NE), epinephrine (E) and isoproterenol. Tissue slices from an animal were equilibrated in a perfusion chamber for 2 hrs in media 199. Using separate tissue samples for each chemical, dose response curves were conducted (6 replicates/curve). Significant increases of LHRH were observed with 10^{-7}M NE and 10^{-8}M E. LHRH release was also slightly increased by isoproterenol (10^{-7}M).

Two experiments were conducted to determine if reproductive aging affects response of hypothalamic tissue to catecholamine challenge in vitro. In Experiment 1, hypothalamic slices from young hens (8 months old) and aged hens (2 yr) were challenged with NE(10^{-7}M). Slices from aged hens responded with a lower release of cLHRH-I than young hens. In Experiment 2, tissues from young (2 months) and aged (3 yrs) male Japanese quail were compared for cLHRH-I response to NE (10^{-6}M). Again, tissue from aged animals showed a lower response. Supported in part by Univ. of MD AES grant (MAO) and USDA 88-37242 (MAO).

474.3

CHARACTERIZATION OF GLUTAMATE RECEPTORS IN THE NEUROENDOCRINE HYPOTHALAMUS R.B. Meeker, R.S. Greenwood and J.N. Hayward, Department of Neurology and Neurobiology Curriculum, University of North Carolina, Chapel Hill, NC 27599.

NMDA and non-NMDA glutamate receptors were identified in the rostral hypothalamus-preoptic area (neuroendocrine hypothalamus) of the rat using [^{3}H]glutamate binding to washed membranes. Glutamate (1 mM) and 2-amino-5-phosphonovaleric acid (APV; 0.2 mM) were used to define specific total binding and specific non-NMDA binding, respectively. Non-NMDA type receptors greatly exceeded the number of NMDA receptors in the hypothalamus by a factor of approximately 10-fold. Saturation binding data revealed the presence of a single class of non-NMDA binding with a K_D of 0.2-0.4 x 10^{-6} M and a B_{max} of 130-150 pmoles/mg tissue. NMDA binding sites were barely detectable with an approximate K_D of 0.2 x 10^{-6} M and a B_{max} of 16-28 pmoles/mg tissue. This distribution of receptor subtypes contrasted with the hippocampus from the same brain where similar levels of of both NMDA and non-NMDA glutamate receptors were observed. Water deprivation for 48 hrs resulted in an increase in the number of non-NMDA receptors in the hypothalamus with no change in the K_D. Thus, the rostral hypothalamus appears to contain mostly non-NMDA receptors. A functional link between these receptors and the vasopressin neuroendocrine system which regulates water balance is suggested by the increase in receptors in response to water deprivation.

Supported by NIH Javits Award NS 13411

474.4

METABOTROPIC GLUTAMATE RECEPTORS ARE EXPRESSED IN PRIMARY CULTURES OF HYPOTHALAMIC NEURONS. M.A. Sortino, G. Aleppo*, F. Nicoletti and P.L. Canonico°. Institute of Pharmacology, University of Catania, School of Medicine, Catania, and ° Chair of Pharmacology, University of Pavia, School of Dentistry, Pavia, Italy.

Glutamate has been found to play an important role in neuroendocrine regulation in the hypothalamus. The control of neuroendocrine secretion by glutamate has been related mainly to activation of ionotropic glutamate receptors that are linked to the opening of ion channels. Thus, quisqualate, kainate and N-methyl-D-aspartate (NMDA) have been found to stimulate luteinizing hormone-releasing hormone (LHRH) and somatostatin release from the hypothalamus *in vitro*. We have previously shown that metabotropic glutamate receptors, whose activation results in the stimulation of phosphoinositide (PPI) hydrolysis, are present at the hypothalamus, and exhibit a typical developmental profile that may suggest a role for these receptors in the maturation of hypothalamic neurons. In the present study we have measured PPI hydrolysis induced by glutamate in primary cultures of hypothalamic neurons. Glutamate, as well as quisqualate, a metabotropic receptor agonist, produced a concentration-dependent stimulation of PPI turnover (as assessed by measuring [^{3}H]inositol monophosphate production) that appeared after 7 days of maturation in culture and was still present at 14 DIV. Ionotropic receptor agonists such as kainate, NMDA and α-amino-3-hydroxy-5-methyl-5-isoxazolpropionate (AMPA) were inactive. At 14 DIV, 100 μM glutamate induced a stimulation of 168 ± 18% of control whereas 100 μM norepinephrine activated PPI turnover by 233 ± 22%. Simultaneous addition of glutamate and norepinephrine resulted in an additive effect. The presence of glutamate metabotropic receptors in primary cultures of hypothalamic neurons, together with the lack of data regarding an involvement of this receptor in the control of neuroendocrine secretion, suggest a role for the metabotropic glutamate receptor in the maturation of hypothalamic neurons as already shown in other brain areas.

474.5

EFFECT OF SUSTAINED COLD EXPOSURE ON SOME PARAMETERS OF HYPOTHALAMIC-PITUITARY FUNCTION: *IN VIVO* AND *IN VITRO* STUDIES G. Cizza [1*], K. Fukuhara [2*], P.W. Gold [1] G.I. Perini [1], M.A. Kling [1] NIMH[1], NINDS[2], National Institute of Health (NIH) Bethesda 20892, USA,

The hypothalamic-pituitary-thyroid axis plays a major role in the metabolic response to cold exposure. We have previously demonstrated that the incubation temperature can modify the *in vitro* hypothalamic secretion of TRH, a peptide involved in the thermoregulation processes (Neuroscience 20th Meeting 1990;16 (1) 397 abstract # 168.7). In the present study we assessed how cold exposure affects hypothalamic-pituitary function. To perform this task, we exposed S-D male rats 4 months old to 4° C for 24 hours. At the end of the cold exposure the cold exposed animals and an adequate number of controls were rapidly decapitated, their blood collected and plasma kept at minus 20° C for subsequent hormonal determinations. Hypothalami from both cold-exposed and control animals were rapidly excised and incubated one per well in a 48 well-plate. After 2 h of preincubation, the hypothalami were exposed to plain media (M 199) in the first three wells, than to KCl 60 mM to assess the maximal secretory capacity, as well as the tissues' viability. The cold-exposed animals showed a significant increase in plasma TT_4 (8.49±0.50 vs 11.66±0.24 μg/dl; $p \leq .0001$ t test), whereas plasma TSH was unchanged (4.07±0.24 vs 4.51±0.26 ng/ml). Consistently with the plasma TSH, the *in vitro* TRH secretion both in basal conditions and under KCl-challenge was virtually the same (basal 34.75±1.80 vs 36.65±1.19 pg/well/20'; KCl 56.03±3.80 vs 55.85±4.35 pg/well/20'). All the explants responded to KCl stimulation, indirectly confirming tissues viability. Also plasma corticosterone (B) was increased in cold exposed animals (40.94±25.11 vs 134.14±17.95 ng/ml; $p \leq .009$ t test) whereas ACTH was unchanged (54.48±19.60 vs 66.29±24.85 pg/ml). We hypothesize that the TT_4 and B plasma increase observed in the animals exposed to cold is a residual effect of a concomitant activation of thyroid and glucocorticoid system, probably by noradrenergic system. The absence of modifications in plasma TSH and ACTH, as well as TRH *in vitro* secretion suggest that the activation of both axes takes place after less than 24 h of cold exposure.

We wish to thank the National Pituitary Agency for providing the materials used in the TSH assay.

474.6

ALPHA-2 ADRENERGIC INPUT IN LOCUS COERULEUS MODULATES THE RELEASE OF ADRENOCORTICOTROPIN IN CATS. D. E. Carlson and D. S. Gann. Depts. of Surgery and Physiology, Univ. of Maryland Sch. Med., Baltimore, MD 21201.

Previous evidence suggested that noradrenergic turnover in the vicinity of the ventrorostral locus coeruleus (vLC) increased in response to hemorrhage. To investigate the possible role of this response in the control of ACTH release, microinjections (100 nl/min for 2 min) of several agents were made at 42 sites in 21 cats anesthetized with chloralose. All sites were tested with L-glutamate (150 mM, GLU) and vehicle (VEH), 38 were tested with the α1 agonist, phenylephrine (1 mM, PE), and 18 were tested with the α2 agonist, clonidine (1mM, CLON). Plasma ACTH was measured by radioimmunoassay. Responses were tested statistically by repeated measures ANOVA. GLU at 9 sites in the region of the vLC elicited a significant increase in ACTH at 4 and 6 min ($P<0.01$) from the onset of injection. Injection of VEH at these sites and of PE at 8 of these 9 sites elicited no change in ACTH. Both of these latter responses differed from that to GLU ($P<0.01$). Injection of CLON at 6 sites that included all 4 of the sites in vLC that were tested with CLON and 2 additional sites immediately dorsal to vLC elicited a significant decrease in ACTH at 4 and 6 min ($P<0.05$) from the onset of injection that differed from the responses to VEH and GLU ($P<0.05$). Injections of all agents at sites remote from the region of the vLC elicited no change in ACTH. An increase in noradrenergic turnover in the vLC may provide inhibitory α2 modulation to neurons in the vLC that were shown previously to be activated by hemorrhage. This input may serve to modulate the influence of the vLC on ACTH release. Supported in part by NIH grant DK-26831.

474.7

CORTICOSTERONE MODULATES HIPPOCAMPAL PYRAMIDAL CELL ACTIVITY. S.G. Beck and K. Choi*, Department of Pharmacology, Loyola University Chicago Stritch School of Medicine, Maywood, Il 60153.

Adrenalcorticoid steroid receptors are present in hippocampal pyramidal cells. Intracellular recording techniques in hippocampal slices were used to measure chronic and short term effects of corticosterone on membrane properties of CA1 pyramidal cells and on $5HT_{1A}$-mediated hyperpolarization. Rats were adrenalectomized (ADX) for two weeks or ADX and implanted with a CT pellet (ADX+CT) to mimick basal CT levels. Blood samples were taken at the time of sacrifice to measure plasma CT and ACTH levels. During the experiment CT was present or not present in the stock buffer. Cell measurements included resting membrane potential, resistance, tau, spike characteristics, fast afterhyperpolarization (AHP) amplitude, and slow AHP amplitude and duration. Cells from ADX rats with undetectable CT levels had a lower membrane resistance than cells from ADX rats with CT levels less than 1 μg/dl, ADX+CT rats or ADX rats with CT in the buffer. The presence of CT in the buffer decreased the amplitude of the slow AHP in cells from ADX rats.

The Emax of the $5HT_{1A}$ hyperpolarization was larger with CT in the buffer for cells from ADX or ADX+CT treated rats. The EC_{50} and slope were not affected by CT. These results indicate that short term CT, not chronic CT, modulates cell characteristics and 5HT mediated responses. USPHS grants MH41917, NS28512, KO2-MH00880 to SGB.

474.8

CORTICOSTERONE REDUCES HIPPOCAMPAL GLYCOGEN CONTENT IN VIVO AND IN CULTURED ASTROCYTES. G.C. Tombaugh, R.A. Swanson, and R.M. Sapolsky, Dept. of Biol. Sci., Stanford Univ., Stanford, CA 94305, and V.A. Med. Ctr., Dept. of Neurology, UCSF, San Francisco, CA 94121.

Glucocorticoids (GCs) can exacerbate hippocampal neuronal injury following a variety of metabolic insults. The mechanism underlying this endangerment is unknown, but is thought to involve an impairment of metabolic energy potential. Given that these steroids can depress glucose uptake and enhance hypoxic vulnerability in hippocampal astrocytes, and that these cells represent the principal storage site for glycogen in the brain, we asked whether GC exposure could influence hippocampal glycogen content in vivo and the intracellular levels of glucose and glycogen in astrocyte cultures in vitro. Rats were adrenalectomized (ADX) for 3 days and received a single s.c. injection of corticosterone (CORT; 10mg/1ml). Brain tissue was fixed in situ by focused microwave radiation 1h, 6h, or 24h later. Hippocampal astrocyte cultures, derived from E18 rat fetuses, were grown for 10-11 days in DMEM with 10% FCS and 25mM glucose. Cells were refed for 24h with Krebs Ringer phosphate (5mM glucose) with or with 100nM corticosterone and then harvested for glycogen assay. Hippocampal glycogen content fell to 83% of the ADX control ($p<.01$) 6h post-injection and returned to control level by 24h. In contrast, cortical glycogen content remained unchanged at 6h but was significantly elevated at 24h. In hippocampal astrocytes, CORT reduced glycogen content to 62% of control ($p<.001$) without affecting intracellular glucose. These data suggest that GC secretion may enhance neuronal vulnerability in the hippocampus by causing a reduction in glial energy stores.

474.9

SECRETION OF MELANIN CONCENTRATING HORMONE FROM CULTURED RAT HYPOTHALAMIC CELLS. D.G.Parkes*, J.L.Nahon* K.A.Klivington and W.W.Vale. The Salk Institute, La Jolla, CA 92037 and IPMC, Sophia Antipolis, France.

Melanin concentrating hormone (MCH) is a cyclic nonadecapeptide present within the lateral hypothalamic region of the rat, man and guinea pig brain, defining a group of cell bodies with extensive projections throughout the brain. This suggests that MCH may play an important role in certain brain functions. In order to establish that the peptide is secreted by mammalian cells, we have examined the regulation of MCH secretion from primary hypothalamic cultures of 7 day old rats, employing a sensitive radioimmunoassay for MCH. Secretion was measured over a 6 hour time period. Basal secretion of MCH after 5 days of culture was 54±8 pg/dish/6h, 98±16 pg/dish/6h after 8 days, and increased to 190±11 pg/dish/6h after 10 days in culture and remained at this level for up to 2-3 weeks. On day 8 of culture, the cyclic AMP stimulant, forskolin (10μM), increased secretion to 146%. The cAMP analogue, 8-Br-cAMP (10mM) increased secretion to 573% of control, and 8-Br-cyclic GMP increased secretion to 261%. The phorbol ester, TPA (10nM), increased secretion to 133%. Cell content of MCH on days 5, 8, 10 and 12 of culture was 21±2, 89±14, 47±12 and 48±6 pg/dish respectively, and this was not significantly changed by any treatment. These results suggest that hypothalamic cells in culture do indeed produce MCH, and that this secretion may be regulated by multiple second messenger systems.

474.10

FUNCTIONAL CHANGES OF HYPOTHALAMIC CRF AND OPIOID NEURONS AFTER ADRENALECTOMY AND/OR CASTRATION. O.F.X.Almeida*,M.S.Harbuz*,C.Stein, E.A.Linton* & S.L.Lightman*. Max Planck Inst.for Psychiatry,Munich,FRG; Westminster Hospital, London,UK; University of Reading,UK.

The influences of short- and long-term adrenalectomy and castration (or both) upon CRF mRNA levels, CRF peptide levels, and endogenous opioid peptide (EOP) content in the hypothalamus, and basal and CRF-stimulated EOP release in vitro, were examined in male rats. Gonadal and adrenal steroids regulated the activity of these peptidergic systems in terms of peptide synthesis, storage pools and secretion. The steroids were also found to alter the sensitivity of EOPergic neurons to CRH. In some cases, evidence was obtained for an interaction between gonadal and adrenal steroids in determining these events (seen as additive or counteractive effects). A finding of major interest was that the response of these peptidergic systems was markedly influenced by the duration of steroid deprivation,the results of chronic treatment often being opposite to those of acute treatment. There was no simple relationship between peptide synthesis, storage and release, even within a single type of neuron.

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474.11

CENTRAL ADMINISTRATION OF NEUROTENSIN STIMULATES THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS AND IS BLOCKED BY THE CRF ANTAGONIST CRF 9-41. W. Rowe, Y.Viau, M.J. Meaney, R. Quirion. Douglas Hospital Research Ctr., Dept. of Psychiatry, McGill University, Montreal H4H 1R3, Canada.

The role of neurotensin (NT) as a neuromodulator in the CNS has focused mainly on the mesocorticolimbic dopaminergic system where NT receptors are localized on the majority of DA neurons. However, NT may also serve a neuroendocrine role as a modulator of HPA activity. Intraventricular (icv) administration of NT has been previously reported to increase circulating ACTH and corticosterone (B) levels (Gudelsky et al., Neuroendocrinology, 1989). We investigated the temporal profile of NT's effects as well as its possible mechanism of action. NT injected icv in freely moving adult male rats caused significant increases in plasma ACTH and B compared to saline treated controls: peak ACTH; 420 ± 64.7 vs 256 ± 30.6 pg/ml, respectively, peak B; 56.5 ± 8.5 vs 27.6 ± 2.75 μg/ dl, respectively. NT treated animals showed higher ACTH and B levels, 66% and 100 %, respectively, up to 4 h following injection: ACTH; 273.9 ± 18.4 vs 168.4 ± 9.7 pg/ml/min, respectively, B; 38.7 ± 6.4 vs 19.7 ± 1.1 μg/dl/min, respectively. Pretreatment with α-helical CRF (1 μmol/kg bw) attenuated the ACTH response to NT administration: 137.7 ± 47.4 pg/ml/min. These findings suggest that NT's actions occur, in part, at the level of the PVN to regulate the release of CRF. In support of this, it has recently been shown that immunoreactive (ir) NT is co-localized with parvocellular irCRF neurons of the paraventricular nucleus (PVN) projecting to the median eminence (Ceccatelli et al., Neuroendocrinology ,1989).

474.12

SEASONAL ALTERATIONS IN HYPOTHALAMIC NEUROTENSIN CONCENTRATIONS. G. Bissette, B. Levant, B. Banks and C.B. Nemeroff. Depts. Psychiat. and Pharmacol., Duke Univ. Med. Ctr., Durham, NC 27710

Neurotensin (NT) is a tridecapeptide neurotransmitter found in mammalian brain and gut. In the brain, NT is widely distributed within and outside of the hypothalamus, and is often found in close anatomic proximity to and colocalized with dopaminergic systems. The concentration of NT in the nucleus accumbens and striatum is increased by administration of antipsychotic drugs and certain "sigma" receptor antagonists. Previous work in this laboratory indicated that endogenous concentrations of NT were not constant throughout the year. To test this hypothesis, ten male Sprague-Dawley rats were killed at the middle of each month from July 1989 through July 1990. Brains were removed, frozen and subsequently dissected at one time. After dissection, hypothalami were extracted in HCL and NT was measured in duplicate aliquots by a single sensitive and specific radioimmunoassay. Values are reported as pg NT/mg protein. The seasonal concentrations of NT in the hypothalamus varied across the year with the nadir occurring in August through October and the apex in December through April (one-way ANOVA). The concentration of NT in December, March and April was significantly different from the concentration in August, September and October. Hypothalamic neurotensin concentrations exhibit seasonal concentration differences that must be taken into account in planning and interpreting the results of these types of radioimmunoassay experiments. (Supported by NIMH grant MH-39415).

474.13

CHANGES IN CALMODULIN COMPARTMENTALIZATION IN MDCK CELLS INDUCED BY MELATONIN. G. Benítez-King,* L. Huerto-Delgadillo,* and (1)F. Antón-Tay. Depto. Neurofarmacología, DIC, Instituto Mexicano de Psiquiatría. (1) Depto. Biología de la Reproducción,CBS, Universidad Autónoma Metropolitana-Iztapalapa. México, D.F. MEXICO.

It has been shown that incubation of MDCK cells with 10^{-9} M of melatonin (MEL) is followed by cytoskeletal rearrangements related to changes in total cell calmodulin (CAM) levels. In the present work, we studied CAM subcellular distribution in control or 10^{-9} M MEL treated MDCK cells. Double staining immunofluorescence method showed that CAM in control MDCK cells appeared as fluorescent spots at cell periphery. In contrast, MDCK cells cultured during 6, 12, 24 hrs. or 4 days with MEL, showed in addition CAM fluorescent spots distributed both all over the cytoplasm and the nucleus. These CAM rearrangements, were reverted 6 hrs. after MEL withdrawal. Moreover, RIA CAM measurements showed that in 10^{-9} M MEL treated cells, membrane bound CAM content was increased by 40 %, while cytosolic CAM decreased by 40% with respect to control cells. These results support that MEL interacts with CAM and suggest that rhythmic CAM compartmentalization could occur in phase with the MEL rhythmic secretion.

474.14

THE EFFECT OF STEROID REPLACEMENT ON ATRIAL NATRIURETIC POLYPEPTIDE (ANP) CONTENT OF THE HYPOTHALAMUS IN ADRENALECTOMIZED RATS. L.Y. Ma*, X.D. Yang*, M.L. Zhang*, S.Q. Xi*, Z.M. Qi*, G.L. Sun*, L. Liu*, with the advice of A.N. Epstein, E. Stellar. Tianjin Medical College, P.R. China and University of Pennsylvania.

We have recently demonstrated that ANP is present in the brain (hypothalamus) and plays a role in the release of the anterior pituitary hormones. Nothing is known concerning the role of the adrenal steroids on ANP synthesis or release in the hypothalamus. In this study Sprague-Dawley rats were divided into 5 groups: 1, Sham-operated (Sham); 2, adrenalectomized (ADRX); 3, ADRX and receiving Dexamethasone (Dex), (10 or 20 μg/100 g b.w./24 hr, s.c.); 4, ADRX and receiving deoxycorticosterone acetate (DOCA), (1 or 2 mg/24 hr, s.c.); 5, ADRX and receiving both Dex and DOCA as described above. Twelve days after surgery, the rats were decapitated and blood was collected for Na^+, K^+, Cl^- and AVP measurement, and the hypothalamus were removed for ANP content. The results showed no differences in plasma Na^+, K^+, Cl^- or AVP due to the adrenalectomy. The ANP content of the hypothalamus were lower in ADRX than in Sham, but the ADRX that received either DOCA, Dex, or DOCA and Dex were higher than Sham. Our results suggest that the adrenal steroids may directly increase ANP synthesis in the hypothalamus.

474.15

GLUTAMATE AGONISTS DO NOT INHIBIT MELATONIN PRODUCTION IN THE RAT PINEAL GLAND *IN VITRO*. L. Kus, R.J. Handa, J.A. McNulty. Department of Cell Biology, Neurobiology, and Anatomy, Loyola University, Maywood, IL 60153

We have previously described a saturable, stereospecific, pH, time, and temperature dependent binding site for [^{3}H]glutamate in the rat pineal gland. The function of this binding site is unknown. In the present study we examined the effect of glutamate agonists on the adrenergic stimulated secretion of melatonin using an *in vitro* perifusion system (Acusyst-S-APS 10, Endotronics). Pineal glands were perifused with an artificial CSF at a rate of 200 µl/min., and subsequent melatonin release measured by RIA (Guilday). Before drug treatment pineal glands were preincubating for a period of 4 hours by which time melatonin output reached basal levels. The addition of L-glutamate (10^{-3}M) to the media prior to stimulation with (-)isoproterenol ((-)Iso) and L-phenylephrine (L-PE) inhibited melatonin production. L-glutamate did not affect melatonin production when pineal glands were stimulated with (-)Iso alone. The addition of the glutamate agonists NMDA, quisqualate, kainate, and trans-ACPD (10^{-4}M) before (-)Iso and L-PE administration did not inhibit melatonin production. This suggests that glutamate's effects are not mediated by any of the known glutamate receptor subtypes. Simultaneous administration of L-glutamate with (-)Iso and L-PE did not inhibit melatonin production suggesting that glutamate's effects may be mediated via a conversion product (GABA?) of glutamate. Supported in part by Sigma Xi, Grants-in-Aid of Research and NSF #BNS-88-01726.

474.16

EFFECT OF MULTIPLE INJECTIONS OF ISOPROTERENOL ON PINEAL MELATONIN PRODUCTION IN TURKISH HAMSTERS. S.M. Hong, R.D. Rollag*, and M.H. Stetson*. Univ. of Delaware, Newark, DE 19716 and Uniformed Services Univ. for Health Sciences, Bethesda, MD 20816.

In previous studies we found that pineals of the Turkish hamster (Mesocricetus brandti) are responsive to single injection of adrenergic agonists only in the later dark phase. In order to further examine the factors involved in pineal melatonin production, two or three isoproterenol (ISO, 1 mg/kg) injections were given at 2 h intervals either in late afternoon or at midday, respectively. Animals given ISO injections at 1600 and 1800 h showed higher melatonin level (p=0.056, t-test) at 2200 h and lower level (p=0.008, t-test) at 2400 h than in saline injected animals. Pineal melatonin levels of animals that received three ISO injections at 1200, 1400 and 1600 h were no different than in saline groups. In comparison, pineals of the golden hamster (Mesocricetus auratus) responded to three ISO injections around noon or two ISO injections right before the dark onset with a significant elevation of melatonin production. These results showed that neither long term exposure (6 h) to ISO around noon, nor extended (4 h) exposure to the beta-adrenergic agonist prior to dark onset was sufficient stimulus to mimic the endogenous melatonin peak in the Turkish hamster. Whether other neurotransmitters are involved in pineal melatonin production in this species needs to be investigated. (supported by NSF Research Grant DCB87-14638)

474.17

MELATONIN MODULATION OF HYPOTHALAMIC FUNCTION AND ESTROGEN RECEPTOR EXPRESSION (ER) IN LSH/SsLak HAMSTERS. N.Lawson, B.Wee, C.Castles*, D.Blask$_1$, B.Benson$_1$, and S.Hill* Tulane Univ. Sch. of Medicine, New Orleans, LA 70112, and 1 Univ. of Arizona College of Medicine, Tucson, AZ 85745.

Short photoperiod or exogenous melatonin administration induces a state of anestrous in LSH/SsLaK female hamsters, which is associated with diminished gonadotropin levels. The neuroendocrine control of reproductive physiology is affected by gonadal steroids, particularly estrogen, which controls gonadotropin release through feedback mechanisms mediated by hypothalamic neurons containing ER. We therefore tested the hypothesis that the pineal hormone, melatonin, may alter hypothalamic sensitivity to estrogen by modulating ER expression. Regularly cycling females in long photoperiod (14L:10D) were bilaterally ovariectomized and then given late afternoon injections of melatonin (25 ug/day) or saline for 10 weeks, after which they were sacrificed. Melatonin treatment significantly suppressed FSH, LH, and prolactin serum levels, as determined by RIA. Brains from both groups of animals were analyzed by immunocytochemistry using the human ER monoclonal antibody H222 (Abbott) and DAB. A 50% decrease in the density of ER immunoreactive cells was noted in the medial preoptic area of melatonin treated animals. These data suggest that melatonin in female hamsters suppresses gonadotropin and prolactin levels, inducing anestrous possibly via its suppression of hypothalamic ER expression.

474.18

EFFECT OF ATP-SENSITIVE K+ CHANNELS ON DOPAMINE INHIBITION OF PRL SECRETION AND SECOND MESSENGER SYSTEMS IN MMQ CELLS, A PITUITARY CLONE NATURALLY EXPRESSING D2 RECEPTORS. G. Schettini, O.Meucci,C.Ventra,A.Scorziello*,M.Grimaldi*,E. Landolfi*,Dept.of Pharmacology, II School of Medicine, University of Naples ITALY .

In this study we used glibenclamide (Glib) to evaluate the role of ATP-sensitive K+-channels in dopamine (DA) inhibition of PRL secretion measured either in mixed pituitary cultures or at single lactotroph cell level, by means of reverse hemolytic plaque assay. The effect of Glib on free cytosolic calcium ([Ca++]i) and adenylate cyclase (AC) activity was also tested. The percentage of PRL-secreting cells was significanlty increased by Glib in a dose dependent manner (1nM-10uM). This effect was completely abolished by DA (1uM). The increase of PRL-secreting cells induced by forskolin was not modified by Glib pretreatment, while the dopaminergic inhibition of PRL was reduced by the blockade of K+(ATP)-channels. Glib neither affected basal AC activity nor modified DA inhibition of the AC enzyme. Finally, Glib induced a dose- and time-dependent membrane depolarization and increased [Ca++]i levels but did not prevent DA-induced membrane hyperpolarization and [Ca++]i reduction.

474.19

INTRACEREBROVENTRICULAR ADMINISTRATION OF BOMBESIN BLOCKS THE ESTROGEN-INDUCED AFTERNOON PROLACTIN SURGE. J.T. Pan and L.M. Mai* Inst. of Physiology, Natl. Yang-Ming Med. Coll., Taipei, Taiwan, R.O.C.

Bombesin, a putative peptide neurotransmitter, has been shown to inhibit both basal and stress-induced secretion of prolactin. It's mechanism of action may be via activating the hypothalamic tuberoinfundibular dopaminergic (TIDA) neurons. Two doses of bombesin, 0.15 and 0.75 µg, were administered i.c.v. at 1200 h in long-term ovariectomized, estrogen-treated rats. Hourly plasma samples were taken from 1100 to 1900 h through indwelling intraatrial catheters. The large dose of bombesin completely blocked the estrogen-induced afternoon prolactin surge, while the small dose just delayed the surge for an hour. Co-administration with [Leu^{13}-ψ(CH$_2$NH)Leu^{14}]-bombesin, a bombesin receptor antagonist, bombesin was no longer effective in blocking the prolactin surge. On the other hand, in bombesin-treated rats, a single injection of sulpiride (1 µg/rat, i.v.) at 1400 h also restored the prolactin surge. We conclude from the results that bombesin acts through specific receptor to activate the TIDA neurons and in turn inhibits prolactin secretion. (Supported in part by NSC80-0412-B010-55).

474.20

EFFECTS OF PASSIVE IMMUNONEUTRALIZATION OF PROLACTIN ON THE ACTIVITY OF TUBEROINFUNDIBULAR DOPAMINERGIC NEURONS. A.E. Fleckenstein, T.W. Toney, C. Gopalan, K.J. Lookingland and K.E. Moore. Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI 48824

This study examines the effects of prolactin (PRL) antisera (raised in rabbits) on tuberoinfundibular dopaminergic (TIDA) neuronal activity as estimated by measuring in the median eminence the concentrations of 3,4-dihydroxyphenylacetic acid (DOPAC), or the accumulation of 3,4-dihydroxyphenylalanine (DOPA) 30 min after administration of a decarboxylase inhibitor. One to 20 hrs after i.v. administration of PRL antisera TIDA neuronal activity was decreased in gonadally intact female, but not male rats. PRL antisera had no effect on TIDA neuronal activity in ovariectomized female rats, but blocked the stimulatory effect of estrogen (Silastic capsules containing 37.5 ug/ml; s.c.; 3 days) on these neurons. These results suggest that sexual differences in TIDA neuronal activity are due, in part, to the stimulatory effects of estrogen on PRL secretion, which in turn tonically activates TIDA neurons in female rats. In male rats, haloperidol (1 mg/kg; s.c.) increased the activity of TIDA neurons 12 hr later, and this effect was blocked by PRL antisera. These results confirm that the stimulatory effect of this dopaminergic receptor antagonist on TIDA neurons is mediated by PRL. Taken together, these results reveal that immunoneutralization with PRL antisera is a useful alternative to hypophysectomy in studies designed to identify PRL-mediated regulation of TIDA neurons (supported by ADAMHA grant MH 42802).

474.21

BOTH FOOTSHOCK STRESS AND DEFEAT DECREASE PLASMA PROLACTIN IN MALE HAMSTERS. K.L. HUHMAN[1], E.H. MOUGEY, & J.L. MEYERHOFF. [1]PO Box 4010, Dept. of Biology, Georgia State Univ., Atlanta, GA 30302-4010; Walter Reed Army Institute of Research, Washington, D.C. 20307-5100.

Although stress increases plasma prolactin (PRL) levels in male rats, it was reported that neither handling nor ether exposure affected plasma PRL in male hamsters. We have found increases in the stress-respondent hormones beta-endorphin, adrenocorticotropin and cortisol following exposure to acute and repeated social conflict, but only in hamsters that become submissive. We have now examined PRL responses following social conflict (resident-intruder paradigm). For comparison, we have also examined the effect of exposure to a footshock stressor. Plasma concentration of PRL was measured by RIA using antisera raised against hamster PRL. We found that footshock markedly decreased plasma PRL. Moreover, following both acute and repeated agonistic encounters, plasma PRL was significantly decreased in submissive hamsters. Thus, it appears that the PRL response to some stressful stimuli in golden hamsters may be anomalous.

474.22

ESTROGEN AND AN ESTROGEN-BSA CONJUGATE MEDIATE RAPID PROLACTIN RELEASE IN GH_3/B6 CELLS. T.C. Pappas, T. J. Collins*, C. S. Watson*. Dept. of Human Biol. Chem and Genetics, Univ. of Texas, Med. Br., Galveston, TX 77550.

Rapid release of prolactin (PRL) in GH_3/B6 cells in response to 17 ß-estradiol (E_2) or estradiol conjugated to bovine serum albumin (E_2-BSA) was investigated by radioimmunoassay. Attached cells cultured in defined or serum supplemented media were treated with steroid or vehicle, and PRL release into the media was assayed. PRL release in cells treated with 10 nM E_2 was increased over vehicle controls as rapidly as 1 min. At 5 min, however, there was no significant difference. At 1 min, the PRL release response was saturated at 10 nM E_2. E_2-BSA was effective in eliciting both 1 and 5 min PRL release over BSA controls at a concentration as low as 1 ng/ml (estimated maximum E_2 concentration 0.3 nM), with response saturation at 10 ng/ml; thus, immobilization of the steroid bound at the cell membrane increases the sensitivity of the response. The rapid time course of this PRL release enhancement as well as the ability of the BSA-linked ligand to elicit this response suggests a membrane site of action for estrogen.

474.23

BRAINSTEM ESTROGEN RECEPTOR- AND DOPAMINE-β-HYDROXYLASE-IMMUNOREACTIVITY IN MALE AND FEMALE RATS. D.H. Olster, J.D. Blaustein and A.P. Jones. Harbor/UCLA Medical Center, Torrance, CA 90502, University of Massachusetts, Amherst, MA 01003, and Pitzer College, Claremont, CA 91711.

Sexually differentiated, steroid-induced estrous behavior and gonadotropin secretion may include a noradrenergic component. Uptake of radiolabelled estradiol by brainstem noradrenergic neurons has been reported (Heritage et al., 1980). We compared brainstem localization of estrogen receptor- (ER-) and dopamine-β-hydroxylase-immunoreactivity (DBH-IR) in gonadectomized male and female rats. ER-IR cells were found in close proximity to DBH-IR cells, primarily in the rostral-most portion of the locus coeruleus. Cellular colocalization of DBH- and ER-IR was observed, but very rarely. Furthermore, no obvious sex differences in DBH- or ER-IR were found in the brainstem. These data suggest that effects of estradiol on noradrenergic activity may not be mediated directly by ERs in brainstem noradrenergic neurons in male or female rats. (Supported by NIH HD 23483, NS 19327 and RSDA MH 00885.)

474.24

A PLACENTAL FACTOR INCREASES TYROSINE HYDROXYLASE (TH) ACTIVITY IN TUBEROINFUNDIBULAR DOPAMINERGIC (TIDA) NEURONS. L. A. Arbogast, M. J. Soares* and J. L. Voogt. Dept. of Physiology, Univ. of Kansas Med. Ctr., Kansas City, KS 66103.

Rat choriocarcinoma (Rcho) cells, which have morphological features similar to trophoblast giant cells and produce placental lactogen I, were used to investigate placental effects on TIDA neuronal activity and prolactin (PRL) release. Rcho cells (100,000 cells/15µl/rat) or media, were injected i.c.v. to ovariectomized rats 65 h before sacrifice. Subsequently, bromocriptine (BROMO;3 mg/kg; s.c.) or vehicle was injected 50, 38, 26, 14 and 2 h before sacrifice. Four groups were used: 1) media + vehicle (control), 2) media + BROMO, 3) Rcho cells + vehicle and 4) Rcho cells +BROMO. *In vitro* TH activity was assessed by incubating hypothalamic explants with brocresine, a dihydroxyphenylalanine (DOPA) decarboxylase inhibitor. The accumulation of DOPA in the median eminence, which contains nerve terminals for the TIDA neurons, was determined by HPLC. DOPA accumulation was suppressed to 60% of control (7.7±0.6 ng/mg protein/30min) in BROMO-treated rats and was increased 2-fold above control in Rcho- and Rcho+BROMO-treated rats. In a second group, TH mRNA signal levels in the arcuate nuclei, which contain the perikarya of the TIDA neurons, were determined by *in situ* hybridization with a 35S-labeled cRNA probe for TH. TH mRNA signal levels were markedly reduced in BROMO-treated rats, but this effect was partially reversed in the Rcho+BROMO-treated rats. Serum PRL (ng/ml) was 16.6±2.5 in control rats, and was reduced to 1.0±0.1 or 1.6±0.4 in BROMO- and Rcho-treated rats, respectively. CONCLUSIONS: 1) Rcho cells produce a factor(s) which increases TH catalytic activity and is capable of reversing the BROMO-induced suppression of TH activity, in part, by a stimulatory effect on reduced TH mRNA levels and 2) factor(s) produced by Rcho cells may suppress PRL release, in part, by a dopaminergic mechanism. Supported by HD24190 and HD07368.

474.25

INTRAVENOUS m-CHLOROPHENYLPIPERAZINE INCREASES PLASMA PROLACTIN AND HYPOTHALAMIC SEROTONIN IN CONSCIOUS RATS. M. Baumann and J. Rutter. Dept. of Biological Sciences, Rutgers University, Piscataway, NJ 08855.

While it is generally accepted that m-chlorophenylpiperazine (mCPP) is a direct serotonin (5-HT) receptor agonist, this drug also releases 5-HT from brain slices *in vitro* (Pettibone and Williams, 1984). We employed the techniques of intracranial microdialysis and intravenous blood sampling to investigate the mechanism of mCPP-induced prolactin (PRL) release *in vivo*. Rats were fitted with indwelling jugular catheters, and microdialysis probes were inserted through guide cannulae aimed at the ventromedial hypothalamus. After overnight perfusion of the probe *in situ*, dialysate samples were collected at 30 min intervals and immediately assayed for 5-HT by HPLC-EC. Serial blood samples were withdrawn just prior to and at 30, 60 and 90 min after drug (or vehicle) injection. Plasma was assayed for PRL by RIA.

Intravenous mCPP (2.0 mg/kg) evoked a dramatic 8-fold increase in dialysate 5-HT that was maximal at 30 min, and was maintained for at least 120 min. In the same rats, plasma PRL was significantly elevated 30 min after mCPP. Dialysate 5-HT and plasma PRL were not altered by iv saline (1.0 ml/kg). Pretreatment with the 5-HT reuptake inhibitor fluoxetine (10.0 mg/kg, ip) blocked the mCPP-induced rise in dialysate 5-HT, but did not reverse the stimulatory effect of mCPP on plasma PRL. Fluoxetine alone elicited a modest 2-fold increase in dialysate 5-HT without affecting plasma PRL. Our results suggest that mCPP enhances extracellular levels of 5-HT by a presynaptic mechanism involving the 5-HT uptake carrier. The PRL-releasing action of mCPP appears to be independent of the presynaptic drug effects (Supported by the Bureau of Biological Research., Rutgers Univ.)

474.26

PROLACTIN RELEASE IN GOLDEN HAMSTERS IS UNDER DOPAMINERGIC INHIBITION. T.O. Moore, K.L. Huhman[1], E.H. Mougey and J.L. Meyerhoff. Neuroendocrinology Branch, Dept. of Medical Neuroscience, Walter Reed Army Institute of Research, Washington, D.C. 20307-5100. [1]P.O. Box 4010. Georgia State University, Atlanta, GA 30302-4010.

Exposure to stressors produces either no effect or decreases plasma levels of prolactin (PRL) in male hamsters. This is in marked contrast to the response in the rat, in which stress increases plasma PRL. As a first step in exploring the neuroendocrine regulation of PRL secretion in hamsters in vivo, we sought to establish whether PRL secretion was under dopaminergic inhibition. Accordingly, we treated hamsters with Domperidone, a peripherally-active dopamine receptor blocker which reaches the anterior pituitary gland and has been used successfully to block dopaminergic inhibition of PRL release. Injections of vehicle, or 0.01, 0.1 or 1.0 mg/kg of Domperidone were given intra-peritoneally to male golden hamsters. One hour after the injections, trunk blood was collected in heparin, centrifuged and plasma frozen. Plasma PRL was measured by RIA, using antisera raised against hamster PRL. Domperidone elicited dose-related increases in plasma PRL, reaching 130 ng/ml at the highest dose. Thus, although the PRL response to stress in male hamsters is anomalous compared to the rat, its release does increase rapidly after blockade of dopaminergic inhibition.

474.27

CLONING OF THE HISTIDINE DECARBOXYLASE (HDC) GENE AND REGULATION BY ESTRADIOL IN RAT BRAIN. C.A.Zahnow, D.E.Millhorn, and D.R.Joseph. Depts. of Physiology and Pediatrics, University of North Carolina, Chapel Hill, NC 27599.

HDC catalyzes the single step synthesis of histamine from L-histidine. To study the effects of HDC regulation on the neuroendocrine system we have cloned 9kb of the rat histidine decarboxylase gene with HDC cDNA (PNAS 87:733, 1990) as probe and have identified the transcription start site by primer extension. Sequence data have revealed a putative promoter region which includes a TATA-like element,and sequences resembling hormone response elements. Experiments were conducted to determine whether the HDC gene is regulated by estrogen. Sprague-Dawley rats were ovariectomized and after 14 days were treated with 10ug of estradiol in 100ul sesame oil. Whole brains were removed and processed for in situ hybridization, Northern blot analysis and enzyme assay. After estradiol treatment, both the mRNA and HDC activity were elevated as compared to vehicle only controls. Experiments are in progress to define the level at which regulation occurs. (Supported by grants from the North Carolina Biotechnology Center and Glaxo Pharmaceuticals.)

474.28

RELAXIN IN THE CEREBROSPINAL FLUID OF FEMALE RATS. LJ Parry, RS Poterski*, C Wasnidge*, AJS Summerlee. Biomedical Sciences, Ontario Veterinary College, Guelph, Ontario, N1G 2W1, Canada.

Experiments were done to investigate whether the 6kD peptide hormone relaxin (**RXN**) injected into the peripheral circulation, enters the CSF in urethane-anaesthetized female rats. Rats were injected with either 5µg porcine **RXN** (in 0.1ml saline: n=64) or saline (0.1ml: n=61) into the carotid artery. A single 100µl sample of CSF per rat was withdrawn from the cisterna magna at either 0,1,5,10 or 20 mins following treatment; samples were pooled from each group. Porcine **RXN** concentrations were measured in the pooled CSF samples (n=8/group) by specific RIA (O'Byrne & Steinetz, 1976). **RXN** was not detected in the CSF either before treatment or in CSF samples from saline-treated animals. In contrast, significant ($P<0.05$:ANOVA) elevations in CSF **RXN** concentrations were observed in all **RXN**-treated animals. The presence of **RXN** in CSF samples was confirmed by SDS-PAGE separation of the CSF samples. Porcine **RXN** was identified only in CSF samples of **RXN**-treated animals by Western Blot analysis using a porcine **RXN** antibody (supplied by DG Porter). These data are the first demonstration that peripherally circulating **RXN** enters the CSF despite its 6kD molecular weight. Supported by NSERC (Canada) and OMAF.

474.29

FUNCTIONAL SUBPOPULATIONS OF INDIVIDUAL PANCREATIC B-CELLS SHOW DIFFERENTIAL RESPONSIVENESS TO CARBACHOL. M. Hiriart, M.C. Ramirez-Medeles‡,M.C. Fernandez* and G. Rosas*. Dept. Bioenergetica, I.F.C., UNAM;°Dept. Sist.Biol.UAM-X, Mexico

It is well known that acetylcholine modulates insulin secretion. We have used a reverse hemolytic plaque assay to study the effect of carbachol (CCh) in cultured rat B- cells, in different glucose concentrations.

CCh (100 nM) increased the percentage of plaque-forming cells in 5 and 10 mM glucose by 85 and 8%, respectively. However, in 15 mM glucose it was decreased in 18% (n=4).

Recently, we described functional subpopulations of B-cells, based on the amount of insulin secreted by single cells (Hiriart and Ramirez-Medeles, Endocrinology, in press). We classified them as: small (SP), medium (MP) and large (LP) plaque-forming cells.

In 5 mM glucose, mainly SP and MP cells were detected. CCh increased the percentage of MP cells and recruited LP cells. In 10 mM glucose, the three subpopulations were observed, CCh also augmented the percentage of MP and LP cells. In contrast, CCh in 15 mM glucose decreased the percentage of MP and LP cells.

The results suggest that the effect of CCh in low glucose is greater, because more cells are recruited to secreting activity. Moreover, B-cells subpopulations with higher secretion rates may be preferentially stimulated by carbachol.

474.30

PERIPHERAL-TYPE BENZODIAZEPINE RECEPTORS AND REGULATION OF STEROIDOGENESIS IN RAT BRAIN MITOCHONDRIA. L.D. McCauley*, N.C. Lan, J.M. Tomich[1], J.E. Shively[2], K.W. Gee. Department of Molecular Pharmacology and Toxicology, [1]Children's Hospital, University of Southern California, Los Angeles, CA 90033. [2]Beckman Research Institute of the City of Hope, Duarte, CA 91010.

Recent evidence has suggested that steroid biosynthesis may occur in central nervous system tissue. Increased pregnenolone synthesis can be induced via a benzodiazepine (BZ) receptor located on the outer mitochondrial membrane. To evaluate the synthetic capabilities of brain mitochondria isolated from male Sprague-Dawley rat brains, ligands with varying affinities for the peripheral-type BZ receptor on the mitochondrial membrane were examined for their ability to stimulate pregnenolone production from cholesterol. Diazepam, Ro5 4864 and PK 11195 increased steroidogenesis by at least two-fold whereas clonazepam had no significant effect. In addition, the ability of steroidogenesis activator peptide (SAP) and three partial fragments of diazepam binding inhibitor were evaluated for activity. All peptides significantly stimulated cholesterol conversion to pregnenolone in brain mitochondrial preparations. Furthermore, SAP displayed competitive kinetics against Ro5 4864 as determined by Scatchard analysis. The K_d for [^{3}H]Ro5 4864 binding in the presence of SAP was calculated to be 5.69 nM, about twice that of control [^{3}H]Ro5 4864 binding. These results suggest that the brain may be capable of *de novo* synthesis of steroids and that peptides and small molecules such as Ro5 4864 may share a common site of action. (Supported by NIH grants NS25986 and NS24645)

NEURAL-IMMUNE INTERACTIONS: INTERLEUKINS AND NEURAL FUNCTIONS

475.1

INTERLEUKIN-1 RECEPTOR ANTAGONIST PROTEIN (IRAP) EQUALLY INHIBITS IL1α- AND IL1β-INDUCED ACTH SECRETION AND THYMOCYTE PROLIFERATION. S.G.Matta, K.M.Linner* and B.M.Sharp*. Endocrine-Neurosci Res Lab, Dept of Medicine, Hennepin Cnty Med Ctr and U of MN, Mpls, MN

Interleukin-1 (IL1) stimulates the release of ACTH via a central mechanism. Whether apparent differences in efficacy of IL1α vs IL1β are due to two different receptors has not been clarified. We sought to address this from a functional perspective, by examining the inhibition by IRAP of ACTH secretion and thymocyte proliferation in response to rhIL1α vs rhIL1β. To determine an equieffective dose for ACTH release in rats, IL1α was given i.v. (25-200 ng/200 g b.w.) or proximate to the median eminence (intra-ME; 1-75 ng/0.5 µl) and compared to IL1β (i.v. 25 ng/200 ng and intra-ME 10 ng). I.V. IL1α 200 ng was equieffective to IL1β 25 ng: peak ACTH responses (pg/ml; mean ± sem) were 520±78 and 489±69, respectively. Intra-ME IL1α 75 ng (276±58 pg/ml) was equieffective to IL1β 10 ng (273±35 pg/ml). To examine the interaction between IRAP and IL1, studies determined that the IC_{50} for IRAP delivered i.v.prior to i.v. IL1α 200 ng or IL1β 25 ng were 2.5 µg or 5.5 µg, respectively. Intra-ME IRAP 1 µg prior to ME IL1α or IL1β also reduced the ACTH response (pg x min/ml):

Dose (ng/0.5 µl)		(-) IRAP	(+) IRAP	% reduction
Buffer		3132±365	3940±301	-
IL1α	200	6777±842	2316±368	100
	75	4890±660	3170±568	100
IL1β	25	13125±918	1079±989	31
	10	11780±1068	6785±669	67

In the rat thymocyte co-mitogenesis assay, the proliferative response to 5 ng/ml IL1α or β was reduced by IRAP (IC_{50} of 12.5 and 25 µg/ml, respectively). In summary, IL1α was less potent than β regarding ACTH secretion, but equipotent in thymocyte proliferation; however, at equieffective IL1 doses, IRAP was similarly effective in both assays. Therefore, it appears that one CNS receptor for IL1α and β, which is similar to the thymocyte receptor, mediates ACTH secretion.

475.2

SYSTEMIC INTERLEUKIN-6 INDUCTION BY CENTRAL INTERLEUKIN-1: NEURAL-IMMUNE MODULATION A. Manfridi*, C. Gemma* M. Sironi*, A. De Luigi*, P. Ghezzi* and M. G. De Simoni. Istituto di Ricerche Farmacologiche "Mario Negri", via Eritrea 62, 20157 Milan, ITALY.

Some of the activities of IL-1 are known to be mediated by IL-6. Intracerebroventricular (i.c.v.) administration to rats of 200 ng of human recombinant ß IL-1 results in a more marked induction of IL-6 compared to systemic injection. This effect is antagonized by the i.c.v. administration of the IL-1 receptor antagonist (IL-1ra, 20 µg). Induction of serum IL-6 by central IL-1 is greatly attenuated by naloxone (10 mg i.p.) pretreatment and by 6-OHDA or 5,7-DHT lesions, suggesting the involvement of opioid peptides and brain monoamines in the induction of circulating IL-6 by central IL-1. Induction of IL-6 was potentiated in adrenalectomized and in hypophysectomized rats indicating that the hypothalamus-pituitary-adrenal axis provides inhibitory signals for this IL-1 central effect. Neither MSH (30 µg icv), nor anti-CRF antibody, compounds known to inhibit several IL-1 activities, could antagonize IL-6 induction, but rather enhanced it. Intracerebroventricular administration of endotoxin (2.5 µg) also induced a preferential induction of circulating IL-6.

Centrally mediated induction of IL-6 indicates the existence of a pathway that could explain how infections or lesions confined to the CNS result in systemic alterations.

475.3

INTERLEUKIN (IL)-1ß DEPOLARIZES MEMBRANE POTENTIALS OF HYPOTHALAMIC SUPRAOPTIC (SON) NEURONS IN RAT SLICE PREPARATION. H. Yamashita, Z. Li* and K. Inenaga*, *Dept. Physiol., Univ. Occupational and Environmental Health, Sch. of Med., Kitakyushu 807, Japan.*

Recent studies have shown existence of IL-1ß immunoreactive neurons in the hypothalamus and elevation of plasma AVP and OXT levels induced by IL-1ß injected intravenously. To provide the direct electrophysiological evidence that IL-1ß affects the activities of hypothalamic neuroendocrinological cells, intracellular recordings were made from SON neurons in brain slices (400µm) of adult male Wistar rats. Total 42 (consisting of 29 phasic and 13 non-phasic) neurons were tested. Their resting potential, membrane resistance and action potential are, respectively, 58.6±1.1 mV, 160.8±9.0 MΩ and 68.2±1.3 mV (Mean±SE). Application of human recombinant IL-1ß at 1.2×10^{-8} M to bath medium for 2 min depolarized membrane potentials (3.39±0.46 mV) with obvious increase of discharges in 25 (59.5%) SON neurons. In other 11 (26.2%) neurons, biphasic responses, depolarization followed by hyperpolarization were observed. The depolarizing effect of IL-1ß usually started 30 s after drug application and ended within 10 min. Such effect could not be blocked by TTX (9/9) but was completely abolished by salicylic acid (4/4). Only 4 neurons were hyperpolarized (2.50±0.96 mV) and the remaining (2 neurons) were not affected by IL-1ß. These results show that IL-1ß directly excites SON neurons through some process involved in prostaglandin metabolism and suggest that IL-1ß may modulate hypothalamic AVP and OXT secretion. (This work was entrusted to UOEH by Sciences and Technology Agency of Japan. Dr. Li was supported by Sasakawa Foundation.)

475.4

INTERLEUKIN 1-BETA INCREASES INTRACELLULAR CALCIUM AND ALTERS THE ELECTROPHYSIOLOGY OF CULTURED CEREBELLAR PURKINJE NEURONS. N. Di Julio*, A. Urrutia* and D. L. Gruol. Dept. of Neuropharmacology, Research Institute of Scripps Clinic, La Jolla, CA 92037.

Interleukin-1-beta (IL-1β) mediates signaling between cells of the immune system. IL receptors are located in the cerebellum (Farrar et al, J. Immunol. 139:459-463). To examine whether IL-1β mediates immune system communication with the nervous system, the effects of IL-1β on intracellular calcium levels and the electrophysiological properties of cultured cerebellar Purkinje neurons (PNs) were studied.

Changes in intracellular calcium ($[Ca^{2+}]_i$) of PNs (14-24 days in vitro) after exposure to IL-1β were measured with Fura-2 Ca^{2+} imaging. Pressure ejection from a pipette or bath application of IL-1β (10 or 100 units/mL) significantly increased $[Ca^{2+}]_i$ in the PN somas (n=36) and PN dendrites (n=15) an average of 76% and 42% above resting, respectively ($P < 0.05$, paired t-test). The mean latency was 4.5 minutes and the average time to peak $[Ca^{2+}]_i$ was 13 minutes (n=32). Partial recovery (25%) was observed in an average of 16 minutes after the peak response (n= 32). Application of antibody-neutralized IL-1β produced a 9% increase in $[Ca^{2+}]_i$ above resting compared to a 100% increase above resting with active IL-1β.

In electrophysiological studies, IL-1β depressed current evoked spiking, the afterhyperpolarization evoked at the termination of a depolarizing pulse and input resistance measured in the hyperpolarizing range of membrane potentials. These data demonstrate that factors secreted by immune cells can alter the physiological function of this CNS neuronal type. (*Supported by MH47680*)

475.5

INTERLEUKIN-1 BETA INHIBITS FIRING OF RAT CEREBELLAR PURKINJE CELLS IN SITU. S.R. White, D.D. Crossley* and J. Smith. Dept. of VCAPP, Washington State Univ., Pullman, WA 99164.

The cytokine interleukin-1 beta (IL1b) has been proposed as a messenger between the immune system and the central nervous system (CNS). It is released by macrophages during the immune response and by activated astrocytes and ameboid microglia during CNS injury. IL1b binding sites are distributed heterogeneously in the CNS and are particularly densely concentrated in the cerebellar cortex (Farrar et al., *J. Immunol.*, 1987, 139:459). This study examined the effects of local application of IL1b on the firing rate of cerebellar cortex Purkinje cells (PC) in chloral hydrate- or urethane-anesthetized rats. IL1b (1.5 nM, 0.4 - 4.8 pg) applied by micropressure ejection produced a dose-dependent inhibition of PC firing that recovered over time. This inhibition was observed in 77% of the PC that were tested and resembled the inhibition that was produced by micropressure ejection of norepinephrine. Equivalent ejections of the vehicle control solution had no effect on PC firing. These results indicate that IL1b can alter neuronal excitability in a region of the CNS that contains dense IL1b binding sites, and support the hypothesis that IL1b may have a neuromodulatory effect in the CNS.

475.6

DIFFERENTIAL EFFECTS OF CENTRAL AND PERIPHERAL INJECTION OF INTERLEUKIN-1ß ON BRAIN *C-FOS* EXPRESSION AND HYPOTHALAMO-PITUITARY-ADRENAL AXIS ACTIVITY. Serge Rivest*, German Torres and Catherine Rivier. The Salk Institute, The Clayton Foundation Laboratories for Peptide Biology, San Diego, CA 92186.

The present study investigated the neuroanatomical sites involved in the stimulatory effects of interleukin (IL) on the activity of hypothalamo-pituitary adrenal (HPA) axis. *C-fos* expression was used to identify the neurological activity of various brain areas following iv or intracerebroventricular (icv) injection of IL-1ß. Intracerebroventricular injections were done by means of a permanent cannula implanted in the right lateral ventricle, while peripheral administrations were carried out through a jugular catheter. Blood samples were taken before, as well as 30 and 120 min after icv or iv IL infusion. Immediately therafter the rats were anesthetized with pentobarbital, then perfused transcardially with 150 mL of heparinized saline followed by 4 % parafolmaldehyde in 0.1 M sodium phosphate buffer. Brains were removed, postfixed for 1 hr, then placed in 30 % sucrose in 0.01 M sodium phosphate buffer until the brains sank. Thirty micron frozen sections were cut on a sliding microtome and cryoprotected at -70 °C until processed. Approximately every fourth tissue section was processed for *c-fos* expression by an Avidin-Biotin-peroxidase method. Both iv (1 µg) or icv (100 ng) injections of IL-1ß increased plasma ACTH levels. Intracerebroventricular infusion of IL markedly increased *c-fos* expression in the paraventricular nucleus (PVN) and in the arcuate nucleus (ARC) of the hypothalamus. In contrast, iv injection of IL-1ß did not measurably alter *c-fos* expression in the PVN or in ARC, while significantly augmenting the *c-fos* expression in the external zone of the median eminence. These results suggest that iv injected IL-1ß acts at the level of external zone of the median eminence to stimulate the HPA axis activity, while icv administered IL-1ß activates neurons within the PVN.

(Supported by DK 26741 and the MRC of Canada)

475.7

INVOLVEMENT OF THE EPOXYGENASE PATHWAY IN INTERLEUKIN-6 (IL-6) STIMULATED CORTICOTROPIN-RELEASING FACTOR (CRF) SECRETION IN VITRO. K.Lyson and S.M.McCann. Department of Physiology, Neuropeptide Division, U.T. Southwestern Medical Center, Dallas, Tx 75235-9040.

Since we suggested that the epoxygenase (EPO) pathway of arachidonic acid metabolism system may mediate IL-6-stimulated CRF release in vitro, we decided to examine the influence of clotrimazole (CLO), a specific EPO inhibitor, on IL-6-stimulated CRF release from the rat medial basal hypothalamus (MBH). After 30 min preincubation in Krebs-Ringer bicarbonate buffer (KRB) or KRB containing CLO (10^{-9}-10^{-5}M), MBHs were incubated for 30 min with KRB or KRB containing IL-6 (10^{-13}M) and/or CLO. CRF release into the incubation medium was measured by RIA. None of the CLO concentrations used changed basal CRF release significantly. As reported previously, 10^{-13}M IL-6 evoked increased CRF release which was significantly suppressed by CLO in a dose-dependent manner at concentrations ranging from 10^{-9} to 10^{-5}M. The maximal inhibitory effect was observed with 10^{-5}M CLO: KRB=3.14±0.18 (mean±sem pg/mg MBH); 10^{-13}M IL-6=5.52±0.27, $p<0.001$ vs. KRB; 10^{-13}M IL-6 + 10^{-5}M CLO=2.93±0.29, $p<0.001$ vs. IL-6. The results suggest that IL-6 stimulates CRF release from the MBH in vitro by the activation of the EPO pathway.

475.8

INTERLEUKIN-1 EFFECTS ON LH RELEASE: MODE OF ACTION. J.J. Bonavera*, S.P. Kalra, and P.S. Kalra, Dept. OB-Gyn, Univ. Fla. Col. Med., Gainesville, FL 32610

We have reported that central administration of the cytokine, IL-1β, blocked the steroid-induced LH surge in ovariectomized rats apparently due to decreased hypothalamic LHRH secretion via an increase in endogenous opioid activity. To determine the component of LH episodes responsible for the diminution in LH release, we examined the effects of IL-1β on pulsatile LH secretion in castrated male rats. Two weeks after castration, rats received either IL-1β (Eli Lilly, Co., 100 ng/3μl, n=8), or saline (3 μl, n=8) via a chronic IIIrd ventricle cannula (icv). Blood was continuously withdrawn (100 μl/5 min) from indwelling intra-atrial cannulae for 1 h before and 2 h after injection. Plasma LH levels declined gradually after IL-1β administration. Analysis of LH pulsatility (CLUSTER method) revealed no change in LH pulse frequency, however, the decrease in LH levels could be attributed to significantly lower peak heights, amplitude and nadir in IL-1β-treated rats vs controls.

Since IL-1 stimulates release of CRF which, in turn, inhibits LHRH-LH we have tested whether CRF mediates the IL-1β-induced LH suppression. Castrated rats were passively immunized with CRF antibody (courtesy Dr. W. Vale; dil 1:5) injected icv (3 μl) at either 15 mins or at 75 and 15 mins before IL-1β (100 ng/3 μl); control rats received 3 μl normal rabbit serum (n=5-7/group). Neither treatment with CRF antibody blocked the inhibitory effects of IL-1β on LH release. Similarly, the CRF antagonist, α-helical oCRF (Bachem, 100 μg/5 μl, icv) injected 15 mins before IL-1β failed to block the decrease in LH release. These results suggest that (a) IL-1β decreases LH secretion apparently by diminishing hypothalamic LHRH output without affecting the frequency of LHRH discharge, and (b) CRF may not mediate the IL-1β-induced decrease in LHRH-LH release. (Supported by NIH HD 11362).

475.9

INTERLEUKIN-1 (IL-1)ß-INDUCED INTERCELLULAR ADHESION MOLECULE-1 (ICAM-1) EXPRESSION IS BLOCKED BY IL-1ß RECEPTOR ANTAGONIST (IL-1ra).
L. Hong*, M.R. Opp, and J.M. Krueger. University of Tennessee, Memphis, TN 38163

ICAM-1 is a cellular ligand that binds lymphocyte function-associated antigen-1 and mediates lymphocyte-tissue cell contact (1). ICAM-1 expression is important in inflammatory responses and is regulated by cytokines such as IL-1, interferon (IFN) gamma, and tumor necrosis factor-α (TNFα) (1). To study ICAM-1 expression in brain cells, we examined ICAM-1 expression in a human glioblastoma cell line (HTB16) and a neuroblastoma cell line (HTB11) using an ELISA to quantitate ICAM-1. Cells were incubated for 4 to 6 days with various concentrations of recombinant human IL-1ß (rhIL-1ß), rhIFN gamma, and rhTNFα in the absence or presence of IL-1ra (1 or 10 μg/ml). rhIL-1ß, rhIFN gamma, and rhTNFα potentiated ICAM-1 expression in both cell lines in a dose-related manner. The effect of rhIL-1ß on ICAM-1 expression was more potent in HTB16 cells, while that of rhIFN gamma was more potent in HTB11 cells. rhIL-1ß-induced ICAM-1 expression, but not rhTNFα-induced ICAM-1, was completely blocked by 10 μg/ml of IL-1ra. Our findings also indicate that expression of ICAM-1 by neuronal cells is regulated by cytokines, thus suggesting neuronal cells are also a target of binding to lymphocytes and could be involved in inflammatory responses.

1) Wawryk,S.O. et al. Immunol. Rev. 108:135-161, 1989. Supported by: MH47103,MS25378

475.10

CENTRAL IL-1ß INDUCES A RISE IN BLOOD PRESSURE IN ANAESTHETIZED MICE.
RS Poterski*, AJS Summerlee. Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph, Guelph, Ontario Canada N1G 2W1.

We previously reported that central administration of rhIL-1ß induces postimplantation pregnancy loss in mice (Croy et al., PNEI 1990; 3: 242-250). Preliminary data indicates that i.c.v. IL-1ß causes a rise in systemic blood pressure leading to disruption of blood supply to the developing fetus. We tested whether injection of rhIL-1ß (Genzyme, Boston, MA) into the cerebral ventricles of CD-1 non-pregnant mice (7-8 weeks old) would have a pressor effect. Mice were anaesthetized with tribromoethanol i.p. and the left common carotid artery cannulated for direct recording of blood pressure. Each animal was then placed into a steriotaxic frame and a small hole drilled above the left lateral cerebral ventricle. A 10 μl microsyringe was placed with its tip in the ventricle. The effects of IL-1ß (1 ng in 1 μl) i.c.v. were compared with control injections of 1 μl PBS-BSA (vehicle) i.c.v. One minute after injection of IL-1ß, there was a significant ($P<0.05$) rise in systolic and diastolic blood pressure (mode 16-20 mm Hg: range 5-26 mm Hg) in all animals (9/9). In contrast, no change in blood pressure was seen after PBS-BSA i.c.v. The data indicate that rhIL-1ß i.c.v. causes a consistent pressor effect in mice.

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475.11

CONVULSANTS INDUCE INTERLEUKIN-1β mRNA IN RAT BRAIN.
M. Minami*, Y. Kuraishi, K. Yabuuchi* and M. Satoh. Dept. of Pharmacol., Fac. of Pharm. Sci., Kyoto Univ., Kyoto 606, Japan.

To estimate whether changes of neuronal activity could alter the synthesis of interleukin-1β (IL-1β) in the brain, the influences of convulsion evoked by drugs on the mRNA level of IL-1β were studied in the rat. Male Sprague-Dawley rats were injected with kainic acid (KA: 10 mg/kg i.p.) or pentylenetetrazol (50 mg/kg i.p.). The expression of IL-1β mRNA in the eight brain regions, that is, the cerebral cortex (CC), hippocampus (HPP), striatum (STR), thalamus (THL), hypothalamus (HT), midbrain (MB), pons-medulla (PM) and cerebellum (CRB), was examined by northern blot analysis. IL-1β mRNA was induced by KA intensely in the CC, THL and HT, moderately in the HPP and weakly in the STR 2.5 h after the injection, but was not detected in the MB, PM and CRB. Pentylenetetrazol induced IL-1β mRNA intensely in the CC, weakly in the HPP, THL and HT 30 min after the injection. Diazepam (10 mg/kg i.p.) suppressed both the convulsion and the induction of IL-1β mRNA produced by KA. Dexamethasone (1 mg/kg i.p.) suppressed the induction of IL-1β mRNA by KA in the brain, but did neither the convulsion nor the induction of c-fos mRNA following the injection of KA. *In situ* hybridization study confirmed the regional difference in the induction of IL-1β mRNA by KA. These results provide the evidence that intensive neuronal excitation induces IL-1β mRNA in particular regions of the brain.

475.12

INCREASES IN RENAL SYMPATHETIC NERVE ACTIVITY, ARTERIAL BLOOD PRESSURE AND HEART RATE INDUCED BY INTERLEUKIN 1-β IN CONSCIOUS RATS.
KANNAN,H.[1], NAKAMURA,T.[2*], HAYASHIDA,Y.[2*] AND YAMASHITA,H.[1] Dept.Physiol.[1] and Dept.Systems Physiol.[2], Univ. of Occup. and Environ. Health, Kitakyushu 807, Japan

The present study was undertaken to determine the effects of interleukin 1β (IL-1β) on renal sympathetic nerve activity (RSNA), arterial blood pressure (AP) and heart rate (HR) as well as body temperature in conscious rats. Either intravenous (i.v.) or intracerebroventricular (i.c.v.) administration of IL-1β elicited increases in AP, HR and RSNA accompanied by a rise in body temperature. The maximum changes in AP, HR and RSNA occurred 10-15min after i.v. injection of IL-1β (100ng), and 20-25 min after i.c.v. injection (5ng). The responses induced by the i.v.and i.c.v.injections lasted for about 15-30 min and were completely abolished when the animals were pre-treated with a cyclo-oxygenase inhibitor, indomethacin (i.v.,10mg/kg). Moreover, i.c.v. injection of prostaglandin E_2(1μg)produced responses similar to those induced by IL-1β but with a rather short latency. The results suggest that IL-1β auguments cardiovascular and sympathetic outflow through the central action of prostaglandin E_2 in conscious rats. (This work was entrusted to University of Occupational and Environmental Health, using the Special Coordination Funds for Promoting Science and Technology.)

475.13

INTERLEUKIN-1 (IL-1) RECEPTORS IN THE BRAIN-ENDOCRINE AXIS CORRESPOND TO TYPE I BUT NOT TYPE II RECEPTORS : EVIDENCE FROM AFFINITY CROSS-LINKING STUDIES.
D.E. Grigoriadis, T. Takao, E.L. Webster, D.E. Tracey* and E.B. De Souza. The Du Pont Merck Pharmaceutical Co., Wilmington, DE 19880, NIDA/ARC, Baltimore, MD 21224, and The UpJohn Co., Kalamazoo, MI 49001.

IL-1 is a key mediator of the immune response to stress, infection and antigenic challenge. IL-1 also alters neuroendocrine activity through indirect effects in brain and direct actions in endocrine tissues. IL-1 receptors have been identified in brain and in endocrine organs with kinetic and pharmacological characteristics similar to IL-1 receptors in the T-lymphoid cell line EL-4 6.1. Recently, multiple IL-1 receptors, referred to as type I (M.W. = 80 kDa) and type II (65 kDa), have been identified in T- and B/macrophage-cell lines, respectively. In the present study, we have used chemical affinity cross-linking of ^{125}I-recombinant human IL-1α (^{125}I-IL-1α) followed by SDS-PAGE and autoradiography to compare the apparent M.W. of IL-1 receptors in the brain-endocrine-immune axis to that in T- and B-lymphoid cell lines. ^{125}I-IL-1α was incorporated in mouse spleen homogenates into two specific and distinct proteins corresponding to the respective M.W. of type I and type II receptors. On the other hand, ^{125}I-IL-1α was incorporated into hippocampus, anterior pituitary, testis and in AtT-20 pituitary tumor cells exclusively into a single protein corresponding to the higher M.W. type I receptor. IL-1α, IL-1β, and an analog IL-1β^+ inhibited the incorporation of ^{125}I-IL-1α in the brain and endocrine tissues in parallel with their relative bioactivities in the T-cell comitogenesis assay while tumor necrosis factor had no effect. These data demonstrate a similarity between IL-1 receptors in the brain-endocrine axis and type I IL-1 receptors in T-lymphocytes and suggest the importance of the cytokine in coordinating brain-endocrine-immune function.

475.14

TYPE I INTERLEUKIN-1 (IL-1) RECEPTORS IN THE MOUSE BRAIN-ENDOCRINE-IMMUNE AXIS LABELLED WITH ^{125}I-RECOMBINANT HUMAN IL-1 RECEPTOR ANTAGONIST.
T. Takao, R.C. Newton* and E.B. De Souza. The Du Pont Merck Pharmaceutical Company, Wilmington, DE 19880 and NIDA Addiction Research Center, Baltimore, MD 21224.

The cytokine IL-1 has a variety of effects in brain including induction of fever, alteration of slow-wave sleep and alteration of neuroendocrine activity. Previous studies utilizing ^{125}I-recombinant human IL-1α (^{125}I-IL-1α) have identified high-affininty binding sites for IL-1 in brain and endocrine tissues with characteristics of Type I receptors in T lymphocytes. Recently, a recombinant human IL-1 receptor antagonist (IL-1ra) with selective actions at Type I receptors both in vitro and in vivo has been identified. In the present study, we utilized ^{125}I-IL-1ra to further determine the characteristics of IL-1 receptors in the brain-endocrine-immune axis. Specific high affinity ^{125}I-IL-1ra binding (displaceable by 100 nM recombinant human IL-1β) was present in homogenates of mouse (C57BL/6) spleen, testis and hippocampus, with low to negligible binding seen in other brain areas such as the frontal cerebral cortex and hypothalamus. Of note, there was a dramatic species difference in the level of ^{125}I-IL-1ra binding in mouse and rat tissues with barely detectable binding present in the rat; a similar species difference was previously noted for ^{125}I-IL-1α binding. In competition studies in hippocampus, Il-1β and a weak analog Il-1β^+ inhibited ^{125}I-IL-1ra binding in parallel with their relative bioactivities in Type I IL-1 receptor dependent assays; tumor necrosis factor did not inhibit ^{125}I-IL-1ra binding. These data demonstrate comparable binding characteristics of ^{125}I-IL-1α and ^{125}I-IL-1ra to Type I IL-1 receptors in the tissues examined and provide further support for role for IL-1 in coordianting brain-endocrine-immune responses to physiological and pharmacological stimuli.

475.15

OCCURRENCE OF INTERLEUKIN-1 RECEPTOR IN THE RAT AND MOUSE PITUITARY. M. Schultzberg, C. Andersson*, J. Bristulf*, S. Nobel*. Karolinska Institute, Clinical Res. Center., Div. of Med. Cell and Neurobiology, Huddinge Hospital, and Dept. of Biochem., Stockholm Univ., Stockholm, Sweden.

Interleukin-1 (IL-1), a cytokine of multiple actions, has been shown to affect hypothalamic and pituitary hormones, notably with resulting increase in corticosterone production. Studies were carried out in order to investigate the occurrence of IL-1 receptor (IL-1R) in the rat pituitary. Antisera raised in rabbits against synthetic peptides of the murine lymphocyte IL-1R were used in immunohistochemical studies and the polymerase chain reaction (PCR) technique was employed to detect IL-1R mRNA. Antisera to the second loop of the extracellular domain of the protein gave an intense immunoreaction in the cells of the intermediate lobe of both mouse and rat pituitary. In addition, a population of the cells in the anterior lobe were mIL-1R immunoreactive. PCR analysis using primers for human and mouse lymphocyte IL-1R confirmed the synthesis of this protein in the pituitary. Experiments using *in situ* hybridization histochemistry to determine the cellular localization of IL-1R mRNA in the pituitary and to investigate its regulation are in progress.

These findings add morphological and biochemical support to the functional evidence of IL-1 receptors in the pituitary, although the source of IL-1 acting on these receptors remains unclear.

This work was supported by grants from the Swedish MRC.

475.16

INTERLEUKIN-1 COMPROMISES CHOLINERGIC FUNCTION IN FOREBRAIN STRUCTURES *IN VIVO*. L. Ni, R.P. Hart, G. Buzsáki, and G.M. Jonakait, Dept. Biol. Sci. and Center for Mol. & Behav. Neurosci., Rutgers Univ., Newark, NJ 07102

The possible role of immune cytokines in the etiology of Alzheimer's disease (AD) has received increasing attention since the discovery that brain levels of interleukin-1 (IL-1) are elevated in the disease, that IL-1 is localized to plaques, and that IL-1 rapidly increases mRNA coding for the amyloid β-protein precursor. Moreover, we have found that IL-1 significantly lowers cholinergic biosynthesis in cultures of embryonic rat basal forebrain and striatum.

In order to determine whether IL-1 compromises cholinergic neurons in adult rats *in vivo* as well, we infused IL-1β (50 U/day) into the lateral ventricles using a subcutaneously implanted Alzet minipump. Choline acetyltransferase (ChAT) activity was measured as an index of cholinergic function. After 10 days, ChAT activity was dramatically lowered in n. basalis (66±5.4% of saline-cannulated controls) and septal region (78±3.6%). After 14 days, ChAT activity in n. basalis was further depressed (50±2.4%). Modest but significant decreases occurred in the frontal cortex (86±3.0%) and striatum (91±1.3%) and after 14 days in amygdala (85±1.5%) and hippocampus (89±3.1%). ChAT activity remained unchanged in the anterior hypothalamus and spinal cord. Neither substance P in striatum nor tryptophan hydroxylase activity in the midbrain raphe were changed by the treatment, suggesting that IL-1 was somewhat specific in its action on cholinergic neurons.

These data suggest that subchronic exposure to IL-1 challenges cholinergic function differently in different cholinergic populations.

475.17

IMMUNOACTIVE AGENTS STIMULATE SUBSTANCE P IN CULTURED SYMPATHETIC GANGLIA. A. M. Shadiack*, D. Ganea*, R. P. Hart, and G. M. Jonakait, Dept. of Biological Sciences, Rutgers University, Newark, NJ 07102.

Immune cytokines are now known to regulate neurotransmitter gene expression. Our laboratory has recently shown that in explant cultures of superior cervical (sympathetic) ganglia (SCG), the macrophage product interleukin-1 (IL-1) dramatically increases both the levels of substance P (SP) and the mRNA coding for its prohormone precursor, preprotachykinin (PPT). We have now tested other products of activated macrophages, including tumor necrosis factor-α (TNF-α) and IL-6. While human recombinant (hr) TNF-α (20 U/ml) produced a modest doubling of SP, hrIL-6 had no effect on SP expression at any dose tested.

Lipopolysaccharide (LPS, 5μg/ml) from *E. coli*, a strong activator of the immune system, caused a two- to five-fold increase in SP that was inhibited by an IL-1 receptor antagonist (Synergen). These data suggest that SP induction by LPS may be mediated through the production of IL-1 in the culture.

In testing the effects of T-cell products on SP expression, we found that doses as high as 50 U/ml of hrIL-2 (Collaborative Research) or 800 U/ml of murine recombinant IL-4 (Genzyme) failed to alter SP levels. However, in the presence of LPS, a common co-stimulant with other immune cytokines, IL-4 increased SP levels above those induced by LPS alone.

These data taken together provide further evidence for a regulatory interaction between the immune and nervous systems.

(Supported by a grant from the ONR.)

475.18

INTERLEUKIN-2 (IL-2) INCREASES [^{3}H]DOPAMINE RELEASE FROM RAT STRIATAL SLICES: CORRELATION WITH THE PRESENCE OF ENDOGENOUS IL-2, IL-2 RECEPTORS AND IL-2 mRNA IN THE RAT STRIATUM. Paul A. Lapchak, Andrus Gerontology Ctr., USC, Los Angeles, CA, 90089-0191, USA.

Intrastriatal application of IL-2 elicits an asymmetric body posture with long-lasting ipsilateral turning and ipsilateral circling behavior suggesting that IL-2 may be interacting with IL-2 receptors that may be present on dopaminergic neurons of the nigro-striatal pathway. The present study determined whether IL-2 receptors are present in the striatum and whether the striatum contains endogenous IL-2 and IL-2 mRNA. In addition, the effects of IL-2 on dopaminergic neurotransmission in the striatum were determined by assessing the effect of IL-2 on [^{3}H]dopamine (DA) release from prelabeled transmitter stores. Immunoautoradiographic analysis and in situ hybridization indicated the presence of low levels of IL-2, IL-2 receptors and IL-2 mRNA in the striatum. Exogenous IL-2 (10^{-11} to 10^{-9} M) was effective in increasing [^{3}H]DA release (37-52%) from striatal slices. In addition, IL-2 (10^{-11} and 10^{-10} M) increased potassium (25 mM)-evoked [^{3}H]DA release from striatal slices (by 25 and 81 %, respectively). The IL 2-induced enhancement of basal and evoked [^{3}H]DA release from striatal slices was not observed when slices were incubated in the presence of the sodium channel blocker tetrodotoxin (0.1 μM) or when striatal slices were incubated with reduced concentrations of external calcium. In summary, it appears that IL-2 and IL-2 receptors are present in the striatum. Furthermore, this study shows that activation of IL-2 receptors is involved in the regulation of striatal dopaminergic neurotransmission. (Supported by HFSP).

475.19

EFFECTS OF LYMPHOKINES ON GLIAL AND NEURONAL CELLS *IN VITRO*: A ROLE FOR LYMPHOKINES AS MODULATORS OF NEURAL-GLIAL INTERACTIONS. D. M. Araujo and C. W. Cotman, Dept. Psychobiol., Univ. California, Irvine, CA, USA.

Lymphokines, in particular the interleukins (ILs), can be released from microglia and astrocytes in culture. However, the effects of ILs on the function of neuronal and glial cells *in vivo* is not completely understood. The present study characterized the effects of various lymphokines on microglial and astrocyte function in culture, by measuring the uptake of ^{3}H-thymidine and the release of IL-1 and -2 from these cells. Furthermore, effects of these lymphokines on the survival of hippocampal neurons *in vitro* was also assessed. Our results show that IL-3, -6, and other lymphokines enhanced the uptake of ^{3}H-thymidine (by 47-86%), as well as the release of IL-1 and -2 (by 25-120%) in microglial cell cultures. In addition, several "classical" growth factors (GFs) such as NGF, FGF and EGF also increased both measures. Similar results were obtained for astroglial cultures. In hippocampal neurons *in vitro*, the effects of lymphokines were complex: both neurotrophic and neurotoxic effects that were clearly time- and concentration-dependent were evident with various ILs, GCSFs, IFNs and TGFs. With IL-2, significant neurotoxic effects were apparent by 24h in culture. Thus, changes in lymphokine release resulting in altered glial cell function may disrupt central neuronal function. This in turn may be further exacerbated by neuronal factors that affect glial cell function.

475.20

LOCALIZATION OF mRNA FOR INTERLEUKIN-2 (IL-2) IN MOUSE BRAIN BY IN SITU HYBRIDIZATION. F. Villemain, T. Owens*, T. Renno* and A. Beaudet. Neuroanatomy Lab., Montreal Neurological Institute, Montreal, Quebec, H3A 2B4, Canada.

Immunohistochemical studies from our laboratory have previously documented the distribution of interleukin-2 (IL-2) in rodent and human brain (Neurosci. Abstr., 1990, 499.13). In normal CD-1 mice, IL-2 immunoreactivity was found to be concentrated within the arcuate nucleus of the hypothalamus where it is selectively associated with a subpopulation of neurons (Neurosci. Abst., 1990, 499.14). In the present study, we sought to determine whether IL-2 was expressed by these cells, or merely taken up from the bloodstream, by localizing IL-2 mRNA using *in situ* hybridization. We used as a probe the 337 base pair PstI-HindIII fragment from the coding sequence of the mouse IL-2 cDNA. A 330 base pair from a cDNA random sequence was used for negative controls. Hybridization was performed on 20 μm-thick paraformaldehyde-fixed serial sections through the forebrain of CD-1 mice. Transformed murine plasmocytomas producing IL-2 or IL-4 served as positive and negative tissue controls, respectively (Eur. J. Immunol., 1988, 18: 97). IL-2 mRNA was detected in a subpopulation of arcuate neurons, confirming that the IL-2 previously detected in this region by immunohistochemistry was synthetized by those cells. Moreover, IL-2 mRNA was also detected within the hippocampal formation, the medial habenula and the cerebral cortex, in conformity with our earlier immunohistochemical findings in the rat. Taken together, these results provide the first evidence for the expression of IL-2 by neurons in mammalian brain. Supported by the Fondation IPSEN and the MRC.

475.21

IMMUNOCYTOCHEMICAL LOCALIZATION OF INTERLEUKIN-4 (IL-4) IN BRAINS OF BALB/c, NUDE AND SCID MICE. I. Šterzl, N. Sadatyar* and G.P. Kozlowski. Dept. of Physiology, Univ. of Tex. Southwestern Medical Center, Dallas, TX 75235-9040.

Monoclonal antibody 11B11 rat anti-mouse rIl-4 (Ohara & Paul, Nature, 325:537, 1987) was used with biotinylated protein-G (BPG-Zymed Labs) and the ABC Elite kit (Vector Labs). In order to enhance the final reaction product, the sections were sequentially reincubated in BPG and ABC prior to color development. Staining for Il-4 disappeared when sections were incubated in a mixture of purified 11B11 added to rIl-4 produced by Sf 9 cells . Similarly, staining in lymph nodes from control and anti-IgD (induces IL-4) injected animals also disappeared. Although individual patterns varied, wide areas of the forebrain were immunopositive. Intense punctate staining was associated with neuronal perikarya and proximal segments of their fibers in field CA3 of the hippocampus, supracallosal and endopiriform layers of the cortex, olfactory stria and nuc triangularis septi (NTS). Non-neuronal staining occurred in astrocytes, microglia and blood vessels. In the athymic nude mouse, the frequency and intensity of staining in most brain regions were greatly reduced. In contrast to BALB/c mice- CA1 stained more than CA3 and more microglia were positive in the NTS. In the severe combined immunodeficient (SCID) mouse there was no staining of brain structures. Therefore, the degree of immunodeficiency in both of these mutant models determines the presence of Il-4 in brain. Supported by AA-06014.

475.22

DISTRIBUTION OF CD4 IN THE FOREBRAIN OF NORMAL (BALB/c), MODERATELY (NUDE), AND SEVERELY (SCID) IMMUNODEFICIENT MICE G.P. Kozlowski and I. Šterzl. Dept. of Physiol., Univ. Tex. Southwest. Med. Ctr., Dallas, TX 75235-9040.

The human immunodeficiency virus (HIV) binds to the cell surface molecule-CD4, an Ig superfamily molecule. Our aim was to determine the regional presence of CD4 in brain. 40 um thick sections were pretreated with 0.1% triton. Monoclonal antibody GK1.5 rat anti-mouse (αL3T4) rCD4 from E.S. Vitetta (Science 242:1166,1988) was used with biotinylated protein-G (BPG-Zymed Labs Inc) and the ABC Elite kit (Vector Labs). Sections were reincubated in BPG and ABC prior to color development. For negative controls, some sections were preincubated with GP120 (5 μg/ml). Punctate final reaction product marked cell surfaces of neuronal perikarya and fibers in wide regions of the brain. Although there was individual variation among BALB/c animals, strongest stain was in the: nu lat olfactory tract, septohypothalamic nu, bed nu stria terminalis, indusium griseum, hippocampus, supraoptic nu, median eminence and the supracallosal, piriform and endopiriform layers of the ctx. Staining was also associated with astrocytes, microglia and blood vessels. In contrast to BALB/c mice, the frequency and intensity of staining were greatly reduced in brains of athymic nude mice; although, glial staining remained intense. In the severe combined immunodeficient (SCID) mouse there was no staining of brain structures. Thus, the degree of immunodeficiency in these mutant animals parallels the relative presence of brain CD4. Supported by AA-06014.

NEURAL-IMMUNE INTERACTIONS: STRESS AND BEHAVIOR

476.1

cAMP AND INTRACELLULAR CALCIUM LEVELS IN SPLENIC MONONUCLEAR CELLS FROM STRESSED RATS. **E.M. Sonnenfeld and B.S. Rabin, Brain Behavior and Immunity Center, University of Pittsburgh Sch. of Med., Pittsburgh, PA. 15261**

Previous findings have implicated norepinephrine as the neurotransmitter involved in stress-induced splenic lymphocyte immunosuppression. Although work by others indicates that sustained elevations of cAMP tend to suppress DNA synthesis, our findings indicate that this mechanism is not a likely explanation for the splenic lymphocyte immunosuppression induced by stress. The cAMP levels from cells taken from stressed rats were not elevated over levels from control cells. Additionally, when cells were stimulated with Con-A for various times, essentially no differences were seen in cAMP levels between stressed and control lymphocytes. However, initial calcium levels in cells taken from stressed animals were found to be significantly higher than levels in cells from control animals. In addition, the response by fura-2 loaded cells to stimulation with Con-A tended to be different for cells from shocked verses cells from control animals. Further studies are in progress to define the mechanism of stress-induced mitogenic suppression.

476.2

IL-1 EFFECTS ON STRESS-RESPONSIVE AND -NON-RESPONSIVE CRH NEUROSECRETORY AXONS IN RATS. M.H. Whitnall, R.S. Perlstein*, E.H. Mougey[1] and R. Neta*. Armed Forces Radiobiology Research Institute, Bethesda, MD 20889-5145, and [1]Walter Reed Army Institute of Research, Washington, D.C. 20307-5100, USA

VP-containing, stress-responsive and VP-deficient, stress-nonresponsive CRH axon subtypes were identified by EM immunocytochemistry, which was also used to monitor axon activation (vesicle depletion). Five h after ip injection of 3 or 10 μg IL-1α, or 1, 3 or 10 μg IL-1β, into 450g Sprague Dawley rats, there were similar increases in plasma ACTH (to 3 times control) and decreases in prolactin (to 30% of control). Endotoxin (LPS, 0.1 or 1 mg) produced comparable effects. One μg IL-1α did not affect ACTH levels. Five h after 10 μg IL-1β, both VP-containing and VP-deficient CRH axons displayed depletion of secretory vesicles (to 22% and 36% of control, $p<0.01$ for both subtypes). Significant depletion was also seen after 1 mg LPS. Vesicle depletion after 10 μg IL-1α was less consistent, perhaps due to a lower affinity of IL-1α to IL-1 receptors mediating the response. The results demonstrate for the first time that, unlike stress, IL-1 can activate both the VP-containing and the VP-deficient subtypes of CRH axons. In contrast to recent suggestions in the literature, we conclude that the ACTH response to IL-1 involves stimulation of pituitary corticotropes by both CRH *and* VP.

476.3

EFFECTS OF SIGNALLED AND UNSIGNALLED STRESS ON NATURAL KILLER CELL ACTIVITY. J. Irwin and C. Neitzert*, Psychology Dept., Queen's University, Kingston, Ont., K7L 2Y1.

A variety of stressors have been shown to reduce cell-mediated immune functions in humans and in animals. It has been argued that the extent of this immune suppression may be limited by the controllability or predictability of the stressor. In the present study we assessed whether the presentation of a warning signal prior to stress exposure would attenuate or exaggerate the suppression of natural killer (NK) cell activity associated with stress.

NK activity was assessed in male CD-1 mice following exposure to one of two stress conditions. For mice in the Signalled Stress group, 6 sec footshock trials (150 μAmp, 60 sec intertrial interval) were preceded by a 10 sec light and tone CS. Those in the Unsignalled Stress condition also received 6 sec trials of footshock, but the light-tone CS presentations were presented on a random schedule. Two additional groups were included to control for any effects of the light-tone CS itself. To establish the association between the signal and the aversive stimulus, the Signalled Stress group was provided with 10 CS-footshock pairings a day for 5 successive days. The Unsignalled Stress group also received 10 footshock trials over 5 days. On the sixth day both groups received 60 trials of stressor exposure and controls received an equivalent amount of handling. Twenty-four hours later splenic NK activity was assessed with a chromium release assay.

Mice exposed to the CS alone or to Unsignalled shock exhibited a modest reduction in NK activity, suggesting that both conditions were mildly stressful. However, mice in the Signalled Stress condition showed a marked suppression of NK activity. Contrary to the view that a warning signal allows animals to adopt preparatory responses to buffer the impact of the stressor, these data indicate that the signal increases the aversiveness of the situation.

476.4

STRESSOR-INDUCED C-FOS EXPRESSION IN BRAIN STEM LOCI OF THE RAT: A CORRELATION OF NEURONAL ACTIVATION WITH IMMUNE ALTERATION. M.A. Pezzone, W.-S. Lee, G.E. Hoffman, K.M. Pezzone,* and B.S. Rabin, Depts. of Pathology and Physiology, University of Pittsburgh, School of Medicine, Pittsburgh, PA 15261.

Physically aversive stressors such as mild electric footshock suppress the mitogenic responsiveness of peripheral blood and splenic lymphocytes and depress splenic natural killer cell activity. In addition, these same immune alterations are elicited with non-aversive, conditioned stimuli when paired with aversive stimuli. In an attempt to define areas of the brain that influence immune function and respond to stressors, we have previously identified stress-induced c-Fos activated areas of the diencephalon. In accordance with our model, we found that c-Fos was strongly expressed in cells of the periventricular nuclei (some of which contain CRH) and other hypothalamic areas directly associated with autonomic function. In this study, c-Fos induction in the brain stem of the rat was examined in control, shocked, and conditioned animals. C-Fos was strongly expressed in the locus ceruleus (A6), the nucleus of the solitary tract (A2/C2), the ventral lateral medulla (A1/C1), and the periaqueductal gray matter in response to the unconditioned stimulus (US, electric footshock). Conditioned animals showed c-Fos induction in these same areas but to a lesser degree. Control animals exposed to a conditioning stimulus (CS, electronic tone) in the absence of the US, expressed very little, if any, c-Fos activity in the above loci, except for a small degree of expression in the periaqueductal gray matter. These results further confirm the role of autonomic and endocrine pathways as mediators of the stress response and will help to more fully characterize the pathways of stress-induced immune alteration.

476.5

STRESS-INDUCED ALTERATIONS IN INTERFERON PRODUCTION AND IA ANTIGEN EXPRESSION: ATTENUATION BY A B-ADRENERGIC RECEPTOR ANTAGONIST. J. E. Cunnick, G. Sonnenfeld[x], A. V. Armfield[x], P. G. Wood[x], and B. S. Rabin. Brain, Behavior and Immunity Center, Dept. of Pathology, University of Pittsburgh, Pittsburgh, PA 15261.

Previous work in this lab has demonstrated that the physical stress of electric foot-shock can alter splenic mitogen response to Con A and splenic NK activity. However, this stressor does not alter splenic IL-2 production. The present study examined the effects of foot shock on the production of another T-cell produced lymphokine, IFN-gamma. IFN-gamma was induced by Con A stimulation of splenocytes for 48 hr. The splenocytes from shocked rats produced 50% less IFN-gamma as measured by ELISA. The reduction in IFN-gamma could be attenuated in a dose dependent manner by administration of nadolol (a gift from E.R. Squibb) prior to shock. Cell surface expression of class II antigen was also found to be reduced in splenocytes from shocked rats. Expression of class II antigen on macrophages is mediated by IFN-gamma and is important in cell-cell interactions.

476.6

A COMPARISON BETWEEN SPRAGUE-DAWLEY, FISCHER 334, AND LEWIS RATS: CORTICOSTERONE RESPONSE TO ACUTE RESTRAINT STRESS; AND ADRENAL STEROID RECEPTOR LEVELS IN BRAIN AND IMMUNE TISSUE. F.S. Dhabhar, R.L. Spencer, and B.S. McEwen. Laboratory of Neuroendocrinology, The Rockefeller University, New York, NY 10021.

We are examining the hypothalamic-pituitary-adrenal (HPA) axis and its interaction with the immune system of 3 strains of rats [Sprague-Dawley (SD), Fischer 344 (F), and Lewis (L)] known to vary in their reactivity to acute stress. Animals were subjected to 1 h restraint stress, (one AM session & one PM session) to examine diurnal differences in the stress response. At each session, three tail blood samples ("basal," "acute stress," and "recovery") were collected for RIA determination of plasma corticosterone (CORT). There was a significant difference in the CORT response between strains. In AM and PM, the CORT response of F rats was the highest [59.8 μg/100ml (AM); 61.2 μg/100ml (PM)]; that of SD rats was intermediate [21.3 μg/100ml (AM); 26.2 μg/100ml (PM)]; and that of L rats was the lowest [11.4 μg/100ml (AM); 25.6 μg/100ml (PM)]. Also, the L rats did not show a diurnal rise in basal CORT levels. The concentration of Type I (mineralocorticoid) and Type II (glucocorticoid) receptors was measured in the hippocampus, hypothalamus, pituitary, spleen, thymus and peripheral blood mononuclear cells. Type II receptor levels were significantly higher in the hippocampus and spleen of SD, and in the thymus of L. Relative to SD rats, Fischer rats are hyper-responsive, and Lewis rats are hypo-responsive, to acute stress. The consequences of HPA axis differences between strains on habituation to chronic stress and on immune parameters are presently being investigated. (Supported by MH 41298.)

476.7

IMMUNOSUPPRESSION IN MICE INDUCED BY ISOLATED HOUSING: ANTAGONISM BY DIAZEPAM. N. Shanks, C. Renton, S. Zalcman & H. Anisman. Dept. Of Psychology, Carleton University, Ottawa, Canada K1S 5B6.

Noninbred CD-1 mice transferred from grouped to isolated housing exhibited a decrease of the peak plaque forming cell (PFC) response and antibody titers observed 4 days after inoculation with sheep red blood cells (SRBC: 10^6). This effect was not due to the transfer of housing condition, but instead was a consequence of the isolation. Moreover, the reduced immune response was dependent on the duration that mice were isolated prior to inoculation. The maximal decline of the immune response appeared in mice that received 5 to 10 days of isolation, after which the PFC response and antibody titers began to increase, approaching the values of group housed mice. Treatment with diazepam (1.0 mg/kg) for 5 days prior to inoculation and throughout the 4 day period following antigen treatment, antagonized the effects of the isolation treatment. These data suggest that the immunosuppression may have resulted from the anxiety or arousal stemming from the isolation.

476.8

DEPRESSION, STRESS, AND IMMUNE FUNCTIONING IN PREPUBESCENT CHILDREN: EFFECTS ON PHAGOCYTOSIS.
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Previous work has shown there to be an age related effect of depression on the immune system such that in older adults depression was associated with a decrease in immune functioning and in younger adults with an enhancement of immune function as measured by mitogen stimulation of peripheral lymphocytes. To further examine the effects of depression on immune functioning across the life span, subjects and controls between 8 and 12 years of age who were Tanner 1 stage of development were studied. The depressed subjects met criteria for major depressive disorder based on the Diagnostic Interview Schedule for Children (DISC-R). Subjects were studied the same day and time as healthy age, sex, SES, and race matched controls. The depressed subjects were significantly more likely to come from non-intact families. We previously reported a decrease in natural killer cell activity in the depressed children, but higher numbers of lymphocytes and B cells. The present experiment examined granulocyte functioning assessing phagocytosis and killing of Staph a. While there were no overall effects of depression, there were striking age related effects such that the older children demonstrated increased phagocytosis ($T=2.3, p<0.04$) and killing ($T=2.2, p<0.04$). Family composition was shown to be significantly related to granulocytic killing, with those from non-intact families showing decreased activity($T=2.4, p<0.03$). These data suggest that the stress of living in a non-intact family may be associated with altered granulocyte function.

476.9

INTERACTIONS BETWEEN SEX AND HANDLING: THYMIC AND SPLENIC GLUCOCORTICOID RECEPTORS (GCCR), MITOGEN RESPONSES, AND PLASMA HORMONE LEVELS IN ADULT RATS. R.E. Landsman, M. Umali*, B.J. Branch*, J.E. Shyrne*, R.A. Gorski and A.N. Taylor. Lab. of Neuroendocrinology, Brain Res. Inst. and Dept. Anatomy & Cell Biology, UCLA; West L.A. VAMC, Brentwood Div., Los Angeles, CA 90024.

GCCR in immune glands may be involved in hormone-modulated effects of stress on immune responsiveness. The effects of daily handling on thymocyte (TC) and splenocyte (SC) proliferative responses to in vitro mitogen challenge and GCCRs were studied in relation to endogenous plasma hormone levels. Male (M) and estrus female (F) Sprague-Dawley rats were either handled (H) 10-min daily for 20 days (n=3) or unhandled (UH) (n=3) prior to sacrifice on day 80. Significant SEX x HANDLING interactions were found for TC but not SC responses to concanavalin A (CON A), TC- and SC-GCCR, and testosterone (T) levels [TC-GCCR & TC-CON A: UHM > HM or UHF; TC-GCCR: HF > UHF or HM; SC-GCCR: UHF < HF or UHM; T: HM > UHM & UHF > HF]. Handling decreased plasma corticosterone (C) and progesterone (P) levels [C: HF < UHF; P: HM&F < UHM&F]. UH M and F exhibited significant negative relationships between C or P and TC-CON A, TC-GCCR and SC-GCCR, while T correlations were positive. Handling abolished these relationships and reversed T vs TC-GCCR. TC-CON A was positively related to TC-GCCR in UH, but not H, M and F. These results implicate hormone changes in handling effects on GCCR and immunoreactivity. (Supported by NIH HD07228 & HD01182 & VA Medical Res.)

476.10

CORTICOSTERONE RESPONSES TO INTERLEUKIN-1 AND RESTRAINT IN RATS LESIONED WITH 6-HYDROXYDOPAMINE
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Stress and immune activation are associated with increases in plasma concentrations of corticosterone (CS). To determine whether this phenomenon is affected by catecholaminergic innervation of neurons in the hypothalamic paraventricular nucleus (PVN), selective lesions were made, using 6-hydroxydopamine (6-OHDA). Injection of 6-OHDA into the PVN depleted norepinephrine (NE) by 75% and significantly inhibited the increase in CS after human interleukin-1α (hIL-1α 0.5 μg/rat i.p.). The lesions did not affect the adrenocortical response to 20 min restraint. Injection of 6-OHDA into the ventral noradrenergic ascending bundle depleted the hypothalamus of NE to a similar extent. This lesion also attenuated the CS response to hIL-1α, but did not alter that to restraint. These results indicate that the IL-1-induced increases in plasma CS concentrations depend at least partly on catecholaminergic innervation of the PVN, whereas non-catecholaminergic pathways participate in activation of the hypothalamo-pituitary-adrenal axis associated with restraint.

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476.11

ALTERATIONS OF CENTRAL CATECHOLAMINES ASSOCIATED WITH PRIMARY AND SECONDARY IMMUNOLOGICAL CHALLENGE. S. Zalcman, N. Shanks, Z. Merali and H. Anisman. Departments of Psychology, Carleton Univ. and Univ. of Ottawa, Ottawa, Canada.

Central catecholamine variations in CD-1 mice were assessed after primary and secondary inoculation with sheep red blood cells (SRBC; 10^6 cells, ip). At the time of the peak primary and secondary immune responses (4 and 2 days post-immunization, respectively), increased dorsal bundle norepinephrine (NE) utilization was evident. Moreover, like stressors, antigenic challenge provoked variations of mesocorticolimbic but not nigrostriatal dopamine (DA). While the catecholamine changes associated with the primary response were only evident on the day of the peak response, the effects associated with the secondary response persisted for several days. Further studies using in vivo microdialysis in Sprague-Dawley rats confirmed the increased DA release in the nucleus accumbens associated with primary SRBC inoculation. However, this release occurred earlier than that detected in mice using HPLC determinations. These data are consistent with the proposition that the immune system acts as a sensory organ and that the immune response may be interpreted as a stressor leading to central catecholamine alterations.

476.12

TIME-DEPENDENT VARIATIONS OF SELF STIMULATION FROM THE NUCLEUS ACCUMBENS FOLLOWING ANTIGEN ADMINISTRATION. G. MacNeil, S. Zalcman, R. Zacharko and H. Anisman. Dept. of Psychol., Carleton Univ., Canada.

Central nervous system activity may influence immune functioning, and conversely immune activation may affect central neurotransmitter activity. Administration of sheep red blood cells (SRBC), for instance, provokes brain region-specific alterations of norepinephrine and dopamine activity at a time which coincides with the peak immune response. The antigen-induced neurochemical changes were reminiscent of those induced by stressors. The present investigation demonstrated that in CD-1 mice SRBC (10^6) inoculation, like stressor exposure, disrupted self-stimulation responding from the nucleus accumbens, coincident with the peak immune response. That is, self-stimulation was unaffected either immediately, 1 or 2 days after inoculation, but was markedly reduced 3 or 4 days after immunization. These data suggest a functional relationship between the immune system response and central neurotransmitter activity. Essentially, it is thought that immune responses are interpreted as stressors, ultimately leading to variations of central catecholamine activity, hence provoking an anhedonia.

476.13

δ– AND κ–, BUT NOT μ–, SELECTIVE OPIOID AGONISTS INHIBIT SYMPATHETIC POST-GANGLIONIC NEURON–DEPENDENT PLASMA EXTRAVASATION IN KNEE JOINT OF THE RAT.

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Plasma extravasation (PE) was induced in the knee joint of the rat by local infusion of either bradykinin (BK) (160 ng/ml), an inflammatory mediator produced at sites of tissue injury, or capsaicin (5 mg/ml). BK–induced PE is dependent on the sympathetic postganglionic neuron (SPGN), whereas capsaicin–induced PE is dependent on primary afferent neurons and the SPGN. When selective δ– (DPDPE) (10 μM), or κ– (U50,488H) (10 μM) opioid agonists were co-infused, BK–PE was significantly attenuated, and this attenuation was reversed by co-infusion of naloxone (1 μM). In contrast, co-infusion of a selective μ–opioid agonist (DAMGO) (10 μM) did not reduce the PE induced by bradykinin. In contrast, DAMGO, was able to block the PE produced by capsaicin. These results suggest that the inhibition of neurogenic PE by opioids is dependent on the specific opioid receptor subtype and the relative contribution of unmyelinated afferent and SPGN terminals: δ– and κ–selective opioid agonists inhibit SPGN-dependent PE and μ–selective opioid agonist inhibits primary afferent but not SPGN–dependent PE. *Supported by NIH grant AM32634*

476.14

HEART RATE LEVEL, HEART RATE VARIABILITY, AND SPECIFIC ANTIBODY RESPONSE IN FREE-RANGING RHESUS MACAQUES. M. L. Laudenslager, K.L. Rasmussen*, and S. Suomi. Univ. Co. Hlt. Sci. Ctr., Behavioral Immunology Laboratory and NIH/NICHD, Laboratory for Comparative Ethology.

The immune response may be influenced by a number of behavioral factors, mediated through two possible routes, circulating hormonal factors and direct autonomic innervation of lymphoid tissue. The present study related measures of autonomic reactivity to the appearance of tetanus toxoid (TT) specific IgM and IgG appearing in the plasma following TT immunization of juvenile Rhesus macaques free-ranging on the island of Cayo Santiago, P.R. Eleven subjects, 250-377 days old, were captured and briefly fitted with telemetry devices for unrestrained monitoring of EKG. A blood sample was obtained from each subject prior to immunization with TT on the day of release. After 10-15 days, subjects were recaptured and a second blood sample was obtained for the determination of plasma IgM and IgG specific to TT by an ELISA. A strong relationship between IgG levels and heart rate ($r=.86$, $p=.003$) and heart rate variability ($r=-.79$, $p=012$) was noted. Plasma cortisol levels measured 24 hr following the first capture prior to immunization were not related to TT antibodies. (Supported in part by NIH Research Grant MH37373)

476.15

GENETIC DIFFERENCES IN SOCIAL BEHAVIOR: RELATION TO CANCER SUSCEPTIBILITY AND NK CELL FUNCTION

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Much of the psychoneuroimmunology literature has focused on understanding how various social and non-social "states" can influence aspects of immune function. Despite considerable theorizing about the relationship of personality variables to disease susceptibility (e.g., cancer), few studies have related immune function to gentically-determined individual differences that are stable across development ("traits"). We studied the 23rd generation (S_{23}) of ICR mice selectively bred for high and low levels of aggression. The high aggressive line (NC900) shows rapid and repeated attack following social isolation, with little variability observed across generations. Conversely, low aggressive mice (NC100) have departed substantially from the foundational stock (S_0), exhibiting high levels of freezing ("timidity") in response to social contact. This robust and reliable line difference appears to be correlated with differences in midbrain dopamine function. We hypothesized that the genetic differences in social behavior observed in NC100 mice would correlate with increased vulnerability to cancer development and reduced natural killer (NK) cell function. To test these hypotheses we assessed 3-methylcholanthrene-induced tumor development and splenic NK cell activity in the NC900 and NC100 lines. Only 44% of the NC900 mice developed tumors, whereas all NC100 mice had palpable neoplasms. Additionally, baseline NK activity was significantly lower in socially inhibited (NC100) mice versus aggressive (NC900) mice. Conversely, no baseline differences in NK activity were found between NC900 and non-selected, socially isolated mice (NC 600). These latter data complement existing behavioral evidence supporting the unidirectional outcome of the selective breeding program. Our data support the hypothesis that genetic ("trait") differences in social behavior may predict differences in disease susceptibility and immune function. Integration of future neurobiological and immunological findings using this mouse model may provide important information concerning mechanisms of brain-immune interactions. (Supported by the Foundation of Hope and MH45371)

476.16

BEHAVIORAL DIFFERENCES IN LEW/N AND F344/N RATS.

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Recently several differences in the neuroendocrine response to inflammatory mediators have been characterized between F344/N and LEW/N rats. These differences are important because they are related with the differential susceptibility to arthritis across these strains. Previous studies have indicated that this susceptibility appears to be mediated by a deficiency in central corticotropin releasing hormone (CRH) biosynthesis or secretion, and is reflected by a difference in hypothalamic-pituitary-adrenal response during stress in these strains. We have also shown that the LEW/N rat hyperresponds to acoustic startle stimuli whereas the F344/N rat hyporesponds, compared to Sprague-Dawley rats. Consistent with previous studies using various stressors, the F344/N rats exhibit an exaggerated corticosterone response to startle stimuli while the LEW/N rats do not. The current studies sought to further characterize potential behavioral differences in these strains. LEW/N rats consistently ambulate less than F344/N rats in an open field, with the ratio of time spent in inner, as opposed to outer, portions of the field being approximately 0.1 for the LEW/N and 0.4 for the F344/N rats. CRH decreased ambulation in both strains, but did not alter this ratio in either strain. In contrast, LEW/N rats entered the open arms of an elevated plus maze more often than F344/N rats, but the time spent per entry was the same for both strains. These results demonstrate that these strains differ in baseline response on several behavioral measures of response to noxious environmental influences, substantiating the useful ness of behavioral markers for neuroimmunological disease.

476.17
BEHAVIORAL EFFECTS OF REPEATED INJECTION OF INTERFERON-α A/D IN BALB/C MICE. L.S. Crnic and A.L. Dunn*. University of Colorado School of Medicine, Denver, CO 80262.

The hybrid recombinant interferon α-A/D (a gift of Hoffman-La Roche) has antiviral activity and produces liver P-450 depression in mice (Renton et al, 1984) but behavioral effects are unknown. Measures of activity (open field), depression (tail hang), muscle strength (forelimb grip strength) and motor ability (swim posture and endurance with 0%, 3%, and 6% added body weight) were examined in BALB/c mice exposed to 1600 U/g of IFN-α-A/D daily for 5 days (n = 9) or to the IFN vehicle (n = 11). Behavior was tested on each day, except for swimming which was tested on day five.

Open field activity was significantly depressed on all days of testing in the group exposed to IFN ($p < 0.02$). The number of times the head dipped under the water when swimming with 3% added body weight was higher in the IFN treated mice (24.1 vs 8.9, $p < 0.025$). Thus, this human hybrid IFN is behaviorally active in mice. Depression of motor activity is a general effect as seen both in this study and in our prior study with acute doses of non-recombinant mouse IFN-α (Lee BioMolecular, Segall and Crnic, 1990). The generality of the effect is seen across different activity measures in these two studies. Further, swim endurance is depressed by IFN. Supported by grants MH00621, HD04024, and MH15442.

476.18
SECOND-ORDER AND SENSORY PRECONDITIONING OF IMMUNOSUPPRESSION: EVIDENCE FOR COGNITIVE CONTROL. B.J. Kucinski, J.E. Cunnick, and H. Fowler*. Dept. of Psychology and Pathology, Univ. of Pittsburgh, Pittsburgh, PA 15260.

Two experiments evaluated the effects of higher-order conditioning on immune function. In the first, rats were given pairings of stimulus A and shock and then pairings of stimulus B and A but without shock. Control subjects received the same except that B and A were unpaired. After a recovery period, different groups received exposure to A, the paired B (B+), the unpaired B (B-), the training context (X), or no treatment (N) just before sacrifice. Mitogen-stimulation assays of lymphocytes from the spleen and blood and an NK cell assay of splenocytes showed comparably pronounced immune suppression for the A and B+ groups, relative to the B-, X , and N groups, attesting to a second-order effect for B+. These results suggest that immune function is affected by psychological forms of stress that derive from a cognitive processing of events; i.e., B+ implies A and A implies shock. To corroborate this, we conducted a second experiment on sensory preconditioning. That study duplicated the first except that pairings (or unpairings) of B and A occurred prior to pairings of A and shock, so as to preclude association of B+ and A's suppressive effect. In evidence of cognitive control, the results showed comparably pronounced immune suppression for the A and B+ groups, relative to the rest, establishing that "worrisome" thoughts about something, even remote, profoundly affect immune function.

476.19
CLINICAL AND IMMUNOLOGICAL CORRELATES OF ANTI-HIPPOCAMPAL IGG ANTIBODIES IN SCHIZOPHRENIA. K.N. Roy Chengappa, R. Ganguli, L. Wechsler and B.S. Rabin, Immunopsychiatry Program, Western Psychiatric Institute and Clinic, Pittsburgh PA 15213

Raised hippocampal autoantibody levels may occur in a subgroup of schizophrenics characterized by negative symptoms, low IL-2 production and the presence of non-CNS autoantibodies. Using an enzyme immunoassay (ELISA), we tested for IgG autoantibodies to human hippocampus in 99 RDC schizophrenic patients and 81 normal controls matched for age and the presence of autoantibodies to non-CNS antigens. Anti-hippocampal antibody levels were correlated with the severity of positive and negative symptoms, duration of illness and Interleukin-2 (IL-2) production. First episode subjects had lower hippocampal autoantibody concentrations than other patients ($p=0.01$). Acutely ill patients with non-CNS autoantibodies had higher anti-hippocampal antibody levels ($p=0.02$). Negative symptoms were significantly correlated with anti-hippocampal antibody levels in remitted patients ($r=0.44$, $p=0.016$). Patients with anti-hippocampal antibody levels above the 75th percentile had the lowest IL-2 production ($p=0.029$).

476.20
IMMUNE COMPETENCE INFLUENCES SLEEP AFTER MICROBIAL CHALLENGE. L.A. Toth and J.M. Krueger. Univ. Tennessee, Memphis, TN 38163.

To evaluate the role of the immune system in mediating the somnogenic effects of infectious disease (FASEB J 3:2062, 1989), we monitored sleep after Candida albicans (CA) infection in rabbits with altered immune competence. Immune function was enhanced by immunization with killed CA or injection of incomplete Freund's adjuvant (IFA; 0.25 ml/kg SQ), or was suppressed by injection of cortisone (40 mg/kg IM) or cyclosporin (25 mg/kg SQ). Cortisone markedly attenuated the fever, increased slow-wave sleep, increased delta-wave amplitude during sleep and acute phase responses that were induced by CA alone. Other regimens were ineffective. Theta-wave amplitude during wakefulness decreased slightly after CA alone and markedly after CA with IFA, but increased after CA with cortisone, cyclosporin or immunization. We conclude that the somnogenic effects of infectious challenge can be altered by manipulation of the immune system. Supported by NS-26429 and NS-25378.

476.21
ANOREXIC EFFECTS OF INTERLEUKIN-1 (IL-1) ARE MEDIATED PERIPHERALLY. S. KENT, K.W. KELLEY* and R. DANTZER*. INSERM U.176, rue Camille Saint-Saëns, 33077 Bordeaux Cédex, France (SK and RD); Dept. of Animal Sciences, Univ. of Illinois, Urbana, IL 61801 (KWK).

IL-1 is a cytokine that is released by activated macrophages and monocytes and mediates many of the local and systemic responses to inflammation. Although both peripheral and central injections of IL-1 produce anorexia, it isn't clear whether this effect is mediated peripherally, centrally, or both. Recently, a specific antagonist for the IL-1 type 1 receptor has been characterized and cloned (IL-1ra; Synergen, Boulder, CO). We have used this protein to determine the site of action for the effects of IL-1 on feeding. Male rats were food restricted and trained on an operant schedule (FR10) for food reinforcement. Rats were tested for 5 min before injection of recombinant human IL-1β (Glaxo, Geneva; 4 µg ip or 40 ng icv)) and then retested 1, 2, 4, 8, and 24 h later. Both ip and icv injections produced profound decreases in responding, with maximal effects 2-4 h post-injection. IL-1ra pretreatment (8 mg/kg ip or 80 µg/kg icv) completely blocked these effects when administered by the same route. In contrast, icv IL-1ra was unable to block the anorexic effects of ip IL-1. IL-1ra had no effects by itself. These results are in agreement with previous suggestions that the anorexic effects of IL-1 are mediated peripherally.
(Supported by DRET (RD) and ONR (KWK))

477.1

EFFERENT PROJECTIONS FROM THE LATERAL PARABRACHIAL NUCLEUS AS DEMONSTRATED BY PHA-L. RM Slugg and AR Light. Dept. of Physiology, UNC-Chapel Hill, Chapel Hill, NC 27599.

To determine the projection sites of nocireceptive and thermoreceptive neurons, discrete injections of PHAL were placed in PBL following the recording of unit activity from the injection micropipette. These injections indicated the presence of two major ascending pathways. 1) The medial pathway branched at the pontomesencephalic junction and coursed bilaterally along the ventrolateral edge of the central gray. This pathway projected heaviest bilaterally into the ventrolateral PAG immediately rostral to the injection site. The parent axon then ascended rostrally into the central gray matter and split into two pathways, one which continued rostrally and gave rise to numerous terminations in the midline thalamic nuclei. The other turned ventrally and terminated densely in the ventromedial hypothalamus, but was observed only from injections confined to the lateral half of PBL. 2) The lateral pathway was ipsilateral only and ran diffusely through the central tegmental tract with occasional terminations. These fibers then branched in the rostral half of the lateral hypothalamus, and both terminated heavily in this region and also crossed the ansa peduncularis to enter the amygdala where terminations were observed equally in the central and medial nuclei. Supported by grants PHS DA04420 and NS16433.

477.2

DISTRIBUTION OF NEUROTENSIN-LIKE IMMUNOREACTIVITY IN THE CAT'S BRAINSTEM AND THALAMUS.

D. Harder, C. Vahle-Hinz and K.-D. Kniffki. Physiologisches Institut, Universität Würzburg, D-8700 Würzburg, Germany.

To further characterize the diencephalic regions involved in nociception the neuropeptide content of the thalamus and its afferent systems were studied in the cat. Neurotensin (NT) has been associated in the rat with visceral sensory pathways, probably including systems involved in nociception.

Deeply anesthetized adult cats were perfused transcardially with 4% paraformaldehyde and 0.2% picric acid. Serial frontal sections were reacted with an antibody against NT (Incstar, 1:6000) using the peroxidase-antiperoxidase (PAP) method (2nd antibody: goat-anti-rabbit, Dakopatts; 3rd antibody: PAP complex, Dakopatts).

Several regions with different densities of NT-like immunoreactive (NT-LI) fibers were found in the brainstem and the thalamus. Dense staining was seen in all parts of the parabrachial n. (PB), the n. coeruleus and the periaqueductal gray of the brainstem as well as in the hypothalamus, whereas moderate staining was observed in the n. of the solitary tract (NTS), the marginal layer of the caudal n. of the spinal trigeminal complex and the principal trigeminal n. At the thalamic level moderate immunoreactivity was present in the habenula and in a region extending from the subparafascicular n. to the midline, apparently including parts of the principal ventromedial n. and the dorsal hypothalamus. In addition, scattered NT-LI fibers were found in the oral n. of the spinal trigeminal complex and in all tegmental fields of the brainstem and in the thalamus within the external medullary lamina ventral of the ventrobasal complex, the mediodorsal n. (MD), the fields of forel and the zona incerta.

The results indicate that NT might play a role in the transmission of visceral and possibly nociceptive information in the cat, since NT-LI fibers were observed in the NTS (medulla), the PB (pons) and the MD (thalamus).

477.3

RECIPROCAL CONNECTIONS BETWEEN THE MEDIAL PREOPTIC AREA (MPO) AND THE VENTRAL MEDULLA: A WGA-HRP AND PHA-L STUDY. Tilat A Rizvi, M Ennis, M T Shipley and M.M. Behbehani. Depts. Anatomy & Physiology. Univ. Cincinnati. Coll. Med., Cincinnati, OH 45267.

Anatomical studies suggest that the MPO and ventral medulla are interconnected. Here, we report on the organization of reciprocal connections linking these basal forebrain and brainstem structures.

WGA-HRP injections into MPO retrogradely labeled numerous neurons in the ventrolateral medulla (VLM) and the midline portion of nucleus raphe magnus (NRM). Both WGA-HRP and PHA-L injections in MPO resulted in dense anterograde labeling throughout NRM; less dense anterograde labeling was also present in VLM. Retrograde tracer injections in NRM produced intense retrograde labeling in median preoptic nucleus (MePO) and MPO. Rostrally, in MPO the majority of labeled cells were located medially; caudally retrograde labeling was concentrated in the more lateral cells. MPO also contained heavy anterograde labeling after WGA-HRP injections in NRM.

The present findings suggest that connections between MPO and ventral medulla are reciprocal but are organized in an asymmetrical fashion: NRM receives heavy inputs from MPO while there is a more moderate projection from MPO to VLM. On the other hand, VLM appears to project more heavily to MPO than does the NRM. These two asymmetrically organized circuits might function to coordinate antinociception and autonomic activity during stereotyped sexual behaviors. (PHS Grants NS20643, NS24698, HL08097).

477.4

ROLE OF NEUROTENSIN NEURONS FROM THE PAG TO THE NRM IN THE RESPONSE TO OPIATE ADMINISTRATION IN THE PAG. M.O.Urban* and D.J.Smith.Anesth. & Pharmacol., WVU-HSC, Morgantown, WV 26506

Some neuronal processes from the periaqueductal gray (PAG) to the nucleus raphe magnus (NRM) contain the tridecapeptide neurotensin (Beitz,J.Neurosci.2:892,1982). When the peptide is injected into the NRM, a dose dependent antinociceptive effect is observed as an increase in tail-flick latency (TFL). Since the NRM functions as a relay involved in opiate effects from the PAG, the role of neurotensin in this relay was studied.

Male Sprague-Dawley rats fitted with guide cannulas over both the PAG and NRM were used. Microinjection of neurotensin into the NRM produces a dose-dependent increase in TF latency with an EC 50 of about 6 nmol. The partial agonist [D-Trp-11]-neurotensin (Quirion et al.,EuropJ.Pharmacol.61:309,1980), at a dose of 3 pmol, antagonized the effect of neurotensin when injected sequentially.

β-endorphin (10 nmol) injected into the PAG produced an antinociceptive response that was inhibited by the injection of the antagonist into the NRM. In contrast, the response to morphine (6 nmol) was potentiated by the antagonist. These results suggest that there may be more than one functional type of neurotensin neuronal projection from the PAG to the NRM.

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477.5

A HIGH THRESHOLD VENTROBASAL THALAMIC POTENTIAL ATTENUATED SPECIFICALLY BY ANALGESIC DOSES OF MORPHINE OR COCAINE. J.A. Kiritsy-Roy, T.J. Morrow and K.L. Casey, Depts. of Neurology and Physiology, Univ. of Michigan and Neurol. Res. Labs., VA Med. Center, Ann Arbor, MI 48105.

Previous studies have analyzed rostrally projecting pain pathways by recording from antidromically identified spinothalamic/spinoreticular tract cells in the spinal cord. We wish to study the neurophysiology and neuropharmacology of supraspinal mechanisms activated by these extralemniscal (EL) pathways. Accordingly, we are developing a model for the study of pain pathways using orthodromic activation of the EL system and its target cells in the thalamus. Studies were undertaken to identify and characterize thalamic field potentials evoked by noxious stimulation in the hindpaw region of the chloral hydrate anesthetized rat (n=15). Slow wave activity evoked by electrical stimulation of the hindlimb was recorded in the ventral posterior thalamus. An evoked potential averaged over 32 stimuli consisted of four components: a negative peak at 11.7±0.4 msec (N12), a positive peak at 23.2±1.2 msec (P23), a negative peak at 50.9±2.2 msec (N51) and a positive peak at 93±3.5 msec (P93). Lesions of the dorsal columns at approximately T6 reduced the amplitude of the N12-P23 complex whereas lesions of the ventral spinal cord (EL pathways) attenuated the amplitude of the N51-P93 components. The lemniscal potential was highly somatotopic, but the EL components were not. The threshold for activation of the EL potentials was 3 to 4 times higher than for the lemniscal potentials. Morphine (1.5-18 mg/kg i.v.) or cocaine (1-4 mg/kg i.v.) produced dose-related suppression of N51-P93 amplitude with little or no effect on the lemniscal components. The effect of morphine was antagonized by naloxone (0.8 mg/kg i.v.).

This ventrobasal thalamic EL potential has physiological and pharmacological characteristics suggesting its' use as a measure of activity in ascending nociceptive systems. (Supported by grants from the Dept. of Veterans Affairs and Bristol-Meyers Squibb)

477.6

ULTRASTRUCTURAL FEATURES OF SPINAL CORD AFFERENTS TO THE INTRALAMINAR AREA OF THE CAT THALAMUS. I.A. Ilinsky[1], R.G. Mackel[2], E. Miyashita[2] and K. Kultas-Ilinsky[1]. [1]Department of Anatomy, University of Iowa College of Medicine, Iowa City, IA, 52242, [2]Rockefeller University, New York, NY 10021.

Topographical distribution of spinothalamic fibers has been investigated in many light microscopic studies (see review by Craig and Burton, Exp. Brain Res. 58:227-254, 1985) which agree that the intralaminar, submedian, posterior complex, ventral posterior and ventral lateral nuclei represent the major domains of spinothalamic projections. Unlike medial lemniscal projections, spinothalamic afferents cluster around motor cortex-projecting neurons in the ventral lateral (VL) and intralaminar (CLn) nuclei (Hirai and Jones, Exp. Brain Res. 71:32-344, 1988). Significantly less is known in regard to fine structural features of their terminals, particularly in the VL and CLn. This study was aimed on identification of spinothalamic boutons in the intralaminar region of the cat thalamus. Multiple injections of a mixture of 10% HRP and 7% WGA-HRP in 2% DMSO were placed bilaterally at C_3-C_5 level of the spinal cord in two adult cats. Anterograde labeling was observed in thin fibers which formed varying density terminal plexuses in the posterior complex, CLn, and VL. However, the densest zone of labeling in both cases was in the postero-medial intralaminar region where CLn borders the parafascicular and mediodorsal nuclei. For ultrastructural analysis, this zone was sampled from serial vibratome sections processed for HRP histochemistry with TMB at pH 6.0 and stabilized with OsO_4. Spinothalamic boutons labeled with HRP were of LR type (large boutons filled with round vesicles) which formed multiple asymmetric synaptic contacts on distal dendrites of projection neurons and vesicle-containing dendrites of local circuit neurons. Occasionally labeled boutons were apposing somata of small cells, however, no synaptic contacts have yet been observed. The majority of the labeled boutons in this thalamic region were a part of glomeruli. Supported by RO1NS24188 and RO1NS26288.

477.7

ULTRASTRUCTURE OF LAMINA I TERMINALS IN NUCLEUS SUBMEDIUS OF THE CAT. A. Blomqvist, A.-C. Ericson*, J. Broman and A.D. Craig. Dept of Cell Biology, University of Linköping, S-581 85 Linköping, Sweden, and Division of Neurobiology, Barrow Neurological Institute, Phoenix, Arizona 85013, U.S.A.

Previous anatomical studies have demonstrated that the nucleus submedius (Sm) is a specific lamina I termination site in the medial thalamus. In the present study, the ultrastructure and synaptic organization of the lamina I terminals in Sm were examined. *Phaseolus vulgaris* leucoagglutinin (PHAL) was injected into lamina I at different spinal levels. PHAL-containing axon terminals in Sm were demonstrated with the peroxidase-antiperoxidase method and analyzed in the electron microscope. Labeled terminal boutons packed with round synaptic vesicles were seen in synaptic contact with dendritic profiles that presumably originate from thalamocortical relay cells. Synaptic contacts were also seen with presynaptic dendrites that in turn synapsed with relay cell dendrites. Examination of serial sections suggests that such serial synapses exist both independently and in the triadic arrangement typical of thalamic sensory relay nuclei. Complex synaptic aggregations in which a relay cell dendrite received inputs from several lamina I afferent terminals and presynaptic dendrites were found. These results suggest that processing of lamina I spinothalamic input to Sm involves afferent inhibition, probably via GABAergic interneurons.

Supported by grants from the NIH and the Swedish Medical Research Council.

477.8

RESPONSES OF RAT NUCLEUS SUBMEDIUS (Sm) NEURONS TO NOXIOUS THERMAL, CHEMICAL AND ELECTRICAL STIMULATION. J.S.Tang*, C.Y.Chiang and J.O.Dostrovsky, Dept. of Physiology, Univ. of Toronto, Toronto, Ontario M5S 1A8, Canada

Recent studies have suggested that Sm may be an important thalamic relay for nociceptive information. The present study investigated the effects of thermal, chemical and electrical stimulation on Sm neurons in urethane/chloralose anesthetized rats. Noxious but not innocuous mechanical stimulation induced responses in 67 of the 86 neurons studied that were histologically verified to be in Sm. Most of these neurons could also be activated by noxious heating (mean threshold 47.6 $\pm$0.4^{o}C), but in many cases the responses to increasing temperatures were not well graded. Many of these neurons were also excited or their response to cutaneous stimulation facilitated by subcutaneous, intramuscular or intraperitoneal injection of 2.5% formalin or hypertonic saline (0.1-0.2 ml). Thirty-eight of 49 Sm neurons tested could be activated by intense electrical stimulation (15-25ms 200 Hz trains, 3 ms pulse duration, 5-10 mA/ 3 s) of tail, paw and vibrissal pad. The latency of the responses ranged from 20 to 500 ms (mean 174$\pm$27 ms, S.E.M.). The conduction velocities of the primary afferents eliciting the responses were estimated for 8 cells by comparing the latencies of the Sm neuron responses to stimulation of the tail at distal and proximal sites and found to range from 0.8 to 33 m/s. The Sm neuron responses to electrical stimulation often appeared only after repetitive train stimulation, and then showed "wind-up", suggesting a polysynaptic pathway to Sm. The response characteristics of the Sm neurons revealed in this study support the hypothesis that Sm may be involved in mediating the affective-motivational aspects of pain. (Supported by NIH)

477.9

NOXIOUS HEAT AND INNOCUOUS COLD PERCEPTION IN MONKEY AFTER INJECTION OF LIDOCAINE INTO THE VPM THALAMIC NUCLEUS. M.C. Bushnell, J.L. Oliveras, N. Bastrash, N. Tremblay, and G.H. Duncan. Fac. Médecine dentaire, Univ. Montréal, Québec, Canada H3C 3J7.

In thalamus, information about pain and innocuous cold is transmitted to the ventroposterior nuclei (VPL and VPM) and to more medial nuclei, such as submedius. The present study evaluates the role of VPM in facial pain and temperature perception, by determining the effect of transient inactivation of VPM on a monkey's detection of changes in intensity of noxious heat and innocuous cold.

Two rhesus monkeys were trained to detect small differences in the intensity of a white light and of noxious heat (near 46°C) and innocuous cold (near 30°C) applied to the facial maxilla. Single cell recordings identified regions within VPM responding to noxious heat and/or cooling. Lidocaine hydrochloride 2% (1.0-5.0µl) was injected into VPM, and the monkey's ability to detect near-threshold changes in stimulus intensity was compared before and after each injection.

In three instances single injections of 1.0-4.0µl lidocaine into caudal VPM were confirmed to block the activity of neurons recorded 2mm from the injection site, but produced no decrement in the monkey's detection ability. Three similar injections, for which neuronal deactivation was not electrophysiologically confirmed, also did not alter thermal detection. However, when two injections were made simultaneously through cannulae separated by 1mm in the AP direction, there was a profound decrease in the monkey's ability to detect intensity changes in noxious heat and a lesser decrease for cold. Nevertheless, electrode recordings showed that these large injections did not deactivate neurons in more medial thalamic nuclei.

These data suggest that VPM plays an important role in thermal perception. However, it appears that deactivation must occur through a large AP extent of VPM to disrupt thermal discrimination, possibly because of overlapping receptive fields along the anteroposterior plane. [Supported by the Canadian MRC.]

477.10

SPINOTHALAMIC (STT) ACTIVATION OF PRIMATE THALAMIC NEURONS IS NOT MODULATED BY GABAERGIC LOCAL CIRCUIT NEURONS. H.J.Ralston, III, A.M. Milroy and D.D.Ralston, Department of Anatomy, University of California, San Francisco, California, 94143

We have examined several hundred labeled STT terminals in macaque monkeys and have contrasted their synaptic interactions with those made by labeled medial lemniscal (ML) terminals. Anesthetized monkeys received microinjections (0.02-0.04µl) of wheatgerm agglutinin horseradish peroxidase (WGA-HRP) into the dorsal horn of the cervical or lumbar enlargements or into the dorsal column nuclei. Following appropriate survival times, the animals were reanesthetized, perfused with aldehydes and thalamic nuclei (VPLc and SG/PO) processed for the light and electron microscopic demonstration of HRP reaction product and GABA immunoreactivity. STT and ML terminals share a similar morphology, being large (approximately 4 µm in diameter) with abundant, rounded synaptic vesicles and numerous mitochondria. ML terminals contact dendritic shafts of projection neurons and, in more than 95% of cases, the GABAergic appendages of local circuit neurons (LCN) which in turn contact the projection cell dendrite to form a triadic relationship and presumably mediate feed forward inhibition. In contrast, STT terminals form axodendritic synapses with projection neurons but rarely (<10% of cases) contact GABAergic LCN's. We conclude that the excitation of thalamic neurons by STT axons, many of which convey noxious information, is rarely subject to GABAergic modulation by thalamic LCN's. Supported by NS 21445 from the N.I.H..

477.11

THE TRIGEMINO-PONTO-AMYGDALIAN PATHWAY DEMONSTRATED BY RETROGRADE TRANSNEURONAL TRANSPORT OF PSEUDORABIES VIRUS. L. Jasmin, J.P. Card† and A.I. Basbaum, Dept. Anatomy and Physiology, UCSF, San Francisco, CA 94143 and †Dupont-Merck, Wilmington, DE 19880

Although limbic structures must contribute to the affective component of the pain response, the circuitry through which nociceptive inputs access these forebrain sites is poorly understood. Recently, Bernard et al (1989) used a combined anterograde and retrograde tracing study that implicated a disynaptic spino/trigemino-ponto-amygdalian pathway (STPA) in the transmission of nociceptive messages. We have initiated a parallel series of studies using an alpha herpes virus (pseudorabies) as a retrograde transneuronal tracer to evaluate the spinal cord and brainstem cells which directly or indirectly access several forebrain sites implicated in pain processing. In the present study we describe results after restricted injections (0.2µl) of the virus into the central nucleus of the amygdala of rats. After different survival periods the rats were perfused with 4% paraformaldehyde and 50µm frozen sections through the brain and spinal cord were immunoreacted with an antiserum directed against the entire virion.

The pattern of retrograde labelling after one day was comparable to that reported in previous studies of the afferent connections of the amygdala, including the lateral parabrachial nucleus, locus coeruleus, periaqueductal gray and dorsal raphe nucleus. At two days we found a more extensive pattern of labelling, in many brainstem structures which are not known to project directly to the amygdala. Consistent with there being an STPA pathway, we found large numbers of cells in the superficial layers of the trigeminal nucleus caudalis and in the paratrigeminal nucleus. Many labelled neurons were also located in the subjacent reticular formation, a region that contain cells which express the c-fos proto-oncogene following noxious stimulation. Scattered cells were found in various laminae of the spinal cord (Later time points are being evaluated). Furthermore, by combining the retrograde transneuronal transport of this virus with noxious-stimulus evoked c-fos expression we believe that a better understanding of how nociceptive inputs access specific regions of forebrain can be obtained.

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477.12

ELECTROPHYSIOLOGICAL EVIDENCE FOR AN INVOLVEMENT OF THE NUCLEUS CENTRALIS OF THE AMYGDALA IN NOCICEPTION. J.F. BERNARD, G.F. HUANG* and J.M. BESSON. Unité de Recherches de Physiopharmacologie du système Nerveux, INSERM U 161, 2 rue d'Alésia F-75014 PARIS, FRANCE.

On the basis of anatomical investigations, we have suggested (Bernard et al., Neurosci. lett., 1989, 100:83-88) that a spino-ponto(parabrachial area)-amygdaloid pathway, originating in lamina I of the dorsal horn, could play an important role in pain processes. This was confirmed by the demonstration that a high proportion of neurons located in the external parabrachial (PBe) area (i.e. external lateral (PBel) and external medial (PBem) subnuclei) and that projected to the nucleus centralis of the amygdala (Ce) was exclusively driven by noxious stimuli. The aim of the present study was to extend our investigation at the level of the Ce. Neurons were recorded in and around the Ce using extracellular micropipettes, in anesthetized rats. The units (n=177) were characterized by their responses to innocuous and noxious natural (mechanical and thermal) and transcutaneous electrical stimuli. A large proportion (80%) of Ce neurons responded exclusively or preferentially to noxious stimuli, either by an excitation (46%) or by an inhibition (34%). The receptive field of both groups of neurons was very large (in most cases all parts of the body). A majority (53%) of neurons responding to noxious stimuli were exclusively and stongly driven by noxious mechanical and thermal stimuli. Transcutaneous electrical stimulation revealed that these latter neurons were driven by Aδ and C fibers. The excited as well as the inhibited neurons exhibited a clear capacity to encode thermal stimuli in the noxious range with a mean threshold between 44 and 45°C. The response of nociceptive excited neurons were depressed by systemic morphine in a dose-related and naloxone-reversible fashion. Thus these results support the conclusion that Ce neurons are involved in nociceptive processes. According to previous study (Bernard and Besson, J. Neurophysiol., 1990, 63:473-490) the spino-parabrachial pathway seems likely to be the major source of nociceptive inputs to Ce.

477.13

IS THERE A NOCICEPTIVE AREA IN SII ? EVIDENCE FROM LASER EVOKED POTENTIALS IN HUMANS.

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In a previous study (EEG J 70, 1988, 429-441), we first found a mid-latency component of the laser evoked potential that projected to the somatosensory cortex. We now describe the scalp topography of this N1 in detail and distinguish it from the vertex potential (N2).

CO_2-laser heat stimuli (15 W, 20 ms, 5 mm beam diameter) where applied to the dorsum of the left hand in 15 subjects. The EEG was recorded from 15 leads, distributed over the whole scalp (bandpass 0.33-200 Hz, linked earlobes reference).

The laser evoked potential began at 140 ms after stimulus onset, with a negativity in contralateral posterior temporal leads (N1). At 170 ms it was significantly larger contra- than ipsilateral to the stimulus (T4 vs. T3). Simultaneously with this posterior temporal negativity, a significant positivity appeared in frontal midline leads. The vertex potential began at about 200 ms and reached its maximum scalp negativity (N2) at 240 ms in the lead FCz. There was no concurrent localized positivity.

The N1 may reflect activity of a tangential generator in or near SII. After electrical nerve stimulation (radial or median nerve) no potential with such a lateral localization could be identified. The N2 topography corresponds to that of other vertex potentials.

477.14

PRIMARY SOMATOSENSORY CORTICAL LESIONS REDUCE THE MONKEYS' ABILITY TO DISCRIMINATE AND DETECT NOXIOUS THERMAL STIMULATION. D.R. Kenshalo, Jr., D.A. Thomas, R. Dubner Neurobiology and Anesthesiology Branch, National Institute of Dental Research, NIH, Bethesda, MD 20892

SI was bilaterally ablated in two monkeys (*M. mulatta*) trained to discriminate and detect noxious thermal stimuli in a reaction time paradigm, where speed of detection (1/sec) was measured. The monkeys discriminated differences of 1.0°-4.0°C, and detected 0.2°, 0.4°, 0.6° and 1.0°C temperature increases. SI lesions significantly ($p < 0.05$) reduced discrimination speeds for both monkeys. Twenty-one weeks after the lesions, discrimination performance had not recovered. Deficits were observed on the heat detection tasks. Detection of these stimuli recovered within 21 weeks, with the exception of the 0.4°C detection task in one monkey. The monkeys also detected innocuous cooling (-0.5°C) and visual stimuli in the same paradigm. After the lesions, detection of visual stimuli remained intact and the detection of the cooling stimulus was unimpaired in one monkey and only slightly impaired for a limited time in the other. The lack of clear deficits in the detection of visual and cooling stimuli indicates that the lesions did not significantly affect motoric, attentional or motivational components of the monkeys' behavior. The findings implicate SI in the neural process underlying the discrimination of noxious thermal stimuli.

PAIN MODULATION: PEPTIDES AND EXCITATORY AMINO ACIDS

478.1

EXPERIMENTAL PERIPHERAL NEUROPATHY ENHANCES EXCITATORY AMINO ACID RELEASE IN THE SPINAL CORD FOLLOWING INTRATHECAL SUBSTANCE P, BUT NOT CHEMICAL NOCICEPTIVE STIMULATION. D. H. Harkness, S. R. Skilling and A. A. Larson. Department of Veterinary Biology, University of Minnesota, St. Paul, MN 55108.

Partial sciatic ligation in rats produces a peripheral neuropathy associated with hyperalgesia and allodynia similar to that seen in human neuropathy. The mechanism by which ligation produces these effects is unknown. Based on evidence that excitatory amino acids play a role in nociception at the spinal cord level and that their release is modulated by the nociceptive transmitter substance P (SP), we examined whether sciatic ligation affects amino acid release in the spinal cord in response to chemical nociceptive stimulation or SP.

Partial sciatic ligation or sham surgery of male rats was done using the method of Bennett and Xie. Seven days later, loop dialysis and infusion cannulae were implanted in the lumbar intrathecal space. The CSF of animals was dialyzed and 2 min samples collected before and after the intrathecal injection of 2.5 nmoles of SP or application of 50% mustard oil to the dorsal surface of the hindpaw.

Mustard oil produced a significant release of both Asp, Glu and Tau, however, there was no significant difference in the release between sciatic ligation and sham operated animals. Likewise, the magnitude of the SP-induced release of Glu or Tau was also not altered by ligation. In contrast, SP-induced release of Asp was significantly enhanced by ligation.

These results are consistent with the hypothesis that the neuropathy associated with sciatic ligation is mediated through SP-induced Asp release. This enhanced release of Asp may reflect an up-regulation of SP receptors secondary to a decreased release of SP from primary afferents. Chemogenic pain-induced release of excitatory amino acids is not significantly changed in peripheral neuropathies. Supported by USPHS Grants DA04090, DA00124, DA04190, DA07234 and CA01342)

478.2

NALOXONE AND NEONATAL CAPSAICIN BLOCK NOCICEPTIVE (MUSTARD OIL)-INDUCED RELEASE OF EXCITATORY AMINO ACIDS FROM THE ADULT RAT SPINAL CORD, BUT NOT THE BEHAVIORAL RESPONSE S. R. Skilling, D. H. Harkness and A. A. Larson. Department of Veterinary Biology, University of Minnesota, St. Paul, MN 55108.

Selective activation of the C-terminal substance P (SP) receptor (NK1) using the SP fragment SP(5-11) produces a naloxone-insensitive release of excitatory amino acids (EAAs) in the dorsal lumbar spinal cord. In contrast, the parent molecule SP(1-11) causes a naloxone-sensitive release of EAAs, possibly through activation of N-terminal SP receptors. To determine if either of these SP-induced changes in EAAs plays a role in nociception, we examined the effect of chemogenic pain stimulation (mustard oil) on the release of EAAs in the dorsal spinal cord before and after systemic naloxone or loss of C-fiber afferents produced by neonatal capsaicin.

Vehicle (50% DMSO) and capsaicin (50 mg/kg s.c.) treated male rats were implanted with a microdialysis cannula in the intrathecal space surrounding the lumbar spinal cord. Twelve hours later, animals were dialyzed and 2 min samples collected before and after application of mustard oil.

Mustard oil (50% mustard oil in ethanol applied to the dorsal surface of the hindpaw) produced a behavioral reaction in rats consisting of caudally-directed biting and shaking of the limb, digging of the bedding and attempts to climb out of the cage. Mustard oil also caused a significant release of Asp, Glu and Tau into the CSF. Release, but not behavior, was blocked by either naloxone pretreatment (10 mg/kg i.p. 15 min prior to mustard oil) or neonatal capsaicin. These results are consistent with the hypothesis that nociceptive stimulation produces an N-terminal SP receptor-mediated increase in EAA transmitter release in the spinal cord and that this release does not appear to be involved in the initial sensation of pain but may play a significant role in its later modulation. Supported by USPHS Grants DA04090, DA00124, DA04190 and CA01342)

478.3

MEDIATION OF KAINATE-INDUCED BEHAVIORAL SENSITIZATION BY SUBSTANCE P (SP) AND SP N-TERMINAL FRAGMENTS IN THE MOUSE.

X. Sun and A. A. Larson. Univ. of Minnesota, St. Paul, MN 55108

Sensitization to the behavioral effects of repeated intrathecal (i.t.) injections of kainic acid (KA) in mice is inhibited by pretreatment with 0.8 μg of capsaicin i.t. The present study was designed to characterize the interaction between SP and KA *in vivo*. Pretreatment of mice at two min intervals with three injections of 7.5 pmoles of SP(1-11) or SP(1-7) potentiated the behavioral response to subsequent injections of KA, while SP(5-11) did not alter the intensity of responses to KA. This suggests that the N-terminal rather than the C-terminal of SP enhances subsequent KA-activity. Sensitization to the behavioral effects of KA was slightly inhibited by pretreatment with either 1 μg of DPDT-SP, a neurokinin antagonist, or even more by 1 μg of d-SP(1-7), an inhibitor of [^{3}H]SP(1-7) binding in the CNS. Coadministration of SP(1-7), not only failed to potentiate KA-activity, but actually inhibited KA-, SP- and NMDA-induced behaviors, an effect that was both prevented and reversed by naloxone. While naloxone attenuated the inhibitory effects of SP(1-7), it prevented, but did not reverse the potentiative effects of SP(1-7) on KA-induced behavior. This suggests a possible link between SP(1-7) and motor activity observed in mice during opioid withdrawal. Supported by USPHS grants DA4090, DA04190 and DA00124.

478.4

EXAMINATION OF THE ROLE OF SPINAL SUBSTANCE P IN TONIC NOCICEPTIVE BEHAVIORS. L.N. Holland and B.D. Goldstein. Department of Pharmacology and Toxicology, Medical College of Georgia, Augusta, GA 30912-2300.

Substance P (SP) in the dorsal horn has been shown to mediate certain types of phasic nociception. Intrathecal administration of SP reduces tail-flick and paw pressure latency in the rat. However, these responses are attenuated following successive administration of SP. We have confirmed this observation and determined that behaviors remain attenuated for up to 150 minutes and that these behaviors correlate with a reduction in the number of SP binding sites and a decrease in SP binding affinity in the dorsal horn. In this study, we examined the role of spinal SP in tonic nociception using a method of SP receptor desensitization. Two models were employed: the formalin test and the monosodium urate (MSU) test.

Male Wistar rats (300-400g) were implanted with intrathecal catheters. SP (0.7mM, i.t.) was administered at 0, 30 and 60 minutes. Rats were then injected into the hindpaw with 5% formalin (100 μl) or into the ankle-joint with MSU (1 mg dissolved in 25 μl 10% Tween 80). Formalin and MSU-induced responses were assessed by measurement of automated stereotypic behaviors and hindpaw pressure ratio, respectively.

In the formalin test, the repeated administration of SP reduced the first phase of pain-related behaviors but had no effect on the second phase response. In the MSU model, the repeated administration of SP did not have any effect on MSU-induced pain-related behaviors.

These data show that alteration of SP receptor activity in the dorsal horn using a method of SP receptor desensitization does not affect tonic nociceptive behaviors. These data suggest that SP in the dorsal horn does not mediate tonic nociception. Supported by the MCG Research Institute.

478.5

INTRATHECAL COADMINISTRATION OF SUBSTANCE P AND NMDA INCREASES THE BEHAVIOURAL RESPONSES TO PAIN IN THE FORMALIN TEST. O.-G.Berge, N.Mjellem-Joly*, A.Lund* and K.Hole. Astra Pain Control, S-151 85 Södertälje, Sweden & Dept. of Physiology, Univ. of Bergen, N-5009 Bergen, Norway.

Excitatory amino acids and substance P are involved in primary afferent transmission of nociception and in spinal processing of nociceptive information. We have studied the spinal action of NMDA and substance P in the formalin test in mice. Formalin (1%) was injected in a volume of 20 µl. Biting or shaking of the injected paw was scored. The substances were administered intrathecally in a volume of 7 µl. A significant increase of the response in the late phase of the formalin test (20-40 min after injection of formalin) was observed after intrathecal coadministration of NMDA (0.125 nmol) and substance P (2 pmol). Single administration of either NMDA or substance P did not induce any change in the response. These findings indicate that combined activation of NMDA receptors and substance P receptors induces a sensitization of the nociceptive response in the formalin test.

478.6

INTRATHECAL COADMINISTRATION OF SUBSTANCE P AND EXCITATORY AMINO ACID AGONISTS IN MICE: POTENTIATION OF A BEHAVIORAL RESPONSE. N.Mjellem-Joly*, A.Lund*, O.-G.Berge and K.Hole. Dept. of Physiology, Univ. of Bergen, Bergen, N-5009 Norway.

The functional interaction between substance P and excitatory amino acid (EAA) agonists in the spinal cord was studied. All substances were injected intrathecally in a volume of 7 µl. A strong potentiation of the behavioral response (biting and scratching) was observed when substance P (2.5 ng) was coadministered with NMDA (0.025 nmol), AMPA (0.0125 nmol) or kainic acid (0.025 nmol). The effect of each drug as well as of the combinations was blocked by the corresponding antagonists injected 5 min prior to the agonists: the selective NMDA receptor antagonist CPP (1 nmol), the non-MNDA receptor antagonist CNQX (2 nmol) and the substance P analog Spantide I (5 µg).

These findings indicate a functional interaction between substance P and EAA neurotransmitters in the dorsal horn of the spinal cord, compatible with the hypothesis that corelease of these substances from primary afferent neurons may enhance nociception.

478.7

INTRATHECAL ANTI-SOMATOSTATIN INHIBITS THERMAL HYPERALGESIA OF THE INFLAMED RAT HINDPAW. R.J.Traub. Dept. of Pharmacology, University of Iowa, Iowa City, IA 52242

Inflammation of a rat's hindpaw results in hyperalgesia to noxious thermal and mechanical stimuli produced in part by central mechanisms. The role of somatostatin in producing this hyperalgesia was examined.

Intrathecal (i.t.) catheters were implanted in male Sprague-Dawley rats and 5 days later the latency for withdrawal of the hindpaw from a noxious thermal stimulus was tested. Hindpaws were tested alternately at 4 min intervals to establish baseline values. Following testing, rats were given 3 i.t. doses over 24 hrs (10 µl/dose) of a monoclonal antibody raised against somatostatin-14 (#Ab607; provided by Dr. John H. Walsh, CURE Antibody Core, NIH Grant DK 17294). Immediately following the last dose, the left hindpaw was inflamed by s.c. injection of 2 mg carrageenan in 0.1 ml saline. The rats were tested again 2 hrs later.

In control rats the withdrawal latency of the inflamed hindpaw was significantly faster than the non-inflamed side. In contrast, there was no significant difference between the mean withdrawal latency of the inflamed and non-inflamed hindpaws in antisera-treated rats. The difference in the size of the inflamed compared to the non-inflamed hindpaw was the same in both groups. The antisera had no effect upon the withdrawal latency of the non-inflamed side compared to baseline values or the non-inflamed control.

The data show that i.t. anti-somatostatin inhibits the hyperalgesia to thermal stimuli applied to the inflamed hindpaw but does not affect acute pain transmission in non-inflamed tissue, suggesting a role for neuropeptides in generating central sensitization and hyperalgesia.

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478.8

PLASMA AND CEREBROSPINAL FLUID SP-LIKE IMMUNOREACTIVITY IN ARTHRITIC AND FREUND ADJUVANT PRETREATED RATS. B. CALVINO*, J.Y. COURAUD*, S. MAILLET*, P. PRADELLES* and J.M. BESSON*. INSERM U161 and CEA, 75014 Paris and Saclay, FRANCE (SPON: European Neuroscience Association).

Behavioral parameters and levels of plasma substance P-like immunoreactivity (SPLI-Pl) were studied in adjuvant induced arthritic rats (AIA), a chronic pain model.

Exp.I: 14, 21 and 42 post-inoculation (PI) days were studied and were compared with the day of inoculation. Levels of SP-like immunoreactivity were also determined in cerebrospinal fluid (CSF) on the same PI days. In arthritic rats SPLI-Pl was enhanced (x4) as early as 14 days PI and remained increased at all stages studied whereas SPLI-CSF was increased (x2) only at 21 days PI.

Exp.II: one group of rats was pretreated with diluted Freund adjuvant before inoculation with the stronger arthrogenic solution, while a control group was pretreated with saline. Rats in the control group developed all symptoms of AIA including chronic pain; SPLI-Pl was strongly increased (x4) and remained at this level as long as 9 weeks PI. In adjuvant pretreated rats, AIA symptoms were strongly reduced and animals developed no pain state. In this group SPLI-Pl was slightly increased (x2) and significantly different from levels of control group.

These data suggest that SP could be distributed in 2 different pools, a peripheral one of inflammatory origin, and a central one which could be more specific to the chronic pain situation.

478.9

EFFECTS OF DELTA-SLEEP INDUCING PEPTIDE ON STRESS-INDUCED ANALGESIA IN RATS. A.S. Wensel*, A.M. Vasquez*, G.A. Olson, and R.D. Olson. Department of Psychology, University of New Orleans, New Orleans, LA 70148.

This study was designed to explore the effects of delta-sleep inducing peptide (DSIP) on stress- and pain-inhibitory mechanisms that are activated in response to environmental stressors. In Experiment 1, DSIP (0 30, 60, or 120 nmol/kg) or naloxone (10 mg/kg) was peripherally (IP) administered to rats before intermittent (ICWS) and continuous (CCWS) cold-water swims. Tail-flick latencies and core body temperatures were taken immediately before and 0, 15, 30, 60, and 120 min after swims. Testing was conducted during the beginning and middle of the light and dark photo-periods. In Experiment 2, animals were retested under matching schedules but without exposure to CWS. In Experiment 1, 60 nmol/kg of DSIP attenuated absolute and relative measures of analgesia 30 min after ICWS without affecting body temperature. The 30 nmol/kg dose had similar but less consistent effects 15 min after CCWS. Naloxone did not reduce analgesia induced by ICWS or CCWS. Experiment 2 failed to demonstrate any direct effects of DSIP upon nocioception or thermoregulation. Although the mechanisms underlying DSIP's anti-stress actions remain unclear, the observed effects of DSIP on CWS-induced analgesia are more consistent with a non-opiate mediated system.

478.10

IONTOPHORETIC CGRP FACILITATES SP AND NOXIOUS STIMULI EVOKED EXCITATIONS OF RAT LUMBAR CORD NEURONS. G.Biella* and M.L.Sotgiu. Dept. Physiopathol.and Therapy of Pain, Fac.of Med.Univ.of Milan and I.F.C.N.-CNR, Milan, Italy.

In vitro preparations and in vivo superfusates have evidenced that coexistence of Calcitonin Gene-related Peptide (CGRP) and substance P (sP) could reflect a functional role in sensory processing of spinal cord networks. We examined in vivo the effects of CGRP and sP administration on both the spontaneous and the noxious and non-noxious evoked neuronal activities in anesthetized rats. Single unit recordings and concomitant iontophoresis/micropressure spotted drug applications (CGRP: 0.5-0.7 µM pH5; sP:10µM pH5) have been performed on wide-dynamic range neurons in lumbar cord. Results from 40 neurons showed that: i) application of sP alone provoked excitation and enhanced the responses to noxious thermal stimuli applied in the periphery ($p<0.001$), ii) CGRP alone was uneffective on the spontaneous activity while enhanced the excitatory responses to noxious stimuli ($p<0.001$), iii) CGRP potentiated the sP induced excitation both on the spontaneous and on the noxious evoked activities ($p<0.001$). This synergism was evident only when sP was applied on a previously running CGRP ejection. iiii) Non-noxious evoked activity remained unmodified by concurrent sP and/or CGRP ejections. It is suggested that when coreleased the CGRP could play the role of "enhancer" of the sP effects in the noxious information encoding.

478.11

MK 801, AN NMDA RECEPTOR ANTAGONIST, POTENTLY REDUCES NOCICEPTIVE BEHAVIORS IN RATS WITH PERIPHERAL MONONEUROPATHY R. L. Hayes[1], J. Mao[2], D. D. Price[3], J. Lu[2], and D. J. Mayer[2]. Dept. of [1]Neurosurgery, [2]Physiology, and [3]Anesthesiology, Medical College of Virginia, Richmond, Virginia 23298

Hyperalgesia in neuropathic pain syndromes could result from either abnormal peripheral nociceptive input or overactivity of endogenous excitatory amino acids at primary afferent synapses. In the present experiment, we examined the effect of MK 801, a non-competitive NMDA receptor antagonist, and a local anesthetic agent, bupivacaine, on thermal hyperalgesia associated with a rodent peripheral mononeuropathy produced by sciatic nerve ligation. Intrathecal injection of MK 801 or perinerve injection of bupivacaine was given on day 3 postsurgery, and thermal hyperalgesia was assessed 24 hrs after injection and on days 5, 6, and 7 postsurgery. Hyperalgesia to radiant heat was significantly reduced 24 hrs after injection in nerve ligated rats receiving either MK 801 (2.5, 5, 10 and 20 nmol) at the lumbar spinal cord, or perinerve injection of 0.5% bupivacaine (0.6 ml), but not in rats receiving saline or MK 801 (10 nmol) at spinal cord thoracic segments (ANOVA, $P < 0.001$). While nerve block or intrathecal MK 801 (2.5 nmol) injection alone on day 3 postsurgery reduced hyperalgesia only within the following 24 hrs, the combination of these two manipulations significantly reduced hyperalgesia for 3 days postinjection (ANOVA, $P < 0.01$). The effect of perinerve block and its potentiation by MK 801 treatment indicates that hyperalgesia after peripheral nerve injury may be induced and maintained by both peripheral and central mechanisms. Our data suggest that NMDA receptor-mediated central mechanisms may be critical to chronic neuropathic pain syndromes, and the pharmacological intervention by excitatory amino acid antagonists may be an important approach for clinical treatment of neuropathic pain.

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478.12

N-METHYL-D-ASPARTATE ADMINISTERED INTRATHECALLY ENHANCES PERSISTENT HINDLIMB FLEXION IN RAT. R.D. Moore*, D.J. Mokler and B.J. Winterson. Departments of Physiology and Pharmacology, University of New England, College of Osteopathic Medicine, Biddeford, ME 04005.

Previously, we have shown that non-competitive NMDA antagonists interfere with the induction of peripherally-induced persistent hindlimb flexion (Anderson & Winterson, Neurosci. Abstr.,1988). The present experiments examined whether intrathecally applied N-methyl-D-aspartate (NMDA) would modulate the development of persistent hindlimb flexion.

Adult Long-Evans rats were anesthetized with Na+ pentobarbital. Saline or 1 mmol NMDA was injected intrathecally in 5 μl volumes via a 27 g needle between T12 and T13. Wound clips were attached to the medial and lateral skin of the thigh. Electrical current (2mA,7ms,100Hz) was applied across the wound clips for 1 hr. Flexion was measured by attaching weight to the hindlimbs until leg lengths were equal. Rats that had received NMDA showed a mean flexion of 20.5g (sem = 2.0g, n = 10), 147% above rats that had received saline (X = 8.3g, sem = 1.4g, n = 7). Rats were returned to individual cages for recovery. At 3 days, rats were reanesthetized, and flexion was again measured. Rats that had received NMDA showed a mean flexion of 15.3g (sem = 1.7g), 110% above rats that had received saline (X = 7.3g, sem = 1.0g). Rats were then spinalized and flexion was measured again. Rats that received NMDA showed a mean flexion of 16.2g (sem = 1.7g), 95% above rats that had received saline (X = 8.5g, sem = 1.5g).

These results provide further support to the hypothesis that the induction of persistent hindlimb flexion is dependent upon spinal NMDA receptors.

(supported by the American Osteopathic Association)

478.13

MK-801 BLOCKS THE INDUCTION OF PERSISTENT HINDLIMB FLEXION DUE TO MUSCLE STIMULATION IN INTACT AND SPINALIZED RATS. M.F. Anderson and B.J. Winterson. Department of Physiology, University of New England, College of Osteopathic Medicine, Biddeford, ME 04005.

We have shown that NMDA antagonists interfere with the induction of peripherally-induced persistent hindlimb flexion (Anderson & Winterson, Neurosci.Abstr.,1988) and that muscle stimulation alone is sufficient to produce persistent flexion comparable to that generated by percutaneous stimulation (Anderson & Winterson, Neurosci.Abstr.,1990). The present experiments examined whether NMDA receptor blockade during muscle stimulation attenuated persistent hindlimb flexion.

In pentobarbital anesthetized adult Long-Evans rats, skin was removed from the thorax and hindlimbs, exposed muscle was wrapped in saline moistened gauze. Rats were kept warm via a thermostatically controlled blanket. Wound clips were applied to the muscle through the gauze, current (2mA, 7ms, 100 Hz, 1 hr) was delivered across the wound clips. Twelve rats were stimulated intact (CONTROL) and 12 rats were spinalized prior to skin removal (SPINAL). From each group, half were treated with the non-competitive NMDA antagonist, MK-801(10 mg/kg,i.p.) 1 hr prior to stimulation (MK-801) and (SPINAL-MK-801). Following stimulation, lidocaine (12.5 mg/kg, i.m.) was injected at the wound clip sites. Flexion was measured for 30-90 min. Flexion of rats pretreated with MK-801 was significantly less whether intact (MK-801: X = 0.0g, sem = 0.0g vs. CONTROL: X = 8.5g,sem = 0.7g) or spinalized (SPINAL-MK-801: X = 0.8g, sem = 0.5g vs. SPINAL: X = 10.8g, sem = 1.9g). These data suggest that 1) persistent hindlimb flexion is mediated through the spinal cord and 2) induction is dependent on NMDA receptor activation driven by muscle afferents. (supported by the American Osteopathic Association)

478.14

EFFECTS OF MK-801 ON BEHAVIORAL HYPERALGESIA AND DORSAL HORN NEURONAL ACTIVITY IN RATS WITH ADJUVANT-INDUCED INFLAMMATION. K. Ren, J.L.K. Hylden, G.M. Williams, M.A. Ruda and R. Dubner, Neurobiology & Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20892.

The involvement of NMDA receptors in a rat model of inflammation and hyperalgesia was evaluated by systemic administration of the non-competitive NMDA receptor antagonist, MK-801. Inflammation was induced by intradermal injection of complete Freund's adjuvant (CFA) into the left hindpaw. Paw withdrawal latency (PWL) from a thermal stimulus was used as a measure of CFA-induced hyperalgesia in awake rats. MK-801 (1.6 mg/kg, i.p.) significantly increased PWLs in comparison to saline-injected rats ($p < 0.05$). Spinal dorsal horn nociceptive neuronal activity was recorded extracellularly in chloralose anesthetized rats. The expanded receptive field size of nociceptive-specific and wide-dynamic-range neurons in the superficial and deep dorsal horn recorded 24 h after CFA injection was significantly reduced to 73 ± 6% ($p < 0.05$) and 74 ± 4% ($p < 0.01$) of control values, respectively, by 2 mg/kg of MK-801 (i.v.). The same dose of MK-801 prevented the expansion of receptive fields 5 to 8 h after CFA injection as compared to saline-injected rats ($p < 0.05$). MK-801 had no significant effect on receptive field size of dorsal horn neurons in rats without CFA-induced inflammation, but blocked a transient expansion of the receptive fields induced by 1 Hz, C-fiber intensity stimulation of the sciatic nerve. Background activity and noxious heat-evoked responses of dorsal horn neurons in rats with CFA-induced inflammation were primarily inhibited while noxious pinch-evoked activity was both facilitated and inhibited by administration of MK-801. These results support the hypothesis that NMDA receptors are involved in the dorsal horn neuronal plasticity and behavioral hyperalgesia that follow peripheral tissue inflammation.

478.15

DELAYED APPLICATION OF MK-801 ATTENUATES DEVELOPMENT OF MORPHINE TOLERANCE IN THE RAT. P. Marek, S. Ben-Eliyahu, A. L. Vaccarino, W. Sternberg, J. Mogil and J. C. Liebeskind. Department of Psychology, University of California, Los Angeles, CA 90024.

The specific NMDA receptor antagonist MK-801 (0.15 mg/kg, i.p.) was administered two hours after morphine injection (20 mg /kg, .ip.), during a four-day period of tolerance induction. Animals receiving delayed injections of MK-801 diplayed significantly less tolerance to morphine than saline injected controls when tested on the fifth day (hot-plate test, 52°C). These results suggest that long-lasting or delayed changes in NMDA receptors may be involved in the mechanism of morphine tolerance. Additionally, MK-801 applied two hours after morphine injection could not serve as a cue for morphine administration, which indicates that the effect of MK-801 was not due to blocking state dependent learning. To examine further the possible effect of MK-801 on the learning component of morphine tolerance, rats were given a single injection of a slow-release suspension of 50 mg/kg of morphine and 0.15 mg/kg of MK-801 and tested for morphine tolerance (15 mg/kg) and naloxone precipitated withdrawal 24 hours later. MK-801 completely blocked the development of morphine tolerance and significantly attenuated withdrawal symptoms (diarrhea, teeth-chattering). These data indicate that the effect of MK-801 is not attributable to blocking the learning component of morphine tolerance. Supported by an Unresricted Pain Research Grant from the Bristol-Myers Squibb Company and NIH grant NS 07628.

478.16

SWIM STRESS PRODUCED ANALGESIA IN THE FORMALIN TEST IS MEDIATED VIA THE NMDA RECEPTOR. A.L. Vaccarino, P. Marek, W. Sternberg and J.C. Liebeskind Department of Psychology, University of California, Los Angeles, CA 90024.

Different forms of stress can produce potent analgesia. In the hot-plate test, a 3-minute swim stress of 32° C produces analgesia which is predominantly opioid in nature. In contrast, the analgesia produced by a 20° C swim stress has a strong non-opioid component which is effectively blocked by the *N*-methyl-D-aspartate (NMDA) antagonist, MK-801. To determine the effect of swim stress in a model of tonic inescapable pain, the analgesic effect of a 3-minute swim stress was assessed using the formalin test. Male Swiss mice (30-40 g) were injected subcutaneously with naloxone HCl (1 mg/kg), MK-801 (0.075 mg/kg) or saline 15 minutes prior to swimming for 3 minutes in water maintained at 20° C or 32° C. An additional group of mice received saline. Following a 2-minute drying off period, the mice were injected with 20 µl of 5% formalin in saline into the plantar surface of one hindpaw. The animal's pain-related behaviour (time spent licking the injected paw) was then continuously rated during the subsequent 10 minutes. Swim stress produced a significant suppression of formalin pain scores relative to non-stressed controls at both the 20° C and 32° C temperatures. No difference was found in the magnitude of analgesia produced at these two temperatures. The analgesia produced in both the 20° C and 32° C swim stress was completely abolished in mice pre-treated with MK-801. Naloxone had no effect. These results suggest that the analgesia produced by swim stress in the formalin test is non-opioid in nature and is mediated at the NMDA receptor. Supported by an Unrestricted Pain Research Grant from the Bristol-Myers Squibb Company and NIH grant NS 07628.

478.17

MK-801 ATTENUATES NON-OPIOID STRESS-INDUCED ANALGESIA IN SELECTIVELY BRED MICE: COMPARISON ACROSS THREE SWIM STRESS PARADIGMS . J.S. Mogil, P. Marek, W.F. Sternberg, I. Panocka and J.C. Liebeskind. Dept. of Psychology and Brain Research Institute, UCLA, Los Angeles, CA 90024.

Recent evidence has implicated excitatory amino acids in the mediation of endogenous mechanisms of pain inhibition. The specific, non-competitive NMDA receptor blocker MK-801 (dizocilpine) has been found to affect opioid-mediated systems of stress-induced analgesia (SIA). We now report that a lower dose of MK-801 selectively attenuates non-opioid forms of SIA. Mice selectively bred for high (HA) and low (LA) swim stress-induced analgesia were tested on the hot-plate (56°C) immediately prior to and 2 minutes after a 3 minute forced swim in 15°C, 20°C or 32°C water. Twenty minutes before testing, animals were administered naloxone (10 mg/kg, i.p.), MK-801 (0.075 mg/kg, i.p.), naloxone + MK-801, or saline. In a separate experiment, mice similarly pre-treated were all given a systemic injection of morphine (10 mg/kg, i.p.). The magnitude of analgesia observed in all experiments was similar. In the HA and C (control) lines, MK-801 attenuated non-opioid SIA selectively. Specifically, MK-801 attenuated SIA induced by 15°C swim, on which naloxone had no effect. Conversely, MK-801 was ineffective on 32°C SIA and morphine analgesia, both of which were reversed by naloxone. MK-801 and naloxone acted additively to reverse 20°C SIA in HA mice. Supported by NIH Grant NS07628 and an Unrestricted Pain Research Grant from the Bristol-Myers Squibb Company.

VISUAL PSYCHOPHYSICS AND BEHAVIOR: HIGHER FUNCTIONS AND MODELS

479.1

INFORMATION TRANSMISSION AND EQUIVOCATION BY SINGLE NEURONS P.H. Bedenbaugh, G.L. Gerstein, *Departments of Physiology and Bioengineering, University of Pennsylvania, Philadelphia, PA 19104*

Neurons both transmit information about their inputs and classify or equivocate them. Equivocation is the extent to which different inputs produce the same output, and can be viewed as a measure of the importance of nonlinear processing by the neuron. We were interested in the relative importance of information transmission and equivocation.

Using computer simulations we generated input data from pools of random Poisson spike trains. Some spike trains were common to all such input data sets, others were unique; the ratio was varied parametrically. Such input data was submitted to a simulated neuron and mutual information was calculated between outputs on separate trials arising from different ratio inputs. Mutual information is a measure of the extent to which one such output could be predicted knowing another. Our neurons were modeled as in MacGregor's SYSTM22 (*Neural and Brain Modeling*, Academic Press, 1988), a simple model with some physiological verisimilitude. We calculated mutual information both with a simple code based on whether the neuron was firing or not, and with a more complex code that took into account instantaneous firing rate, phase, medium term average firing rate, and medium term change in firing rate. The complicated code yielded slightly higher values of mutual information than the simple code. We found that different ratios of common to unique input produced distinct values of mutual information between corresponding outputs. Using trials where the inputs differed but little, the calculated mutual information was much less than one would expect from the similarity of inputs, i.e. a loss of information. We conclude that nonlinear effects and equivocation are important facets of neural function. In fact, for our simulated neuron, equivocation seems to be larger than information transmission. Supported by NIH MH46428. Thanks to D. Kepke and I. Nelken for helpful discussions.

479.2

SYNCHRONIZED OSCILLATIONS DURING COOPERATIVE FEATURE LINKING IN A MODEL OF VISUAL CORTEX. D. Somers and S. Grossberg, Department of Cognitive and Neural Systems, Boston University, Boston, MA 02215

A model of synchronized oscillations in visual cortex is presented to account for neurophysiological findings (Eckhorn, et al, Biol. Cyber., 60, 1988, Gray, et al, Nature, 338, 1989) that synchronization of neural activity may reflect the binding of local feature detectors into a globally coherent perceptual grouping. Grossberg (Biol. Cyber., 23, 1976;1978) had previously predicted that cortical codes would be expressed by resonant standing waves in which cooperatively linked cells oscillate in phase with one another. It was also noted that the standing waves could be replaced by an approach to an equilibrium point if no "slow" variables exist in the network.

Our model utilizes two neural network architectures previously used in modeling pre-attentive vision (Boundary Contour System[BCS]) and attentive visual object recognition (Adaptive Resonance Theory[ART]). The oscillations in our model are not merely assumed to exist as has been done by others, but rather emerge from a system of nonlinear feedback equations in which inhibition acts more slowly than excitation. Our model and computer simulations account for the rapidly synchronizing response of distant visual cortical cells to both single and double bar moving stimuli. The two network architectures make complementary predictions about the processing of disjoint patterns. The "Bipole Cell" architecture of the BCS predicts that, under appropriate stimulus conditions, activity will be induced and synchronized at sites between disjoint patterns, thus performing a pre-attentive boundary completion. Conversely, the "Adaptive Filter" architecture of ART synchronizes, but does not connect, disjoint patterns during attentive object recognition.

Even nearest neighbor and random coupling schemes can generate synchrony for the single bar input, which demonstrates the robustness of the synchrony effect in cooperative networks. These results suggest how research on this topic can be integrated into formal models of visual perception in cortex.

479.3

A NEURAL NETWORK MODEL FOR TEXTURE SEGMENTATION AND PERCEPTION J. Xing, G.L. Gerstein, M.R. Turner, *Department of Physiology, School of Medicine, University of Pennsylvania, Philadelphia, PA-19104*

A neural network is set up to investigate the possible mechanism of texture segmentation in visual cortex, based on M. Turner's algorithm (M.Turner, Biol. Cybern, 55,1986). In this model, there are sixteen neural pools, each receives the output from one kind of Gabor filter; they are tuned to four spatial frequencies and four orientations. Neurons in a pool are arranged into arrays according to their spatial locations. The activity of each neuron is simulated by MacGregor's program PTNRN10. There are both excitatory and inhibitory interconnections among neurons in the same and different pools. The outputs of all the sixteen pools are integrated in a higher layer.

With this model, we tested neural representations in the network for many textures, from regular patterns to complex natural images. "E" represents the sum of excitatory strengths of all connections to that neuron, "I" is the corresponding inhibitory sum. We found that: (1) With appropriate E/I values, all texture images we tested can be segmented; the optimal sets of E/I values fall in the same region for most images; (2) The representations of texture images in the neural network mainly depend on E/I, Boundaries or segmentations could be respectively obtained by varying E/I; however, the spatial distributions of excitatory and inhibitory strengths with distance have little effect on the resulting boundaries or segmentations; (3) The time required for a representation of texture segmentation in the network varies with texture structures; regular, high density textures need less time to be segmented. Generally, the boundary or segmentation can appear in 60 ~ 120ms, and becomes stable after ~ 150ms. We also investigated the mechanism of texture perception. We suggest that the perception of a texture image could involve several different neural representations, which are obtained by the clustering of neural pools with similar firing pattern. Supported by MH46428 and N014-87-K-0766.

479.4

THE EFFECT OF 40 Hz FLICKER ON THE PERCEPTION OF GLOBAL STIMULUS PROPERTIES

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Neurons in the cat visual cortex show oscillatory responses in the range 20-60 Hz. Under certain conditions, influenced by global stimulus properties, neurons in spatially separate functional columns synchronize their oscillatory responses. This has been interpreted as support for the hypothesis that synchronized oscillatory responses serve to establish relations between spatially separate features (Gray *et al.*, 1989). If these coherent oscillations influence perceptual judgements, we reasoned that neuronal oscillations induced by flickering visual targets might have an effect on visual segmentation.

We measured psychophysical performance of human subjects in a texture segregation task, where the texture elements flickered against a gray background at a temporal frequency between 20 and 50 Hz. The stimuli were fields containing line segments whose position was jittered from a regular array. A rectangular patch contained segments differing in orientation from the rest of the field. The subject's task was to indicate whether the rectangle itself was oriented horizontally or vertically. We varied the difference in orientation between the segments inside and outside the rectangle to find a value that just supported reliable performance. We tested four temporal phase conditions: no flicker, synchronized flicker of all texture elements, synchronized flicker of the elements within one region, and unsynchronized flicker of all texture elements.

Performance depends in the expected way on signal strength as determined by contrast. It is, however, entirely independent of the temporal pattern of the flicker. We conclude that either stimulus-induced oscillations do not interfere with other oscillatory responses, or that the physiologically observed oscillatory responses are not necessary for perceptual grouping.

479.5

CHANGES IN GAMMA-BAND ACTIVITY OF RHESUS MACAQUE NEOCORTEX DURING A VISUAL PATTERN DISCRIMINATION TASK.

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Recent findings of stimulus-dependent gamma-band (25-75 Hz) activity in visual cortex have led to questions concerning its functional role in cortical processing. Transcortical field potentials were simultaneously recorded from 16 chronically implanted bipolar electrodes in rhesus macaque monkeys as they performed a visual pattern discrimination task. The electrodes were distributed across the cortical convexity of the hemisphere contralateral to the preferred hand. The monkeys were trained to respond to one stimulus pattern type (4 squares arranged in a diagonal line) by lifting a lever within 500 msec (GO task), and to another type (the same squares arranged in a diamond) by holding the lever for 500 msec (NO-GO task). The gamma band was isolated by digital filtering, and the rms amplitude was computed for each of 141 100-msec-long windows spanning 700 msec post-stimulus. For each task type, the rms amplitude of each window was averaged over all correctly performed trials. The averaged gamma amplitude showed location-specific deviations from baseline. The magnitude and timing of these deviations differed according to task type, the strongest changes occurring before 500 msec in the GO task and after 500 msec in the NO-GO task. These findings suggest that activity in the gamma frequency band in many cortical regions may have a functional role in performance of visual pattern discrimination tasks. (Supported by NIMH Grant MH43370 to EEG Systems Laboratory, San Francisco, CA).

479.6

SPATIO-TEMPORAL DYNAMICS OF STIMULUS-INDUCED VISUAL ATTENTION.

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We have shown that the focus of visual attention is detected by motion sensation in an adjacent line (Hikosaka et al., ARVO '91). When a spot (cue) appears on a screen, followed by appearance of a line, the line is perceived to be drawn from the cued side. This is because the cue facilitates the perception of the nearby portion of the line (Miyauchi et al., ARVO '91).

We now report that the spatio-temporal dynamics of attention can be plotted using a short line (length: ≈0.2deg) as the probe. While the observer fixated on a spot, a cue spot and then a short line appeared. We found that the motion sensation was greater when the line was closer to the cue stimulus. We used a cancellation method to quantify the gradient of attention: the probe line was drawn in the direction toward the cue so as to cancel the attention-induced motion effect. If the attentional effect was large, we needed to draw the line more slowly. The speed of the line drawing was taken to be a measure of local attentional gradient. A two dimensional map of the magnitude of attention was thus obtained. We studied the effects of single and multiple cues. A single cue produced a single peak with quasi-exponential decay outward. The map changed its magnitude and shape after onset of the cue stimulus. Two cue stimuli produced two corresponding peaks, suggesting the presence of multiple foci of stimulus-induced attention. We also studied the spatial and temporal interactions of the two stimuli.

479.7

Lack of spontaneous "popout" in a patient with severe agnosia and achromatopsia

K. Nakayama[1] and G. Plant[2]. Dept. Psychology, Harvard Univ[1]. and Maida Vale Hospital, London[2]

A 51 year old patient suffered bilateral strokes involving the ventral occipito-temporal cortex. He presented with a bilateral superior altitudinal field defect, with normal funduscopy and ERG.

In addition to his achromatopsia (reported by Haegerstrom-Portnoy, et al, ARVO 1991, p.1215), he had severe agnosia, particularly with more complex visual forms such as faces, animals, cars, etc., yet he could read very difficult material, albeit haltingly. Surface representation seemed largely intact insofar as he could recognize Kanisza demonstrations of occluding and occluded contours. Visual acuity at high photopic light levels was normal. Brightness discrimination, 2-D structure from motion, as well as the recognizing of Johanson biological motion figures appeared to be largely intact. In a task where he was to find a backward letter R, he was surprisingly unable to find the letter when it was uniquely marked with an opposite brightness polarity in comparison to the normal distractor Rs. Yet, when explicitly asked to find an opposite polarity letter against others, his performance improved dramatically. Despite this lack of spontaneous "popout" with opposite polarity targets (which was repeatedly observed), the backwards letter R was quickly identified if it was marked by motion against a stationary background.

479.8

FRONTAL ACTIVATION DURING SHIFTS OF VISUAL SPATIAL ATTENTION: A PET STUDY.

S.E.Petersen, M.Corbetta, G.L.Shulman*, and F.M. Miezin Dept. Neurology & Neurol. Surg., McDonnell Ctr. for Studies of Higher Brain Function, Wash. Univ. Sch. of Med., St.Louis, MO 63110.

Theories of attention posit a mechanism that shifts attention in space and is organized in direction and/or field specific coordinates. To examine this idea, normal subjects were scanned with PET activation methodology during a task in which visual field (left, right) and direction of attention movements (left,right) were manipulated in a blocked design. Previously we have reported a superior parietal activation dependent on the field of attention shift. The task display was a horizontal row of small boxes extending into both hemifields. Subjects detected a star presented for 150 ms within one of the boxes. On 80% of trials the order of probed locations predictably followed a particular direction; on 20% random positions were probed (shifting task). A central detection task was run in which peripheral probes were presented but subjects detected the onset of a star in a central box. PET sessions also included a fixation point control with no stimulation as well as a passive control in which the peripheral stimuli were presented but no task performance was required. PET subtraction images were obtained using shifting -, central detection -, and passive - fixation conditions. These images indicated that: 1. A region of the prefrontal cortex was activated when subjects shifted attention in the contralateral visual field, but not during central detection, with minimal activation when the stimuli were passively presented; 2. There was no evidence of activation related to attention shifts in a particular direction. This is similar to the results found in the superior parietal lobe, except that parietal activation was seen in the passive condition. These results are consistent with an interpretation that both parietal and frontal mechanisms contribute to shifting attention in space, but serve different task demands.

479.9

ACTION-BASED SPATIAL ATTENTION IN NORMAL AND HIPPOCAMPAL LESIONED MONKEYS.

Michael Taffe*, Beth O. Moore, Steven P. Tipper* and Gordon C. Baylis. Dept. of Psychology, U. C. San Diego, CA 92093, and Dept. of Psychology, McMaster University (S.P.T.)

Human subjects display an action-based attentional system when tested on a task that requires them to reach for a target stimulus. Distractor objects on the action path towards the target cause very much more interference than those located beyond the target (Tipper et al., *Psychonomic Soc. Meeting*, 1990). In order to prevent inadvertant responses to distracting stimuli on the path to the target, it is hypothesized that subjects use a mechanism of distractor inhibition. We examine the extent to which spatial attention in macaque monkeys is organized in a similar way.

It was found that the pattern of results for both normal and hippocampal-lesioned monkeys was very similar to that seen in normal humans. It is suggested that inhibition of distractor items is a mechanism also utilized in infra-human primates. Hippocampal lesions were found not to affect performance of this task in any way. This suggests that while lesions to the hippocampal system may impair monkeys' ability to make spatially selective responses (Rupniak & Gaffan, *J. Neurosci, 7, 2331*, 1987), spatial attention remains largely intact.

This work was supported by grants from N.I.M.H., The U.S. Office of Naval Research, and N.A.T.O.

479.10

VISUAL SPATIAL ATTENTION DEFICIT IN DYSLEXICS. EVIDENCE OBTAINED FOR COMPUTER PROGRAMS.

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Computer animated programs were prepared to be applied in normal and dyslexic children.The exercises started with a sample figure:faces, letters,numbers or special lines patterns with 1-4 discrimination details. They are followed by 10 fixed or 24 randomized complete or incom plete test figures moving on lines.The images run to velocities of 0.1 to several seconds. A sound and congratulations message is presen-ted if the child counts the correct number of complete figures. Another sounds accompany erroneus answers.

The adequate solution of the task showed age dependence. In this way, normal children were capable to count correctly until 0.1 sec. velocities the simplest figures. But subjets up to 12 years old need 0.5 sec. to answer co-rrectly the more complex exercises.For dysle-xic children it was very difficult to obtain correct answers, even tough the programs were runned very slowly. This results indicate vi-sual spatial attention deficits for disabled readers.

479.11

A HIERARCHICAL MODEL FOR 3D OBJECT RECOGNITION BASED ON 2D VISUAL REPRESENTATION. E.Sklar[1,2], N.Intrator[2*], J.I.Gold[2*], S.Y.Edelman[3*] & H.H.Bülthoff[1,4]. 1Dept. of Cognitive Science & 2Center for Neural Science, Brown University, Providence, RI 02912; 3Dept. of Applied Mathematics & Computer Science, Weizmann Institute of Science, Rehovot 76100, Israel; 4Center for Biological Information Processing, MIT E25-201, Cambridge, MA 02139.

A central question in visual recognition is whether internal representations of 3D objects are themselves 3D and object-centered or 2D and viewer-centered. Recent evidence suggests that recognition performance for some classes of 3D objects is constrained by the 2D properties of the stimulus image. Specifically, [1] the ability to generalize from a few 2D images of a 3D object to novel views is limited (Bülthoff & Edelman, *in press*), and [2] the influence of 3D transformations (affine and non-affine) on recognition is correlated with the induced image-plane distortion and not with the extent of the 3D transformation itself (Edelman & Bülthoff, *MIT-AI Memo* 1239).

These findings are consistent with a representation scheme based on multiple 2D views along with some means for view interpolation (*e.g.* Poggio & Edelman, *Nature*, 343). A computational network model of visual processing for view interpolation has been developed (Intrator & Gold, *in press*) using a biologically motivated learning and self-organization scheme (Bienenstock *&al., J. Neurosci.* 2). The model not only agrees with the psychophysical data, but can be shown to perform a sophisticated statistical analysis for dimensionality reduction (Intrator, *NIPS-90*) through feature extraction. We compare simulation results to human performance for several types of stimuli, and evaluate the predictiveness of the the model's feature analysis.

A possible physiological interpretation of these results is suggested by a 'hierarchical organization model' of visual cortex (*e.g.* VanEssen & Maunsell, *TINS* 6). Key aspects of this model are the increasing receptive field size and stimulus selectivity of cells in the 'higher' visual areas of extrastriate cortex, particularly on the 'form pathway' projecting to inferotemporal cortex. We suggest that a correspondence can be draw between the computational and physiological models at the level of feature extraction / stimulus specificity. Additionally, view generalization may be accounted for by changes in receptive field size and topography in a hierarchical cortical model.

479.12

ORIENTATION BUT NOT PATTERN DRIVES VISUALLY GUIDED PREHENSION IN A VISUAL FORM AGNOSIC. L. S. Jakobson and M. A. Goodale. Univ. Western Ontario, Canada.

We recently described a patient (DF) with visual form agnosia who showed strikingly accurate guidance of hand and finger movements directed at objects whose size, shape, and orientation she failed to perceive (Goodale et al. *Nature* 1991). MRI revealed damage in areas 18 and 19, with considerable sparing of primary visual cortex. The dissociation in visual performance suggests that visual mechanisms underlying "conscious" perceptual judgements normally operate separately from those underlying the "automatic" visuomotor guidance of skilled actions of the hand and limb. We have since shown that DF is unable to use visual pattern to guide her hand movements although she remains sensitive to the orientation of the elements that comprise the pattern. Thus, when presented with a slotted surface in which the slot was cut in the shape of the letter T, DF was able to guide a T-shaped form into the slot on only half the trials. Interestingly, her visually-driven errors were almost always perpendicular to the correct orientation. In other words, the mechanisms controlling her visuomotor behaviour appeared unable to 'discriminate' the stem from the top of the T, even though the hand-held form was rotated to the correct orientation of one element of the T or the other. This result combined with our earlier observations suggests that in normal brains there may be a contribution to visuomotor guidance from the systems underlying pattern recognition although more basic information about object dimensions and orientation can be processed independently from these pattern recognition systems. *Supported by MRC grant #MA-7269 to MAG and an MRC studentship to LSJ.*

479.13

THE EFFECT OF THE LOWER AND HIGHER ORDER VISUAL CORTICAL LESIONS ON ILLUSORY CONTOUR ORIENTATION DISCRIMINATION AND TEXTURE SEGREGATION IN THE CAT. DeWeerd P., Sprague J.M., Vandenbussche E. and Orban G.A., Lab Neuro-en Psychofysiologie, K.U. Leuven, B-3000 Leuven, Belgium and Dept. of Anatomy, Sch. of Med., Univ. of Pennsylvania, Phila., PA 19104-6058, U.S.A.

We have trained 5 cats in orientation discrimination with illusory contours (IC) in which the contour was induced by two sets of circle halves of which the endpoints were either separated by a gap or shifted in phase. Five cats were trained in texture (TEXT) segregation (square of line segments of one orientation surrounded by line segments of different orientation). In three IC- and three TEXT-cats we made bilateral ablations of areas 17 and 18 (type-1 lesion). Performance after lesion was close to chance, with little or no recovery despite intensive retraining for 0.5 to 1 year. In 4 cats (2 IC- and 2 TEXT-cats), we made combined bilateral lesions of areas 7,19,20a,21b,AMLS,PMLS, which receive direct projections from 17 and/or 18 (type-2 lesion). The initial effect (first two months) was as devastating as after type-1 lesions, for both IC- and TEXT-cats. However, retraining allowed 3 of the 4 cats to reattain close-to-normal performance. Our data suggest that the areas destroyed by type-1 and type-2 lesions intervene in the normal processing of illusory contours and textures, and that intactness of areas 17 and 18 is critical in the recovery observed after type-2 lesions.

479.14

THE ROLE OF INFERIOR TEMPORAL CORTEX AND DORSOLATERAL FRONTAL CORTEX IN VISUAL IMAGERY IN MONKEYS. M. Colombo, A. Eickhoff, and C. G. Gross, Department of Psychology, Princeton University, Princeton, NJ 08544.

Four monkeys were trained on a serial-order task (D'Amato and Colombo, J Exp Psychol [Anim Behav], 14, 131-139) to press five simultaneously presented visual stimuli in one specific order, symbolized as A→B→C→D→E. Two monkeys then received bilateral lesions of inferior temporal cortex and two received bilateral lesions of dorsolateral frontal cortex. The frontal lesions had no effect on performance of the serial-order task. In contrast, inferior temporal lesions severely disrupted performance.

After relearning the task postoperatively, the monkeys were given a test of visual imagery in which two of the five stimuli were presented at a time and the subject was required to respond to them in the order in which they appeared in the sequence (e.g. C→E; A→D; B→E). Neither inferior temporal nor dorsolateral frontal lesions impaired performance on this test.

The significance of these findings for understanding the neural mechanisms of visual imagery and their relation to the response properties of inferior temporal neurons is discussed.

479.15

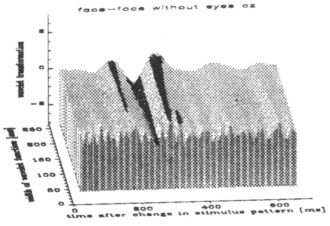
WAVELET METHOD AS THE NEW APPROACH TO ANALYSIS OF EVOKED POTENTIALS IN THE ELECTROENCEPHALOGRAM. A.W. Przybyszewski, Q.-J. Grüsser, Dept. of Physiology, Freie Universität Berlin, Arnimallee 22, 1 Berlin 33, Germany.

A new method in analyzing stimulus-category-related visual evoked potentials (EPs) is demonstrated.

(a) EPs related to face, hand, person and object perception were convoluted with the second derivative of the Gauss function (so-called wavelet function) to obtain wavelet transformation. The width of the wavelet function was varied from 44 to 264ms, resulting in 3D-pictures.

(b) The same procedure was applied for category-specific difference EPs.

(c) To obtain statistical evaluation criteria the individual EPs were randomly shuffled across stimulus categories, and from these "random groups" average EPs were computed. From these "average random EPs" difference curves were computed and treated with the wavelet method as described above.



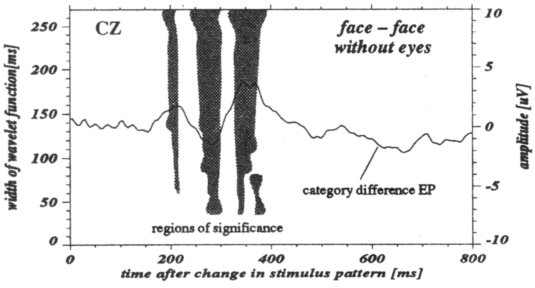
(d) This "statistical error" basis was compared with wavelet transformation of category difference EPs and the range where this difference exceeded ± 3 s.e. – was determined (figure).

(e) This range was mapped on a 2D-diagram ("contour plot"). (see figure in Seidler et al. abstract this meeting). (Supported by DFG grant Gr. 161).

479.16

WAVELET FILTERING APPLIED TO FACE-RESPONSIVE COMPONENTS OF VISUAL EVOKED POTENTIALS. W.S. Seidler*, A.W. Przybyszewski, K.-H. Dittberner, Q.-J. Grüsser. (SPON: ENA). Dept. of Physiol., Freie Universität Berlin, Germany.

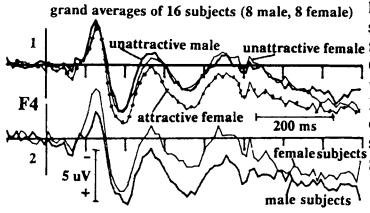
The visual evoked potentials (EPs) in adult volunteers to suddenly changed slides (4 x 6°) were convoluted with the second derivative of a Gauss function (wavelet), the width of which was varied from 44 to 264 ms. We analyzed EP-wavelet transformations of category difference-EPs (face/ face without eyes/ horizontal eyes/ vertical eyes) and found significant differences in time ranges around 160, 200, 270, 300, 360 and 450 ms after stimulus change. (Supported by a DFG-grant Gr.161).



479.17

INFLUENCE OF STIMULUS GENDER AND ATTRACTIVITY ON THE FACE-RELATED COMPONENTS IN VISUAL EVOKED POTENTIALS Margitta Seeck*, S. Bork*, O.-J. Grüsser, Dept. Physiol. Freie Univ. 1 Berlin 33, Germany

The influence of gender and face appearance on visual evoked potentials (EPs) was studied. In 16 subjects (8 male, 8 female, range: 23-31 years) EPs were recorded through the electrodes F3, F4, T5, T6, Cz, Oz, with reference to linked mastoids (intern. 10/20-system). 160 different slides were projected for 2.5-4.5 seconds (random variation). Black/white-photos of "attractive" and "unattractive" male and female faces, which had been previously rated by other independent subjects, served as stimuli. The face-responsive component appearing in the EP (Bötzel & Grüsser, 1989) was confirmed, consisting of a pronounced positive peak at about 200 ms with a consecutive very rapid negativity. Significant differences were found between the stimulus category "attractive females" and the other 3 categories. The presentation of attractive female faces led to larger positive amplitudes beyond 150 ms latency, which were significant at the right frontal electrode F4 and Cz (fig. 1). This result was more pronounced in EPs of male subjects compared to female subjects (fig. 2). DFG grants (Gr161 & Se520)

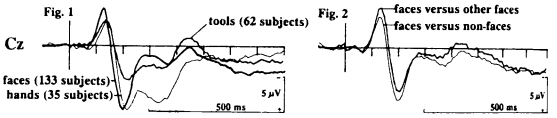


479.18

EVALUATION OF FACE-RELATED EVOKED POTENTIAL COMPONENTS ACROSS DIFFERENT PARADIGMS. Elke Heusser*, S. Bork, A. Brokate, W. Fischer, O.-J. Grüsser, Margitta Seeck, W. Seidler. Dept.Physiol. Freie Univ. 1 Berlin 33, FRG (SPON: EBBS)

Bötzel and Grüsser described components in EEG-evoked potentials (EP) related to the presentation of b/w-photos of faces but not to other meaningful stimuli. Within the last two years this finding could be confirmed in several studies. The largest amplitude of the EP related to faces and the most distinct category-related differences were found at electrodes F3, F4 and Cz. At Cz (reference: linked mastoids) an EEG-response of 3 prominent peaks (N135-P200-N290) was found.

We presented photos of faces in 11 different paradigms to 133 right-handed subjects (50% female, 50% male). In 4 paradigms ("class 1") we compared face- to non-face-stimuli (e.g. body-parts, tools), in 3 paradigms ("class 2"), to other face-stimuli (e.g. different views of the head). The early components related to body-parts were similar to those evoked by faces and revealed a large positive peak at about 200-210 ms. Beyond 210 ms the EPs deviated significantly from "face"-EPs. The EPs related to flowers and tools were very similar and had less prominent and later peaks (Fig.1). Comparing the results between the two classes of experiments, significant differences in "face"-EPs occurred only between 340-440 ms. In the earlier face-specific-components, no difference could be detected (Fig.2). Gender-differences appeared for face-stimuli beyond 340 ms. (DFG-grants Gr161, Se520)



COCHLEA: NERVE RESPONSES, TRANSMITTERS AND SECOND MESSENGERS

480.1

DII LABELING OF THE AUDITORY NERVE IN THE CHINCHILLA AND MONGOLIAN GERBIL. P. R. Schuyler*[1,2], A. Al-Ahmad*[3], S. C. Chamberlain[1,3], N. B. Slepecky*[1], R. L. Smith*[1,3], and R. T. Verrillo[1]. [1]Institute for Sensory Research, [2]Department of Electrical and Computer Engineering, and [3]Department of Bioengineering, Syracuse University, Syracuse, NY 13244

The auditory nerves of chinchillas and gerbils were labeled with minute crystals of DII. In chinchilla, the dye was placed either at the surface of the auditory nerve in the internal meatus or in Rosenthal's canal at various sites along the cochlear spiral. In gerbil, dye was placed only at sites in Rosenthal's canal. Diffusion of the dye occurred in darkness at room temperature for 4-11 months while the tissue was stored in 4% paraformaldehyde in 0.1M phosphate buffer at pH = 7.2. Tissue was then dissected and decalcified with buffered EDTA. Serial 20-µm frozen sections were mounted on gelatin-coated slides. Slides were stored uncovered and frozen for months without loss of fluorescence or resolution.

When DII was placed on the auditory nerve, the neurons of radial fibers to inner hair cells were continuously labeled along a region in the apical half turn of the basal turn; however, no labeled spiral fibers were observed in the organ of Corti. This suggests that the axons of type II ganglion cells are segregated from type I fibers in the internal meatus. In both species, punctate labeling in the spiral ganglion resulted in: a discrete dense band of labeled fibers in the modiolus; a mottled region in the peripheral internal meatus; and a discrete dense band as the nerve trunk enters the cochlear nuclei. The mottled region suggests that a zone of tonotopic scrambling may exist at the base of the modiolus. We examined carefully the labeling of stained fibers in the gerbil cochlear nucleus. No DII was observed surrounding the characteristic microcystic lesions confirming that these structures are postsynaptic and not part of the auditory nerve fibers. The ultimate objective of continuing studies is a detailed map of the tonotopic organization of the auditory nerve in the modiolus, internal meatus, and cochlear nuclei in both species. Supported by NIH grant PO1DC00380 NSF grant BNS8920418, and the Department of Bioengineering.

480.2

MECHANICAL AND NEURAL CONTRIBUTIONS TO PROLONGED RESPONSE LATENCIES IN IMMATURE KITTENS. E.J. Walsh and J. McGee*. Boys Town National Research Hospital, Omaha, NE 68131.

It is well known that neural responses to acoustic stimulation are significantly prolonged in neonatal kittens relative to those observed in adults (Walsh et al., 1986). The source of the prolongation is unknown and both cochlear and neural factors may contribute to the immaturity. Onset latency of the cochlear microphonic (CM) was measured to estimate cochlear delay associated with responses to acoustic stimulation, and auditory brainstem response (ABR) Wave I latency was measured simultaneously to determine overall response latency. The difference between the onset of CM and ABR Wave I latency was taken as an inferential estimate of synaptic and other neural delays. Far-field procedures were used to record CM and ABR so that measurements could be made from individuals longitudinally.

CM and ABR Wave I latencies declined along roughly exponential trajectories and CM acquired adultlike latencies relatively early compared to Wave I (i.e., CM latencies were adultlike by the end of the second postnatal week). At 4 kHz, CM latency accounted for approximately 70% of the overall response delay on the 7th postnatal day, 67% on day 11, and 60% on the 15th day. Similar findings were made for responses to 11 kHz, although CM accounted for approximately 60% of the total delay at both 11 and 15 postnatal days. Thus, preliminary results indicate that mechanical immaturities originating in the cochlea are the primary determinants of Wave I latency prolongation and that neural immaturities associated with synaptic delay and myelination account for the remainder.

(Supported by NIDCD grant #DC01007).

480.3

FUNCTIONAL CHARACTERISTICS OF SPIRAL GANGLION NEURONS AFTER HAIR CELL REGENERATION. R.J. Salvi, S.S. Saunders*, and E. Hashino*, Hearing Research Lab., SUNY University at Buffalo, Buffalo, NY 14214

Recent studies have shown that sensory hair cells in the avian ear can regenerate after being destroyed by ototoxic drugs or acoustic trauma. One of the important questions that needs to be answered is whether spiral ganglion neurons which reinnervate the newly formed hair cells function normally. In the present study, extensive hair cell lesions were produced in adult chickens using kanamycin (KM) (400 mg/kg/d x 10 days). Immediately after KM treatment, the compound action potential (AP) threshold was significantly elevated and the amplitude greatly depressed. The only neurons that responded to sound immediately after KM treatement were those with low characteristic frequencies (CF); however, thresholds of the units were extremely high and their tuning curves were broader than normal. In addition, the spontaneous discharge rates of neurons that were responsive to sound were reduced significantly. Over the next few weeks, the AP threshold and amplitude recovered rapidly and neurons with a broader range of CFs were encountered. Similary, the neural thresholds improved, the tuning curves became more sharply tuned and the spontaneous discharge rates increased with recovery time; however, these measures had not fully recovered by 40 days post-treatment. (Supported by NSF BNS9007822).

480.4

CHARACTERISTICS OF THE RESPONSE RECOVERY FOLLOWING TWO-TONE SUPPRESSION IN AUDITORY-NERVE FIBERS. K.G. Hill*[1] and C.D. Geisler[2]; Research School of Biological Sciences, Australian National University, Canberra City, Australia[1]; Depts. of Neurophysiology and Electrical and Computer Engineering, University of Wisconsin, Madison, WI 53706[2].

During a period of tens of milliseconds following termination of an excitatory, conditioning tone, the neural rate response in an auditory nerve fiber to a second excitatory test tone is reduced, relative to the response to the test tone preserved in isolation. Normal response magnitude to the test tone characteristically recovers as the delay between termination of the conditioning tone and the test tone is increased (Harris and Dallos, J. Neurophys. 42, 1083, 1979). This effect, is also manifest as post-excitatory depression of spontaneous rate.

Immediately following termination of a suppressor tone imbedded in a continuous, excitatory tone at fiber "characteristic frequency (CF)", the CF-driven neural rate is re-established with a characteristic recovery that becomes slower as the level of the suppressor tone is increased above excitatory threshold (Hill and Palmer, Hearing Research, in press).

In a two-tone paradigm, if the suppressor tone is set to that level that elicits a neural rate equal to the steady-state, CF-driven rate, or, if the suppressor tone and the CF tone at corresponding levels are presented in sequence with the suppressor tone leading as conditioner, then a similar delayed recovery of the CF-driven rate is observed. This suggests that recovery of response to the CF tone following presentation of the suppressor, either in the two-tone paradigm or as a prior conditioning tone, shares a common mechanism.

Supported by DC00116 (NIH)

480.5

DEVELOPMENT OF EVOKED MEASURES OF AUDITORY FUNCTION IN THE CANINE NEWBORN. G.M. Strain and B.L. Tedford*. Vet. Physiol., Pharmacol. & Toxicol., Louisiana State Univ. Sch. Vet. Med., Baton Rouge, LA 70803.

Congenital deafness resulting from perinatal degeneration of the stria vascularis occurs in species ranging from human to mouse. Many dog breeds are affected, especially Dalmatians, where the incidence reaches 30%. Prior to studying the perinatal process of hearing loss in Dalmatians we studied the normal pattern of development of auditory function in hearing dogs from birth until after opening of the ear canal using chronically implanted electrodes.

A silver ball electrode was implanted in the ear canal adjacent to the tympanum 24-48 hr after birth in beagle puppies; an L-shaped polyethylene tube was also implanted for presentation of auditory stimuli. Implants were also made in two Dalmatians known to carry the deafness gene(s). Click stimuli (100 μs, 11.4/s, 135 dB SPL; rarefaction [R], condensation [C] and alternating [A] polarity) were used to elicit brainstem auditory evoked potentials (BAEP) and other derived responses; recordings (n=1000 X 2, 10 ms, 150-3K Hz, vertex to tympanic electrode) were made daily until after opening of the ear canals.

Cochlear microphonics (CM) were present 3 days after birth, the earliest time at which recordings were taken. Recognizable BAEP peaks were first present on days 4-6 after birth in response to R and C stimuli, along with N1, the 8th nerve action potential, while responses to A stimuli were not present until 1-2 days later. A similar pattern was observed in one Dalmatian, although possibly delayed. The second Dalmatian showed a CM but no BAEP or N1 up through the age of 9 days when it died; it is assumed that this dog would have been deaf in the implanted ear. The observed sequence of development was earlier than previously reported for the dog (*Acta Otolaryng* 76;1, 1973).

(Supported by LA-SVM 1272, NIH DC01128, and the Dalmatian Clubs of America.)

480.6

MODULAR, EXPANDABLE MICROCOMPUTER WORKSTATION FOR SYNTHESIZING COMPLEX AUDITORY STIMULI, AND FOR RECORDING AND ANALYZING NEUROPHYSIOLOGICAL RESPONSES. J.D. Vrieslander*, J.F. Skovira*, and R.R. Capranica. Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853. Current address for JFS: Engineering Accelerator Systems, IBM Corp., Endicott, NY 13760.

A microcomputer-based system has been developed for fast, digital synthesis of complex sound stimuli, recording of single-unit spike trains, and interactive graphic analysis of response properties. For synthesis and spike recording, the system uses an Apple Macintosh II, a GW Instruments data acquisition card, and a modest amount of interfacing and signal conditioning electronics. The software for analyzing acquired data files runs on any Macintosh.

Stimuli can be computed waveforms or digitized natural sounds; both types are outputted in 16-bit samples at rates up to 100 ksamples/s. The software design is very flexible; by adding new program modules virtually any computable waveform can be synthesized, and any stimulus parameter can be set to a constant or selected as a variable. Experimenters can quickly design a series of parametrically varying stimuli, and immediately generate them under manual or automatic control.

Neural responses are recorded using either of two interfaces. For simultaneous recording from multiple units, spike events can be separated by external threshold discriminators or spike sorters, with timestamps saved for eight independent channels. For single-unit experiments, the spike train waveform is digitized, and the spike times are extracted by seeking pattern matches to a single-spike template. The algorithm uses separately optimized "coarse" and "fine" scans to improve speed and accuracy, and template updating to adapt to slow changes in spike shape. With either interface, spike timing can be as precise as ±5 μs. The pattern-matching scheme outperforms threshold discriminators when there is substantial noise or baseline drift in the spike train waveform.

The workstation has been used successfully in several studies of sound encoding in the peripheral auditory systems of frogs and lizards. Supported by NIH grant NS 09244 and (for JFS) IBM Corp.

480.7

OLIVOCOCHLEAR NEURONS IN RHESUS MONKEY AND CHIMPANZEE. R.N. Strominger, N.L. Strominger, S.M. Silver* and S.M. Parnes*. Department of Anatomy, Cell Biology and Neurobiology and Division of Otolaryngology, Albany Medical College, Albany, NY 12208.

A study was performed of the distribution and morphology of olivocochlear neurons (OCN) in rhesus monkey and chimpanzee after unilateral cochlear injections of HRP.

Two distinct cell clusters were labeled bilaterally in monkey. The predominant cluster, composed of small fusiform neurons, was ipsilateral to the injection just dorsomedial to the lateral superior olive (LSO). Similar neurons were labeled in this area contralaterally, but were less numerous. The second group was a diffuse collection of large multipolar neurons in the region of the ventral nucleus of the trapezoid body (VTB). In one animal the ipsilateral group was larger but in the other the contralateral group had more labeled neurons.

In chimpanzee three distinct cell groups were labeled ipsilaterally. The predominant group was composed of large multipolar neurons in the region of the lateral nucleus of the trapezoid body. A slightly smaller group, composed of intermediate sized neurons, was dorsomedial to the LSO. The smallest group composed of large multipolar neurons was in the VTB. Only 4-5% of some 340 OCN were contralateral; all were in the VTB. The number of OCN in chimpanzee was about a third that of monkey.

480.8

TRANSIENT INTRACRANIAL PRESSURE EVOKES EIGHTH NERVE POTENTIALS IN FROG. R.L. Seaman and J.A. Rasbury*. Dept. of Biomedical Engineering, Louisiana Tech Univ., Ruston, LA 71272.

To evaluate the potential of intracranial pressure to stimulate the inner ear, experiments were performed on Rana catesbeiana (140-250 g, M/F) under ketamine anesthesia at room temperature. After removal of midline skin, muscle, and partial frontoparietal bone, dura was contacted with a vertical probe. An Etymotic ER-3A earphone and an ER-10 microphone were coupled to the left tympanum. Potentials were recorded between electrodes in midline frontoparietal bone and posterior to the left otic capsule.

Transient displacements of the probe evoked tympanum pressures and eighth nerve potentials (EPs). The pressures consisted primarily of damped sinusoids with a frequency of 100-200 Hz. Individual EPs were similar in shape to EPs to acoustic clicks but occurred 1-1.5 ms later. Pressure and EP responses were absent when the driven probe was not touching the flexible floor and were different from responses evoked when the probe contacted bone.

Results demonstrate the ability of time-varying intracranial pressures to stimulate the vertebrate inner ear, most likely through ducts connecting brain and inner ear chambers.

Supported by La. Tech Faculty Development Grant and Center for Rehabilitation Science and Biomedical Engineering.

480.9

P_2 PURINOCEPTORS STIMULATE INOSITOL PHOSPHATE RELEASE IN THE GUINEA PIG ORGAN OF CORTI. A.S. Niedzielski and J. Schacht. Kresge Hearing Research Institute, University of Michigan, Ann Arbor, MI, 48109-0506.

Extracellular ATP is known to bind to P_2 purinoceptors and elicit responses in a variety of cell types, including neurons. Cellular responses to ATP have also been found to be mediated by the phosphoinositide second messenger cascade. In some cases, ATP may act as a cotransmitter in conjunction with acetylcholine. In the mammalian auditory periphery, acetylcholine is believed to be the primary efferent neurotransmitter. Recent studies by Ashmore and Ohmori (J. Physiol. 428:109-131, 1990) have demonstrated that ATP stimulates an increase of intracellular calcium in isolated outer hair cells from the guinea pig cochlea, whereas acetylcholine had a much smaller effect. This raises the possibility that ATP acts as a neuromodulator or neurotransmitter in the cochlea.

Previously, we reported that phosphoinositide hydrolysis is stimulated by muscarinic agonists in the guinea pig organ of Corti, possibly at the level of the outer hair cell. We have now determined that the P_2 receptor agonist ATP-γ-S (200 μM) elicits a 5-fold increase in inositol phosphates in the organ of Corti. Another P_2 agonist, α,β-methylene ATP (200 μM), elicited a 1.8-fold increase. By comparison, 1 mM carbachol increased inositol phosphates 2-fold.

These results suggest new functional roles for the phosphoinositide second messenger system in the cochlea, in addition to its activation via muscarinic receptors. Physiological responses to ATP via phosphoinositide-coupled receptors may include modulation of afferent and efferent neurotransmission.

(Supported by NIH grants DC-00078 and DC-00011)

480.10

CALMODULIN-DEPENDENT INHIBITION OF PHOSPHORYLATION OF TWO SPIRAL GANGLION PROTEINS.
R. Naik*, D. Coling and J. Schacht. Kresge Hearing Research Institute, University of Michigan, Ann Arbor, MI 48109-0506.

Phosphorylation of spiral ganglion proteins by endogenous protein kinase activity was assayed in homogenates from guinea pig inner ear with [γ-^{32}P] ATP. Regulation of protein phosphorylation by second messenger systems was assessed by additions of calcium, calmodulin, cyclic nucleotides and lipids. Phosphorylation of two prominent spiral ganglion proteins, 12 and 81 kD, was significantly reduced in the presence of calcium and calmodulin. This effect was partially blocked by the calmodulin antagonist trifluoperazine. Three models for this regulation are considered involving the stimulation of either protein phosphatase or proteolytic activity or the inhibition of protein kinase activity. In chase experiments designed to test the involvement of phosphatase and protease activity, the time-dependent decrease in ^{32}P label in both the 12 and 81 kD proteins was independent of calcium, calmodulin and trifluoperazine. These data favor regulation by a calcium/calmodulin-dependent inhibition of kinase activity. This type of regulation has been proposed for another 80-87 kD protein, MARCKS (Alberts et al., 1984). The 12 kD spiral ganglion protein appears to be a novel substrate for this unusual mode of regulation.

Supported by NIH research grant DC-00078 and NIH training grant DC-00011.

480.11

MOLECULAR CLONING OF cDNAs FOR G PROTEINS FROM COCHLEAR TISSUES. M.Tachibana*, E.Wilcox*, N.Yokotani*, M.Schneider*, D.Drescher and J.Fex*. Lab. of Molecular Biology, National Institute on Deafness and Other Communication Disorders, Bethesda, MD 20892

G proteins play crucial roles in sensory organs such as retina and olfactory epithelium. Little is known, however, about G proteins in the hearing organ. The purpose of this study was to obtain the primary structure of G proteins in the cochlea by molecular cloning techniques.

Total RNA was extracted from the whole cochlea of the mouse and from the organ of Corti or stria vascularis/spiral ligament of the guinea pig. RNA was reverse transcribed and cDNAs with nucleotide sequences resembling those coding for G proteins were selectively amplified by polymerase chain reaction (PCR), using degenerate primers. Agarose gel electrophoresis revealed two bands of PCR products of approximately 300 and 350 base pairs. Individual PCR products were cloned into pGEM-7Zf(+).

Three G_S-like cDNAs were obtained from the whole mouse cochlea and from the guinea pig organ of Corti. The molecules showed high (>94%) nucleotide similarity to known G_S cDNAs from other tissues. Four G_i-like cDNAs were obtained from the guinea pig stria vascularis/ spiral ligament. Three of these had moderate (74-81%) nucleotide similarity to the known G_i cDNAs from other tissues. One of them showed high (97%) similarity to the G_i cDNA from the macrophage cell line. Using these G protein-like cDNAs as probes, we are now trying to determine full-length sequences of G protein cDNAs from a cDNA library, which we are making from the mouse cochlea.

480.12

MORPHOLOGY AND DISTRIBUTION OF TERMINAL FIBERS IN THE AMPHIBIAN AND BASILAR PAPILLAE. C.Bertolotto*, D.D.Simmons, and P.M.Narins. Dept. of Biology and Brain Research Institute, UCLA, Los Angeles, CA 90024.

There are two distinct auditory organs in the frog: the amphibian papilla (AP) and the basilar papilla (BP). Biocytin injections were made into the posterior branch of the VIII nerve of *Rana pipiens*. Fibers were labeled retrogradely to both auditory organs. Both the AP and BP were sectioned systematically and reconstructed in the light microscope. Additionally, the gross innervation patterns as well as the fiber caliber and myelination in thin sections were also observed. Although labeled fibers in the AP exhibited more morphological heterogeneity than fibers in the BP, the two organs were surprisingly very similar. In caudal regions of the AP, labeled fibers had an average diameter that ranged from 0.65-3.75 μm, and contacted as many as 5 hair cells (HCs). The average number of terminal branches was 8.2 with a branching asymmetry of 0.5. In the rostral regions, the fibers had an average diameter that ranged from 0.75-4.5 μm, and contacted as many as 9 HCs. The average number of terminal branches was 7.5 with a branching asymmetry of 0.6. Labeled fibers in the BP had a more homogeneous morphology. The average diameter of labeled fibers was roughly 4 μm, and they contacted as many as 9 HCs. The average number of terminal branches was 11 with a branching asymmetry of 0.6. The morphological heterogeneity of fibers in the AP may be a function of the systematic place differences in frequency response of the papilla.

(Supported by the Sloan Foundation to DDS and the NIDCD #DC00222 to PMN).

480.13

FIBRONECTIN STAINING OF THE BASILAR MEMBRANE OF YOUNG AND AGED RATS. E.M. Keithley, L. Kitabayashi and N.K. Woolf. Division of Otolaryngology, University of California, San Diego, La Jolla, CA 92093.

Mechanical presbycusis was hypothesized by Schuknecht (Arch Otolaryngol 80:369 '64) to account for age-related hearing loss in excess of that attributable to the loss of hair cells and neurons in the human cochlea. Aged animals also show hearing losses that seem large relative to the loss of sensory-neural elements. It has now been demonstrated that fibronectin is a major component of the basilar membrane (BM) and it is possible that this large (220kD) matrix protein contributes to BM stiffness (Santi et al Hear Res 39:91 '89). Young (2 months) and aged (25 months) Sprague-Dawley rats were perfused with mixed aldehydes and cochlear sections were incubated with biotinylated goat anti-rabbit fibronectin. The BM in the young animals had strong reactivity throughout its length. The old animals showed comparable staining of the BM in the basal turn, but weak or no staining in the apical cochlear turn. It is possible that the decrease in BM fibronectin affects cochlear mechanics and contributes to the observed threshold shifts in aged cochleas.

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480.14

TRANSIENT EXPRESSION OF FIBRONECTIN-LIKE PROTEIN IN THE ORGAN OF CORTI FOLLOWING NOISE EXPOSURE. N.K. Woolf, F.J. Koehrn*, H. Gompers-Foster* and A.F. Ryan. Div. of Otolaryngology, UCSD Medical Center and Veterans Administration Medical Center, La Jolla, CA 92093-9112.

Noise trauma is a significant source of hearing loss and cochlear pathology. While the anatomical and functional correlates of noise exposure are well established, the molecular bases for temporary (TTS) and permanent (PTS) threshold shift are poorly understood.

Mongolian gerbils were exposed to a two-octave (1414-5656 Hz) band of noise for one hour at 110 dB SPL. Auditory brain stem evoked potential thresholds were determined prior to noise exposure, and at either 6 hours, 24 hours or 7 days postexposure. Animals were sacrificed following their postexposure hearing test and processed for immunocytochemical examination. Fibronectin-like protein was detected in Deiters' cells beneath the outer hair cells during periods of TTS at 6 and 24 hours postexposure, but not following recovery from TTS at 7 days. Fibronectin expression reflects either synthesis or uptake of this glycoprotein by Deiters' cells during recovery from TTS.

Supported by DC139 & the VA Research Service.

480.15

DUAL MECHANISMS FOR PRODUCTION OF NEW HAIR CELLS IN REGENERATING AVIAN COCHLEA. Y Raphael* and J.M. Miller. Kresge Hearing Research Institute, University of Michigan, Ann Arbor, MI 48109

Acoustic overstimulation of chick auditory epithelium results in hair cell loss and subsequent replacement by new hair cells (Corwin and Cotanche, 1988; Ryals and Rubel, 1988). Although the complete mechanism by which avian hair cells regenerate is not known, it has been shown that repair is based, at least in part, on production of new hair cells which eventually replace lost cells. The source of new hair cells, however, has not yet been unequivocally established. In the present study we examined the auditory epithelium immediately following pure tone (1.5 kHz, 124 dB, for 10 h) overstimulation. We observed the presence of mitotic figures in the luminal surface, among the cells which constitute the auditory mosaic in the region of short hair cells. Transmission electron microscopic analysis also showed cells which appeared like differentiating hair cells. These cells had a luminal surface, a microvilli cover, dense cytoplasm, basal bodies and a kinocilium. Our preliminary interpretation for this early appearance of cells with this "immature hair cell-like" combination of morphological features is that supporting cells may transdifferentiate to become hair cells without divisions. This suggests there may be at least two different mechanisms for hair cell regeneration in chicks: 1. Supporting cells of the epithelial mosaic divide during or early after the insult and some of them become new hair cells. 2. Supporting cells transdifferentiate to new hair cells.

supported by the Deafness Research Foundation.

480.16

IMMUNOCYTOCHEMICAL LOCALIZATION OF AMPA RECEPTOR SUBUNITS IN THE RAT COCHLEA. R.A. Altschuler, C. Hunter, Y. Raphael* and R.J. Wenthold. Kresge Hearing Research Institute & Dept. Anat & Cell Biology, University of Michigan, Ann Arbor, MI 48109 and Lab. Molecular Otology, NIDCD, NIH, Bethesda, MD 20892.

While the endogenous transmitter of hair cells has yet to be identified, there is considerable evidence that it acts on an excitatory amino acid (EAA) receptor. Recently, a family of EAA receptors (GluR) was cloned (Hollmann et al,1989; Keinanen et al,1990; Boulter et al,1990) . The GluR family is composed of at least four similar members (A-D) which, when expressed in oocytes or cultured cells, can combine to form heteromeric complexes with physiological properties that appear to be related to subunit composition. It is therefore important to know which subunits are expressed in the cochlea and their localization.

Antibodies were made to synthetic peptides corresponding to carboxy terminal sequences for GluR-A, GluR-B/C and GluR-D. The antibodies were applied either to cryostat sections or surface preparations of paraformaldehyde fixed rat cochlea and visualized with immunofluorescence and immunoperoxidase techniques respectively. 0.3% triton-X was used to increase permeability in surface preparations. In cryostat sections immunolabeling of spiral ganglion cells was observed using antibodies to GluR B/C and GluR-D but not with antibodies to GluR-A. Antibodies to GluR-B/C and GluR-D also immunolabeled afferent dendrites at the bases of inner and outer hair cells, visible in both cryostat sections and surface preparations. No labeling in the organ of Corti was observed using antibodies to GluR-A. These results suggest that a similar excitatory amino acid receptor is present at synapses of inner and outer hair cells and that the receptor complex contains GluR-D, and GluR-B/C but not GluR-A.

480.17

FUROSEMIDE OTOTOXICITY IS ENHANCED IN ALBUMIN-DEFICIENT RATS. L.P. Rybak, C. Whitworth,* V. Scott,* A. Weberg*. Dept. of Surgery, SIU School of Med.,Springfield,IL 62794

The analbuminemic rat was developed from Sprague-Dawley rats (Nagase et al., Science 205:590, 1979). The Nagese analbuminemic rat (NAR) has only barely detectable amounts of albumin in its serum. These mutants have no grossly discernible signs of pathology, and the total serum protein concentration is similar to that of normal Sprague-Dawley rats because of an increased globulin fraction. Furosemide is a loop diuretic which is highly protein-bound. Ototoxicity is one of its potential side effects. The purpose of the present studies was to investigate the effect of furosemide on the endocochlear potential (EP) of the Sprague-Dawley and NAR rats. Young adults of either type were anesthetized with Rompun (1 mg/kg). Tracheotomy was performed and the EP was measured with a microelectrode inserted through the round window membrane. After a stable EP was recorded, furosemide 35 mg/kg was injected through a cannula in the jugular vein. Sprague-Dawley rats were found to have very small reduction of the EP (9.46 ± 2.9 mv). In sharp contrast, the NAR's exhibited an extremely large reduction of the EP (88.0 ± 10.0 mv) ($p<0.001$). These findings support the hypothesis that the access of furosemide to its site of ototoxic action in the cochlea depends on the quantity of unbound furosemide in the serum. (This work supported by NIH-NIDCD Grant #DC-00321 and NIGMS #GM-40858).

CHEMICAL SENSES: PERIPHERAL MECHANISMS IV

481.1

RAT TASTE EPITHELIAL cDNA LIBRARY: MOLECULAR GENETIC APPROACH TO TASTE TRANSDUCTION. P.M. Hwang, C.E. Glatt, D.S. Bredt, G. Yellen, R.R. Reed and S.H. Snyder. Dept. of Neurosci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

The mammalian taste receptor cells utilize diverse signal transductory mechanisms for different taste modalities. These are thought to range from direct interactions with apical membrane channels by certain ionic tastants to the generation of intracellular second messenger molecules by other types of tastants. Data from our laboratory as well as from others suggest a role for second messengers in taste signal transduction. However, the scarcity of taste receptor cells has limited biochemical investigations into the mechanisms of taste transduction. In order to circumvent this difficulty we have recently constructed a cDNA library from rat tongue epithelial tissue highly enriched in taste receptor cells, and we have initiated a search for the various genes involved in the cyclic nucleotide and phophoinositide signal transduction pathways in our cDNA library.

We describe the construction and the characterization of the rat taste epithelial library as well as the identification of various signal transductory genes found in it. Also we report the cloning and characterization of a unique potassium channel gene that has high homology to a delayed rectifier potassium channel.

481.2

PARTIAL PURIFICATION AND CHARACTERIZATION OF AN L-ARGININE RECEPTOR/CHANNEL FROM CATFISH TASTE EPITHELIUM. J.G. Brand[1,2,3*], J.H. Teeter[1,2], and D.L. Kalinoski[1], [1]Monell Chemical Senses Center, [2]Univ. of Penn., and [3]Veterans Affairs Administration, Phila. PA 19104.

Behavioral, neurophysiological and biochemical studies suggest that there are independent high affinity receptor sites for the stimuli L-alanine (L-ALA) and L-arginine (L-ARG) in the catfish taste system. Biochemical studies have characterized binding of L-ALA and L-ARG to a sedimentable membrane fraction (P2) from taste epithelium and have begun to characterize L-ALA-stimulated second messenger accumulation in taste tissue. Electrophysiological studies have identified L-ARG-gated cation channels in purified taste membranes. More recent studies have shown that taste stimulus binding to fraction P2 could be selectively inhibited by the lectins DBA, Jacalin, PHA E&L and RCA I. Furthermore, the biotinylated lectins selectively labeled only a few glycoproteins by Western blotting to SDS-PAGE separated taste membranes. We have taken advantage of this selective labeling by lectins to partially purify and reconstitute an L-ARG receptor/channel. Fraction P2 from taste epithelium was extracted using 4% CHAPS. Extracted material was incubated with either PHA E or RCA I coupled to agarose for 90 min at 10°C. The lectin-agarose was poured into a column, washed, and specifically bound material eluted with either the specific hapten sugar or 1 M NaCl. Eluted material was either separated by SDS-PAGE or reconstituted into phospholipid vesicles by extensive dialysis. Phospholipid bilayers into which PHA- and RCA- purified membrane vesicles had been incorporated frequently displayed reversible increases in cation conductance in the presence of micromolar concentrations of L-ARG. The conductance, ion selectivity and concentration dependence of the partially purified L-ARG-activated channels were similar to those observed with native membrane vesicles.

481.3

ANTAGONISM OF THE GERBIL'S SUCROSE TASTE RESPONSE BY 6-CHLORO-N-ACETYL-D-TRYPTOPHAN. William Jakinovich, Jr. Dept. of Biological Sciences, Lehman College and the Graduate School, CUNY, Bronx, NY 10468.

We have discovered a fourth sucrose taste antagonist, 6-Chloro-N-Acetyl-D-Tryptophan (6-CNAT), an amino acid rather than a sugar derivative, which has been partially characterized as follows:

a) In solution, by itself, it does not stimulate the gerbil's chorda tympani nerve.

b) The taste response produced by sodium chloride was unaffected by 6-CNAT.

c) Sucrose responses were inhibited when mixed with 6-CNAT.

Supported by NIH NINCDS grant #DC00434.

481.4

DYNAMIC FILTER PROPERTIES OF CHEMORECEPTOR CELLS IN SIMULATED ODOR PLUMES. J. Atema, R. Voigt* and G. Gomez*. Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA, 02543

Intensity fluctuations in turbulent odor plumes are typically chaotic in time and space dimensions. These stimulus intensity patterns are filtered by a fluid boundary layer before they reach the receptor surfaces. Microvoltammetry allows us to measure a more precise temporal correlation between stimulus arrival and cell response than previously possible. A carbon-tip microelectrode approximately the size of a lobster olfactory sensillum (30 um diameter) was placed into the tuft of sensilla in a recording chamber allowing simultaneous measurements of the chemical signal and the neural response to the stimulus. We used both well-defined pulses and with semi-natural odor plume patterns. The instantaneous firing rate of spikes in relation to the instantaneous stimulus intensity profile allowed us to determine how chemoreceptor cells in situ translate the chaotic chemical intensity signal characteristic of odor plumes into a temporal neural signal pattern (transfer function). The data confirm results from earlier studies on adaptation and disadaptation properties of receptor cells and predictive computer models based on information contained in odor plumes (Moore & Atema, 1988 *Biol. Bull.*). The rate of stimulus onset and its relation to the immediately preceding stimulus history appear to be critical features for these chemoreceptor filters.

Supported by NSF (BNS 8812952) to JA.

481.5

IN SITU RECORDING FROM HAMSTER FUNGIFORM TASTE CELLS: RESPONSE TO SOUR STIMULI. T.A. Gilbertson, P. Avenet, S.C. Kinnamon and S.D. Roper. Dept. of Anat and Neurobiol, Colo St Univ, Ft. Collins CO 80523, and Rocky Mt. Taste and Smell Center, Univ Colo Health Science Center, Denver, CO 80262.

Current transients reflecting taste cell action potentials were recorded in response to sour stimulation using a non-invasive technique (Avenet and Lindemann, Biophysical Abstracts 59:594, 1991). A recording pipette of 100 μm tip diameter which could be internally perfused with sour (acid) stimuli was pressed onto fungiform papillae of whole tongues excised from hamsters. Stimulus solutions contained 30 mM NMDG-Cl, 5 mM HEPES, pH 7.0 or 30 mM NMDG-Cl and various combinations of either citric acid (CA) or HCl. Action currents were 5 to 20 pA in amplitude and their frequency varied in a dose-dependent manner with pH. Shapes of action currents were generally biphasic, but monophasic currents were also recorded. In a single taste bud, CA always gave a stronger response than HCl at an equivalent pH, but NMDG-citrate buffered to pH 7.0 gave no response. Taste buds previously adapted to NaCl (10 mM) still showed responses to CA indicating that different cells are involved in salt and sour responses. Unlike the mudpuppy (Kinnamon and Roper, J. Gen. Physiol. 91:351, 1988), the citric acid response was not affected by KCl or TEA. The response to CA was also unaffected by vanadate, 9-AC, cadmium, DIDS, nor was it affected by exposing the apical membrane to NH_4Cl to modify internal pH. Amiloride (30 μM), however, completely and reversibly suppressed the action currents elicited by CA. An amiloride-sensitive steady state current could also be measured in the presence of CA. These data suggest that sour (acidic) stimulation involves an amiloride-sensitive process and that in mammalian fungiform papillae the transduction mechanism for sour taste does not involve a blockage of apical K-channels. Supported by NIH grants DC00244, DC00766(SCK), DC00374(SDR), andAG06557 (SDR).

481.6

MEMBRANE CAPACITANCE MEASURES ELECTRICAL COUPLING BETWEEN TASTE CELLS. A. R. Bigiani and S. D. Roper. Dept. of Anatomy and Neurobiology, Colorado State University, Ft. Collins, CO 80523, and the Rocky Mt Taste & Smell Center, Denver, CO 80262.

In vertebrate taste buds, intracellular dye injections with Lucifer Yellow have revealed coupling between receptor cells (Yang & Roper, 1987). To study functional intercellular coupling in taste buds, we have developed a new experimental approach consisting of applying patch pipettes onto taste cells in thin (100-200 μm) slices of lingual epithelium from *Necturus maculosus*. In this preparation, interrelationships among cells within the taste bud are preserved. The whole-cell voltage-clamp configuration of the patch recording technique readily allows one to measure the membrane capacitance of taste cells. Membrane capacitance gives an estimation of the cell membrane available to be charged during a voltage step command, including any additional membrane contributed by electrically-coupled cells. We tested whether capacitance measurements monitored functional electrical coupling by perfusing the slice preparation with 1-octanol, a blocker of gap junctions. If taste cells are electrically-coupled via gap junctions, we should initially record high values of cell capacitance which would be reduced by 1-octanol. Indeed, in some experiments, cell capacitance was initially high and decreased when 500 μM 1-octanol was added to the bath. Lucifer Yellow in the patch pipette confirmed that these cells were coupled to adjacent cells. In other experiments, 1-octanol had no effect and these cells were not dye-coupled. These data indicate that functional electrical coupling between taste cells can be monitored by measuring membrane capacitance during patch clamp recordings. This now provides a means to test whether electrical coupling in the taste bud is affected by chemosensory stimulation or by neuromodulators.

481.7

UNILATERAL NARIS CLOSURE IN ADULT MICE PRODUCES A DOWN-REGULATION OF THE OLFACTORY BULB DOPAMINE PHENOTYPE H. Baker[1], D. Stone[1] and J. Maruniak[2]. [1]Cornell U. Med. Coll., White Plains, NY 10605 and Univ. Missouri-Col., Columbia, MO 65211.

Recent studies have shown that unilateral neonatal naris closure, assessed in adult rodents, produces a decrease in the expression of the dopamine phenotype in periglomerular neurons of the ipsilateral olfactory bulb. To distinguish afferent regulation of development from maintenance of phenotype, the current studies investigated the effects of unilateral naris closure produced in adult (4- to 6-month old) CF-1 mice. The changes in the dopamine phenotype, i.e., activity, immunoreactivity and mRNA for tyrosine hydroxylase (TH), the first enzyme in catecholamine biosynthesis, were measured 6-8 months post-closure. TH activity ipsilateral to the closure was reduced to 25% of that in the contralateral side and to 20% of that in sham operated animals. TH immunoreactivity (evaluated with a specific TH-antiserum) and mRNA [demonstrated by in situ hybridization (ISH)] exhibited a similar down-regulation. In contrast, immunoreactivity and mRNA (by ISH) for the GABA synthesizing enzyme, glutamic acid decarboxylase, were unchanged both ipsilateral and contralateral to the closure. These data suggest that maintenance as well as development of the dopamine phenotype are dependent on odor stimulation.
Supported by AG09686.

481.8

STRESS PROTEINS IN BULBECTOMIZED AND CONTROL RAT OLFACTORY EPITHELIUM (OE). V.McM.Carr and A.I.Farbman. Dept. of Neurobiology & Physiology, Northwestern Univ., Evanston, IL 60208.

We have examined the immunohistochemical reactivity of monoclonal antibodies directed against a 70 kDa heat shock protein (HSP70) and ubiquitin in the OE of control and unilaterally bulbectomized (OB-X) rats. These proteins are expressed rapidly and transiently in response to a variety of stresses. OE from Bouin's-perfused unoperated rats showed widely scattered HSP70-positive olfactory sensory neurons in the apical half of the OE. This localization and their absence from OB-X OE at 10 d. postop. indicates they are very mature neurons. Following OB-X, reactivity to ubiquitin and HSP70 appears in the supporting cells at 2 and 6 hrs postop, respectively. These initial responses were bilateral and also occurred in sham operated controls. However, by 24 hr supporting cell reactivity occurred on the OB-X side only. By 48 hr postop this supporting cell reactivity had disappeared. The results support other data showing rapid, transient stress protein responses in other systems (Brown, Neurosci.Res.,27:247,1990).

Supported by NIH Grant #DC 00347.

481.9

FLUORESCENT DYE (DII) REVEALS THE TASTE BUD CELLS AND AFFERENT INNERVATION IN THE LINGUAL EPITHELIUM OF THE MEXICAN SALAMANDER, AXOLOTL: A CONFOCAL LASER SCANNING MICROSCOPIC STUDY. T. Nagai. Dept. of Physiology, Teikyo Univ. Sch. of Med., Tokyo 173, Japan

Use of salamanders with large taste buds of a simpler structure has promoted cellular studies of taste receptor cells. However, little is known about afferent innervation to the cells. In a whole mount preparation of the anterior of the lingual epithelium, the overall innervation pattern of the glossopharyngeal nerve (IX) was clearly shown by the fluorescent carbocyanine dye, dioctadecyl-tetramethylindo-carbocyanine perchlorate (DiI) applied to the IX nerve stump. Beneath the epithelium, the labeled nerve fibers expanded horizontally to form a mesh-like structure, from which fascicles of the fibers extended outward toward the epithelium to innervate taste buds. Numerous taste buds (10/square mm at the tip) were labeled possibly transneuronally. In sectioned preparations, a single or several labeled cells with apical elongation toward a taste pore, showing a typical taste cell, was seen. In-between the distributed taste buds, fine fibers apparently formed free nerve endings within the epithelium.

482.1

Responses of subthalamic nucleus cell to pallidal stimulation and dopamine receptor blockade *J.R. Hollerman and A.A. Grace,* Departments of Behavioral Neuroscience & Psychiatry, Center for Neuroscience,University of Pittsburgh, Pittsburgh, PA 15260.

The subthalamic nucleus (STN) provides excitatory drive to the output nuclei of the basal ganglia, and to dopamine neurons in the midbrain. In this study, we recorded the response of subthalamic cells to stimulation of the globus pallidus in anesthetized rats in order to identify and characterize STN neurons. In addition, the influence of dopaminergic transmission on STN cell activity was assessed by recording the responses of individual STN neurons to acute administration of the dopamine receptor blocker haloperidol (HAL). In peristimulus time histograms, three types of response to pallidal stimulation could be identified: 1) excitation (lasting from 2-10 msec post-stimulus) followed by inhibition (from 10-40 msec, N=6), 2) inhibition (from 2-20 msec) followed by excitation (from 20-40 msec, N=5) and 3) inhibition only (from 2-40 msec, N=3). These responses most likely reflect and interaction between an inhibition mediated by pallidosubthalamic fibers (Rouzaire-Dubois, 1980) and a short latency excitation resulting from stimulation of excitatory corticosubthalamic fibers passing through or near the globus pallidus (Kitai and Deniau, 1981). In contrast, the longer latency activation observed may be a "rebound" excitation. Administration of HAL (cumulative dose of 0.8 mg/kg, i.v.) caused an increase in the firing rate of 2 cells, a decrease in the firing rate of 4 cells and no change in the firing rate of 3 cells. In 4 cells tested following HAL administration, i.v. apomorphine (0.8 mg/kg) reversed the change in firing rate caused by HAL.

482.2

NEOSTRIATAL UNIT ACTIVITY RELATED TO MOVEMENT PREPARATION IN A GO/NO-GO TASK IN THE CAT. J.W. Aldridge, J.F. Thompson*, E.A. Walters*, J.M. Groh and S. Gilman. Dept. of Neurology, Univ. of Michigan, 1103 E. Huron, Ann Arbor, MI 48104.

The functional role of single units in the putamen (PUT) and caudate nucleus (CN) in relation to the preparation of visually cued reaching movements was studied. Two cats were trained in a GO/NO-GO visual discrimination task. The animals sat on a force platform that had sensors for each limb, head movements and contact with the food delivery apparatus and target display. Visual cues were presented after a control period in which all limbs had to remain in contact with the force platform. Correct actions were: 1) GO cue - touch display with right forepaw; 2) NO-GO cue - maintain steady contact with force platform. Single units were recorded with microelectrodes inserted through a recording chamber. Changes in discharge rate were identified by a statistical comparison of epochs in the control and cue-to-movement periods. Significant rate changes in the GO task were detected in 31.9% of PUT (30/94) and 24.2% of CN (98/405) units. Lateral CN regions had more responsive units (32.1% vs 17.4%) than medial regions. Discharge changes prior to movement were usually (60.0% PUT, 56.1% CN) more tightly coupled to the stimulus than to the up-coming movement. Units with vigorous GO cue-related activity were usually (72.2% PUT, 54.5% CN) activated in the NO-GO task. Units with a weak link to the GO cue were rarely activated in the NO-GO task and units responsive to the NO-GO cue alone were uncommon. These findings suggest that, in the behavioral setting of a GO/NO-GO motor task, neostriatal activity is strongly related to preparation for executing or withholding movement. Furthermore, unit discharge related to the sensory cues is strongly linked to the context of movement requirements in the task. Support: NIH grant NS19613 and United Cerebral Palsy Foundation.

482.3

SYNAPTIC COOPERATIVITY MAY ARISE FROM THE FAST INWARD RECTIFICATION IN NEOSTRIATAL SPINY CELLS. C.J. Wilson, Dept. of Anatomy and Neurobiology, University of Tennessee, Memphis. TN, U.S.A.

Each neostriatal spiny neuron receives thousands of excitatory synaptic inputs from afferent fibers, which somehow act together to determine the timing and duration of the episodes of firing that are characteristic of these cells. Computer simulations of spiny neurons including large numbers of afferent synapses were employed to examine the degree to which the known morphological and biophysical properties of the cell may contribute to interactions among concurrently-active excitatory synaptic inputs.

Despite the location of excitatory synapses on dendritic spines, local dendritic EPSPs were extremely large when more than a few percent of synapses were activated in a clustered fashion on a portion of the dendritic field. Clustered activation of the dendrites was very ineffective in firing action potentials due to current attenuation by the classical synaptic nonlinearity. Synaptic activation distributed over the dendritic field produced much smaller local PSPs which summated more linearly and so were much more effective in exciting the cell. Simulation of the fast inwardly rectifying conductance known to be present on neostriatal neurons had little effect on clustered synaptic input. With distributed synapses inward rectification produced a cooperativity in which an excitatory input was more effective if it occurred concurrently with a limited number of others. There was an optimal number of inputs, beyond which additional synapses again had diminishing effects. The optimal number of inputs depended upon the size of the synaptic conductance change, the membrane potential and the activation curve for the inward rectifier, but invariably resulted in an EPSP amplitude that approximated spike threshold at the soma. Supported by NIH grant NS20743.

482.4

ENHANCEMENT OF MOTOR CORTEX EVOKED NEURONAL RESPONSES IN THE SUBTHALAMIC NUCLEUS FOLLOWING GLOBUS PALLIDUS LESION IN RATS. Lawrence J. Ryan and Kevin B. Clark*. Department of Psychology, Oregon State University, Corvallis, OR 97331-5303.

The reciprocal connections of the globus pallidus and the subthalamic nucleus provide an important basal ganglia site for interaction of information arising from the cortex. Motor cortex information arrives via projections to the neostriatum or the subthalamic nucleus, whereas limbic cortex information arrives only via the neostriatum. The effect of globus pallidus lesion on the response of subthalamic nucleus neurons to motor or prelimbic cortex stimulation was examined.

Large kainic acid lesions of the globus pallidus, much of the neostriatum and the lateral portions of the thalamus were made in 5 rats. Smaller lesions, which partially damaged the globus pallidus and involved either the neostriatum or the lateral thalamus were made in 8 rats. Two groups of naive rats (n= 9 and 6) were examined. Extracellular recordings of antidromically activated (from globus pallidus or substantia nigra) subthalamic neurons were made. Primary motor and prelimbic cortex were stimulated with 0.3 and 0.7 mA (0.2ms) single pulses.

GP lesions increased 1) the high frequency bursting of subthalamic neurons, 2) the duration of excitation elicited from motor cortex [0.7 mA: Large lesions - X(SEM,n cells)=58.5(3.46,42) ms; Small lesions - 62.9(7.18,36); Cont.1 - 24.5(3.25,29); Cont.2 - 42.47(1.63,33)], 3) response amplitude (# action potentials evoked per stimulus) [0.7mA: Large lesions - 1st Excitation - 1.17(0.08,29), 2nd excitation - 9.59(0.92,42); Small lesions - 1st - 1.43(0.26,19), 2nd - 5.87(1.36,36); Cont.1 - 1st - 1.0(0.15,15), 2nd - 2.02(0.28,36); Cont.2 - 1st - 0.54(0.09,24), 2nd - 1.48(0.28,23) and 4) the percentage of subthalamic cells that responded to motor cortex stimulation (Large lesions - 98%; Small Lesions - 61%; Cont.1 - 59%; Cont.2 - 58%). Large lesions eliminated responses to prelimbic cortex.

Thus globus pallidus modulates subthalamic neuronal responsiveness to afferent input arriving from the motor cortex and acts as a conduit for information from limbic areas to reach the subthalamic nucleus.

This research was supported by grant MH 45341 (to LJR) from the N.I.M.H.

482.5

MOTOR AND OCULOMOTOR SEQUENCING AND NEURAL ACTIVITY IN THE CAUDATE NUCLEUS OF MONKEYS. Y. Jurquet* and J.P. Joseph. Vision et Motricité, INSERM U94, F-69500 Bron, FRANCE

Neural activity in the head of the Caudate Nucleus of a behaving monkey was investigated during performance of a sequencing task derived from a paradigm used by Barone and Joseph in Prefrontal Cortex (EBR, 1989, 312-333). Basically, during a central fixation task, the animal had to remember the order of illumination of three fixed peripheral targets which were briefly illuminated in a random order. After a delay, using the same order, the animal had to orient successively towards each target and press it with an arm movement.

A) Visual responses to target onset usually consisted in a brief activity which depended on 1) the location of the target, 2) its rank (first or second) in the sequence, and 3) if the rank was second, on the location of the first target. In many cells, if the animal correctly encoded onset of a target but was prevented to use this information in the orienting and key-pressing task (by the experimenter who aborted the trial), then onset of the target was not re-encoded in the course of an identical trial executed 10 sec later. However, if identical trials separated by 10 sec. were successively presented to the animal and were all executed, then the information regarding location and rank of the targets was encoded each time. B) Many neurons were activated in relation to target acquisition by the gaze and/or by the arm. The main finding is that the activation depended not only on the parameters of the movements but also on the temporo-spatial characteristics of the whole sequence and on their rank in the sequence.

482.6

EFFECTS OF UNILATERAL NEOSTRIATAL LESIONS ON CUE- AND MOVEMENT-RELATED RESPONSES OF PALLIDAL NEURONS DURING A TRIGGERED HEAD MOVEMENT IN THE RAT. S. Usui, T.W. Gardiner, & S.T. Kitai Dept. Anatomy & Neurobiology, College of Medicine, University of Tennessee, Memphis, Memphis, TN 38163.

In previous work, we have demonstrated that neurons in the globus pallidus (GP) and neostriatum (Str) of the rat exhibit both cue- and movement-related changes in firing rate during performance of a trained head movement. GP appears to receive primarily inhibitory input from Str and excitatory input from the subthalamus, and in turn sends efferents to the entopeduncular nucleus, substantia nigra reticulata, and subthalamus. These circuits may participate in the control of sensory-triggered head movements. We examined the effects of unilateral Str lesions on behavioral performance and neuronal activity in GP during the head movement task. Rats were trained to hold their heads centered and then move to a left or right target in response to an auditory cue. Subsequently, unilateral lesions were made by injecting quinolinic acid (QA, 30-50 nmol) into lateral Str. Reaction and movement times were measured both before and several days after the lesion. To date, single-unit recordings have been obtained from 96 GP neurons. Thirty-four units have exhibited task-related changes. Seventeen units (50% of task-related neurons) altered their firing rates in association with cue presentation, whereas only movement-related changes in activity were observed for the remaining 17 units. The data obtained thus far contrasts with our previous data from intact rats in which nearly 80% of task-related units in GP exhibited changes in firing rate with cue presentation. Supported by NS-26473 and NS-20702.

482.7

THE RELATION OF PALLIDAL ARM NEURON DISCHARGE TO THE AMPLITUDE AND VELOCITY OF MOVEMENT. R.S. Turner and M.E. Anderson Depts of Psychology, Physiol. and Biophysics, Rehab. Medicine, and Regional Primate Research Center, University of Washington SJ-40, Seattle, WA 98195

Pallidal arm neuron discharge is often related to movement amplitude or velocity (Georgopoulos et al., 1983). We addressed two questions: (1) is pallidal activity better related to movement amplitude or velocity, (2) do movement amplitude effects interact with other characteristics of movement related changes in discharge?

Pallidal neurons were recorded from the external and internal segments (GPe and GPi) of two monkeys while they performed a two dimension reaching task. This required hand movements across a surface to targets in 8 directions and 3 distances from a central start position. Arm neurons were identified by: (1) task related changes in firing, and (2) responses to passive manipulation of the arm.

A high proportion of the neurons had task related changes in firing that were scaled with movement amplitude. Fewer were related to movement velocity. Of the 30 GPe and 12 GPi arm neuron studied during movements to three target distances, 67% had a linear relation between the average firing rate during the movement time and movement amplitude. All neurons in which more than 4 movement directions were tested (8 neurons) had a significant movement amplitude effect. The amplitude effects were independent of the magnitude, sign, and directionality of a neuron's task related discharge. Firing rate was better related to movement amplitude than peak velocity in 71% of the cases studied. Partial correlation coefficients confirmed this last finding.

Thus, pallidal activity is preferentially and independently related to movement amplitude. Supported by NS15017, RR00016 and H133B00081.

482.8

CHRONIC MULTI-CHANNEL SINGLE-UNIT RECORDINGS FROM 6-OHDA-TREATED RATS DURING L-DOPA-INDUCED CIRCLING. S.F. Sawyer, C.D. Myre and D.J. Woodward. *Department of Cell Biology and Neuroscience, University of Texas Southwestern Medical Center, Dallas, TX 75235*

Damage to the dopaminergic nigrostriatal projection in rats following intracerebral injection of 6-hydroxydopamine (6-OHDA) constitutes a useful animal model of Parkinson's disease. We have previously reported altered rates and patterns of neuronal activity in the neostriatum of 6-OHDA-treated rats. In this study, neostriatal unit activity was monitored following administration of L-dopa in unilaterally dopamine-depleted rats. Male Sprague-Dawley rats received an injection of 6-OHDA (8 µg in 4 µl) into the left medial forebrain bundle. Two arrays of 16 microwires were implanted in the basal ganglia, with at least one array positioned in the left neostriatum. Damage to the dopaminergic nigrostriatal projection was assessed by behavioral testing and contralateral circling to apomorphine (0.25 mg/kg, i.p.) or L-dopa (50 mg/kg, i.p.). Microwire localization was determined from X-rays and histological reconstruction. Spike activity of up to 8 neurons was recorded concurrently for weeks following 6-OHDA administration. Neurons in dopamine-depleted neostriatum tended to have higher rates and more bursty patterns of spike activity compared to neostriatal neurons in intact animals. L-dopa administration (50 mg/kg, i.p.) had heterogeneous effects on neuronal activity in the neostriatum: some neurons exhibited lower firing rates and less bursty patterns that resembled those found in neurologically-intact animals, whereas other units displayed an increase in spike activity that was correlated with circling behaviors. Preliminary studies indicate that neither SCH 23390 (0.3 mg/kg, i.p.; D1 antagonist) nor sulpiride (30 mg/kg, i.p.; D2 antagonist) blocked circling induced by L-dopa, and did not profoundly modify L-dopa-induced alterations of neostriatal spike activity. Administered together, the antagonists blocked both rotation and alterations in unit activity. Further studies are underway to investigate the basis of heterogenous responses to L-dopa. *Supported by DA-05352, MH-44337, AFOSR 90-0416 and Biological Humanics Foundation.*

482.9

ACTIVITY OF DOPAMINE NEURONS IN MONKEYS LEARNING AND PERFORMING COGNITIVE TASKS. W. Schultz*, T. Ljungberg* and P. Apicella* (SPON: European Neuroscience Association). Institut de Physiologie, Univ. Fribourg, CH-1700 Fribourg, Switzerland

Cognitive deficits are major symptoms of Parkinson's disease. Previously we showed that dopamine (DA) neurons in behaving monkey respond to attention-generating and motivating stimuli. We now tested DA neurons in cognitive tasks.

In spatial delayed responding, one of two horizontally arranged lights was illuminated for 1 s as instruction. A central light appearing 2 s after instruction offset triggered a reaching movement toward a lever indicated by the instruction light. Correct responses were rewarded with a drop of juice. Typical DA neuron impulses were recorded during and after task conditioning from areas A8, A9 and A10 in 2 Macaca fascicularis monkeys. During conditioning, 29%, 21% and 21% of 28 neurons responded phasically to instruction stimuli, trigger stimulus and delivery of reward, respectively. Responding neurons were located throughout areas A8, A9 and A10, although reward responses predominanted in A10. After task acquisition, the fraction of neurons responding to instruction and trigger stimuli increased (38% and 49% of 73 neurons, respectively), whereas reward responses diminished (11%).

In delayed alternation, the rewarded lever was alternated after each successful movement, and instructions were absent. Of 88 DA neurons, 65% and 52% responded to the trigger and reward, respectively. Trigger responses were lower before task acquisition and after overtraining, and reward responses diminished after overtraining. None of the DA neurons in either task showed delay-related sustained activity typical for striatum and frontal cortex.

These data demonstrate that DA neurons respond to the most significant attentional and motivating stimuli during performance of a cognitive task. Their activity does not reflect the specific cognitive components of the tasks, such as preparation of movement, expectation of external stimuli, expectation of reward, or working memory. Rather, DA neurons appear to participate in underlying attentional and motivational processes necessary for cognitive performance.

482.10

LEARNING-RELATED PLASTICITY OF STRIATAL FORELIMB NEURONS IN THE AWAKE, UNRESTRAINED RAT. R.M. CARELLI and M.O. WEST. Dept. Psychology, Rutgers Univ., New Brunswick, NJ 08903.

Long-Evans male rats (n=5) were chronically implanted with a detachable microdrive to record forelimb neurons in the dorsolateral striatum during a tone-discrimination task involving lever-pressing with the contralateral (right) forelimb for water reinforcement. To reduce trial-to-trial variability in reaching toward the lever, rats were trained to stand facing the lever, with the right forepaw on the floor in order to initiate tone onset at the start of each trial. Prior to each session, neurons related to forelimb movement were identified by sensorimotor exam. Increased discharge time-locked to the lever-press (relative to baseline firing when the forepaw was motionless on the floor) was termed "Signal:Baseline" (S:B). We have previously reported that forelimb neurons discharged in relation to the lever-press during early sessions (S:B range 2-10) but in no instance did a forelimb neuron discharge during the same skilled movement during repeated sessions (S:B=1) despite the fact that EMG activity showed consistent relationships to the lever-press across all sessions (Neurosci.Abs. 16: 233, 1990). Within-subject analysis revealed significant correlations between S:B and behavioral measures indicative of learning the task. However, S:B was not consistently correlated with the same aspects of learning between subjects. Thus, the change in forelimb neuronal firing is interpreted as a learning-related plasticity, reflective of learning about static conditions relevant for movement. As the subject learns that the context and contingencies governing a particular movement remain constant from trial-to-trial and session-to-session, the striatal representation of that movement gradually declines to a level indistinguishable from baseline. A similar decline would not be expected in sensorimotor cortical representations of the movement, which are projected to frontal cortical areas which receive convergent input from the striatum (via the pallidum and thalamus). This implies that a unique quality of the striatal contribution to motor programming regards the degree of constancy in sensorimotor contingencies relevant for movement. Supported by NSF BNS-8708523, DA 04551 and PHS RR 07058-21.

482.11

A COMPARISON OF PREMOVEMENT NEURONAL ACTIVITY IN MONKEY NEOSTRIATUM AND SENSORIMOTOR CORTEX. R. J. Nelson and T. W. Gardiner. Dept. of Anatomy and Neurobiology, College of Medicine, University of Tennessee, Memphis, 875 Monroe Avenue, Memphis, TN 38163.

Premovement activity (PMA) of primary somatosensory (SI) and primary motor (MI) cortical neurons often begins prior to EMG activity when monkeys perform sensory triggered movements. Recent studies suggest that some neostriatal neurons also discharge early in similar tasks. PMA was compared for cortical or neostriatal neurons recorded from five adult rhesus monkeys (*Macaca mulatta*) trained to make wrist flexion and extension movements in response to vibratory stimuli to the palm or to visual cues. They were also trained to maintain their wrist position following the same vibratory cues used in the movement task. Accordance with the *NIH Guide for Care and Use of Laboratory Animals, revised 1985* was maintained. To date, 27 area 3b, 63 area 1, 84 putamen and 29 caudate neurons have been analyzed. In general PMA for both putamen and caudate neurons occurred significantly earlier (means 110-170ms) before movement than PMA for area 3b and 1 neurons (means 70-110ms), depending upon movement direction and cue stimulus type ($p<.05$; single factor ANOVA; Scheffé comparison test). Neostriatal PMA occurred significantly earlier than that for MI neurons during extension but not flexion movements. One third of the neostriatal PMA neurons showed qualitatively different activity patterns immediately after, as compared with before movement onset. Over 85% of cortical PMA neurons showed early activity that was sustained for ≈100ms after movement onset. The magnitude of PMA from background for area 1 neurons was significantly smaller during vibratory as compared with visually cued trials. This was not the case for MI, area 3b or neostriatal neurons. Like the SI neurons, neostriatal PMA neurons rarely showed vibratory stimulus related responses in the no-go task if these responses were not present in the movement task.

These results suggest that: 1) PMA in neostriatal neurons can occur prior to that in MI and SI, 2) unlike area 1 neuronal PMA, decreased PMA magnitude in vibratory-cued trials is not commonly seen for neostriatal PMA neurons and 3) neostriatal PMA neurons could influence cortical neuronal PMA via demonstrated pallidothalamocortical circuitry projecting ultimately to MI and SI. Support by **NIH Grant NS26473.**

482.12

SYSTEMIC AMPHETAMINE INCREASES MOTOR-RELATED FIRING OF NEURONS IN THE DORSOLATERAL STRIATUM OF THE FREELY-MOVING RAT: L.L. Peoples*, D.J. Woodward#, C.F. Flaherty, M.O. West. Rutgers U., New Brunswick, NJ; #Anat., U. of Texas Southwestern Medical School, Dallas TX.

The present study characterized the effects of intraperitoneally administered d-amphetamine (amph) on the activity of single cells in the dorsolateral striatum of freely-moving rats. Male Long-Evans rats were chronically implanted with detachable microdrives positioned for recording in the dorsolateral striatum (+2.0 to 0.0 mm A-P from Bregma, 3.5 to 4.5 mm from midline, and 3.0 to 6.0 mm from skull, Paxinos and Watson, 1986). In each of 12 experiments, a given cell was first recorded after a saline injection and then subsequently recorded after an amph injection (0.5 or 1.0 mg/kg). To control for the potential effects of amph-induced motor behaviors on the activity of striatal cells, pre- vs post-amph comparisons of firing rates were made during behaviors that remained stable across the pre- and post-drug periods. Specifically, comparisons were made across pre- and post-drug periods in which the rat either: 1) locomoted, at a constant rate on a treadmill which rotated on a 30 sec on/30 sec off schedule or 2) engaged in specific body part movements with amplitudes and velocities unaffected by amph. The activity of 10/12 cells was positively correlated with locomotion (i.e., firing rates were greater during TM locomotion than they were during rest). Moreover, 3 cells exhibited firing patterns positively correlated with a specific movement of a particular part of the body (i.e., firing rates were greater during the specific movements than they were during any other movement). As compared to saline, amph consistently increased the mean firing rates exhibited during locomotion, as well as that exhibited during specific body part movements. Moreover, for the three cells that exhibited a specific movement correlate, amph-induced increases in firing rates were greater during the correlated movement than during any other movement. Previous experiments indicate that the striatum is involved in the transduction of amphetamine's pharmacological actions into stereotyped motor output. The present data strongly suggest that the cellular correlate of this transduction process, in the dorsolateral striatum, is enhanced excitation. Supported by DA04551, DA-02338, AA3901, and the Biological Humanics Foundation.

482.13

COCAINE EFFECTS ON SINGLE NEURONS IN THE DORSOLATERAL STRIATUM OF FREELY-MOVING RATS: T. Mittler*, L.L. Peoples* and M.O. West. Dept. Psychol,Rutgers U.,New Brunswick, NJ.

The present study was designed to characterize the effects of the psychomotor stimulant cocaine on neuronal processing related to motor activity. Adult male Long-Evans rats were chronically implanted in the dorsolateral striatum (at Bregma A-P, 4.0 mm from midline, 4.5 mm from skull, verified histologically) with bundles of 6-10 microwires (62μ diam., stainless steel, teflon coated). To differentiate cocaine's effects on neuronal firing from drug induced motor effects on neuronal firing, pre- vs. post-drug comparisons were made with behavior constant across conditions. This was achieved by forcing locomotion on a treadmill which was activated periodically (20 sec. on/40 sec. off), and by video analysis. A computerized system that time-stamped each neural discharge and each video frame allowed comparison of neuronal activity pre- vs. post-drug both within and between different motor behaviors. After a saline injection (IP) a firing rate baseline was recorded for 1 hr., followed by an IP cocaine injection (10 mg/kg) and a 3 hr. recording. Preliminary results (from 8 units in 4 rats) show that most neurons increased firing during locomotion, while either increasing or decreasing firing in response to cocaine. Supported by DA04551 and PHS RR-07058.

482.14

BIPHASIC EFFECT OF SUBTHALAMIC NUCLEUS ACTIVITY ON SUBSTANTIA NIGRA DOPAMINE NEURON FIRING. I.D. Smith and A.A. Grace, Deptartments of Behavioral Neuroscience and Psychiatry, Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.

The subthalamic nucleus (STN) is known to play a major role in the regulation of movement by the basal ganglia. Both anatomical and electrophysiological studies show that the rat STN directly modulates basal ganglia output via an excitatory projection to the entopeduncular nucleus and the substantia nigra zona reticulata (SNzr). Furthermore, several studies suggest that the STN also projects to dopamine (DA) neurons of the substantia nigra zona compacta (SNzc), although the net influence of the STN on DA neuron activity remains unclear. Our prior investigations have shown that the STN is involved in the induction and maintenance of burst firing in SNzc DA cells. However, chemical (Robledo and Feger; *Brain Res. 518*, 1990) or electrical activation of the STN rarely elicits a clear effect on DA cell activity. In this study, we examined the effects of $GABA_A$ antagonist and agonist infusion into the STN on SNzc DA neuron firing rate and pattern.

Microinjections of the GABAA antagonist bicuculline methiodide (200 μM, 200 μl) into the STN resulted in an immediate 70.5±24.5% decrease in DA cell firing frequency (N=6). Although these neurons recovered their baseline firing rates within 7 to 16 minutes following the injection, there was an 81.8±26.8% increase in the prportion of spikes fired in bursts. This is consistent with our finding that electrical stimulation of the STN results in a population increase in burst firing. In contrast, injection of the GABAA agonist muscimol into the STN (200 μM, 200 μl) caused a rapid and reversable 15 to 150% increase in DA neuron firing rate (N=7).

Thus the STN appears to exert a dual action on SNzc DA cells: 1) an inhibition possibly mediated through STN excitation of the inhibitory SNzr or globus pallidus projections to the SNzc, and 2) a facilitation of burst firing which may be a direct effect of excitatory STN afferents. The activation of the STN in DA depleted animals shown by metabolic mapping studies could contribute to the elevated level of DA neuron burst firing reported in 6-OHDA treated rats (Hollerman and Grace, 1990).

(Supported by USPHS NS19608, MH42217, MH45156 and MRC of Canada)

482.15

ELECTROPHYSIOLOGICAL RECORDINGS OF PALLIDAL NEURONS IN RATS RUNNING IN PLACE ON A TURNTABLE TREADMILL. P. Patino, E. Kriek, and C.R. Freed. Div. Clinical Pharmacol., Univ. of Colo, Hlth. Sci. Ctr., Denver, CO 80262.

Previous studies in our laboratory have shown that neurons in ventromedial striatum of the rat increase their firing when animals run in place on a turntable treadmill for a water reward. Globus pallidus (GP) is an important part of the output pathway of the striatum. With the circular treadmill, we have now recorded pallidal neurons in moving animals. Both intact animals and animals with unilateral dopamine depletion of striatum were studied using chronically implanted 18 micron stainless steel electrodes insulated with Parylene C. In intact animals, basal firing rates ranged from 23 to 62 hz and responded to changes in motor activity in 83% of cases. 28% of cells increased firing rate more than 100% during running while 55% of cells reduced firing rate at least 50% during locomotion. In dopamine depleted animals, GP neurons were slower under resting conditions - 5 to 30 hz - and fired mainly in bursts. Only 33% of cells showed a change in firing during movement. In summary, GP neurons fire rapidly in intact animals at rest and show complex changes in firing with movement. In animals with unilateral dopamine depletion, there is a marked reduction in firing rate and a loss of movement related change in cell firing.

482.16

OCULOMOTOR ACTIVITIES IN THE MONKEY SUBTHALAMIC NUCLEUS M. Matsumura,J. Kojima,T.W.Gardiner AND O. Hikosaka Lab. of Neural Control, National Institute for Physilogical Sciences,Okazaki, 444,Japan.

The subthalamic nucleus sends excitatory signals to the substantia nigra pars reticulata, which exerts tonic inhibition on saccade burst cells in the superior colliculus. To investigate the nature of the subthalamic signals we recorded single cell activities in monkey subthalamic nucleus in relation to visuo-oculomotor tasks. Of 265 neurons tested, 95 were related to the tasks, while 66 neurons responded to active or passive body movements. The task-related activities were classified into: eye fixation(n=22), saccade(n=22), visual(n=14), target-reward(n=29), and lever-release(n=49). Eye fixation activity was sustained spike activity occurring while the monkey was fixating on a light target during the task; it ceased when the target went off and the monkey made a saccade. Saccade activity was a phasic increase of spike frequency time-locked with a saccade made in the task. It usually peaked after saccade onset. The activity had optimal directions, mostly toward the contralateral side. Visual response had receptive fields, most frequently centered on the contralateral parafoveal region. Its latencies were 70-120 ms. Target-reward activity was sustained spike activity preceding and dependent on expectation of reward. These visuo-occulomotor neurons were located mostly in the ventral part of the subthalamic nucleus, whereas skeletomotor-related neurons were in the dorsal part. These resuslts suggest that the subthalamic nucleus acts to maintain eye position on an object of interest or recover eye fixation once a saccade is executed.

BASAL GANGLIA AND THALAMUS IV

483.1

DISSOCIATION OF OPPOSITE EFFECTS OF D1 AND D2 DOPAMINE AGONISTS FOLLOWING LESION OF THE EXTERNAL PALLIDAL SEGMENT IN THE MONKEY. M. Filion, L. Tremblay, B. Gomez-Mancilla, M. Matsumura and P.J. Bédard. Neurobiology Res. Ctr, Laval Univ., Québec, Qué., Canada.

Kainic acid was used to destroy exclusively neurons in nearly two thirds of the external segment of the globus pallidus (GPe) unilaterally in a cynomolgus monkey. Ventral and rostral parts of the structure were spared. Neurons of the caudate nucleus and putamen were also destroyed but only in a small area above the GPe. The internal pallidal segment (GPi) was totally intact. Within the first 24 hours, generalized hypokinesia, bradykinesia, and flexed posture appeared. The signs gradually decreased in severity, became localized to the side contralateral to the lesion, but persisted until the animal was sacrificed, 19 days after the lesion. In particular, the upper limb remained flexed. Spontaneous circling toward the lesion occurred from day 5 to 11. From day 9 to 15, drugs thought to act specifically on either D1 (CY-208243) or D2 (LY-171555) dopamine receptors were tested. The D1 agonist decreased whereas the D2 increased the hypokinesia. Only weak doses of the D1 agonist (0.1 mg/kg) decreased the hypokinesia. At stronger doses (0.5 mg/kg), the drug acted like the D2 agonist, which induced the same effect at both weak and strong doses (0.01 and 0.1 mg/kg). The present results suggest that effects of D2 receptors activation are weakened by selective GPe lesion, unmasking opposite effects induced by D1 receptors activation and mediated mainly through the GPi (Gerfen et al., Science (1990) 250: 1429-32). Electrophysiological correlates of these results are currently under study.
(Supported by the FRSQ and MRC of Canada).

483.2

DIFFERENTIAL EFFECT OF D1 AND D2 DOPAMINE RECEPTOR STIMULATION ON SOMATOSTATIN AND NEUROPEPTIDE Y LEVELS IN RAT STRIATUM. T.M. Engber, R.C. Boldry, S. Kuo* and T.N. Chase. ETB, NINDS, NIH, Bethesda, MD 20892.

Little is known about the influence of dopamine on striatal medium aspiny neurons, a type of interneuron which expresses both somatostatin and neuropeptide Y (NPY). We have examined the effects of dopamine denervation and D1 or D2 dopamine receptor stimulation on the levels of somatostatin and NPY in the striatum. Rats with a unilateral 6-hydroxydopamine lesion of the median forebrain bundle were treated for 7 days with either the D1 agonist SKF 38393 (12.5 mg/kg/day) or the D2 agonist quinpirole (1 mg/kg/day) on either an intermittent (once daily i.p. injection) or continuous (via osmotic pump implanted i.p.) regimen; lesioned control animals were given vehicle solution by both injection and infusion. Rats were sacrificed 3 hours after the last injection, and both striata were dissected using the nuclei punch method; somatostatin and NPY levels were measured by radioimmunoassay. Striatal dopamine denervation had little effect on the levels of either somatostatin or NPY. In comparison with controls, intermittent SKF 38393 treatment caused a substantial reduction in the content of both somatostatin (61%) and NPY (57%) in the denervated striatum; continuous SKF 38393 decreased NPY to a lesser extent (39%) but had no effect on somatostatin. Intermittent quinpirole decreased both somatostatin (35%) and NPY (27%) content relative to control values. Continuous quinpirole, on the other hand, increased the levels of both somatostatin (43%) and NPY (39%) These results suggest that striatal medium aspiny neurons are influenced by both D1 and D2 receptor stimulation and that they respond differentially to continuous and intermittent D2 stimulation.

483.3

DIFFERENTIAL EFFECT OF SUBTHALAMIC NUCLEUS LESION ON D1 AND D2 AGONIST-INDUCED ROTATION IN 6-HYDROXYDOPAMINE LESIONED RATS. J.J. Anderson, T.M. Engber, and T.N. Chase. ETB, NINDS, NIH, Bethesda, MD 20892.

Evidence suggests that activity of the pathway from the striatum to the lateral globus pallidus (LGP) may be modulated by dopamine primarily via D2 receptors. The subthalamic nucleus (STN) receives a large inhibitory input from the LGP. Increased excitatory drive of the STN to the substantia nigra pars reticulata and medial globus pallidus, as an indirect result of nigrostriatal dopamine cell loss, may contribute to motor impairments observed in Parkinson's disease. The effect of STN lesion on D1 agonist SKF 38393 and D2 agonist quinpirole-induced rotation was examined in rats with unilateral 6-hydroxydopamine lesion of the medial forebrain bundle. Ten days following infusion with 6-hydroxydopamine, the nigrostriatal lesion was verified by examining the rotational response to apomorphine (0.05 mg/kg sc). A homogeneous group of responders was selected and half of these animals were microinjected with the neurotoxic agent quinolinic acid (60 nmoles/0.2 ul phosphate buffered saline vehicle; pH 7.4) in the STN. The other half were microinjected with 0.2 ul vehicle in the STN and served as controls. After 7 days, the rotational response to SKF 38393 (1.5 and 5.0 mg/kg sc) and quinpirole (0.1 and 0.5 mg/kg sc) was examined. In STN-lesioned rats, both SKF 38393 and quinpirole-induced rotations were markedly reduced relative to controls in a dose-dependent manner with a significantly greater reduction seen in quinpirole turning (93% reduction) compared to SKF 38393 turning (69% reduction). These results indicate that lesion of the STN differentially affects D1 and D2 agonist-induced rotational behavior in 6-hydroxydopamine lesioned rats.

483.4

EFFECTS OF KAPPA OPIOID AGONISTS ON ROTATIONAL BEHAVIOR IN 6-HYDROXYDOPAMINE-LESIONED RATS R.C. Boldry, T.M. Engber, and T.N.Chase. Experimental Therapeutics Branch, NINDS, NIH, Bethesda, MD 20892.

The effects of dopamine receptor activation in the striatum are mediated by projections to the globus pallidus and the substantia nigra pars reticulata. Recent studies have suggested that D2 receptors are predominantly expressed on neurons which project to the globus pallidus while D1 receptors are primarily located on the striatonigral projections. The opioid peptide dynorphin is thought to act as a neurotransmitter in the striatonigral projection and to produce its effects by activating kappa opioid receptors. To evaluate the role of dynorphin receptor activation in motor function, we have examined the effects of the kappa agonists U50,488 (2.5, 10 and 25 mg/kg, s.c.) and spiradoline (0.5, 1 and 5 mg/kg, s.c.) on the rotational behavior elicited by D1 and D2 dopamine agonists in rats which were unilaterally lesioned with 6-hydroxydopamine. Both U50,488 and spiradoline dose-dependently decreased the rotational response to the D1 agonist SKF 38393 (1.5 mg/kg, s.c.). Conversely, neither drug affected the total number of turns produced by the D2 agonist quinpirole (0.1 mg/kg s.c.). However, changes were observed in the time-course of quinpirole-stimulated rotation following kappa agonist treatment at the highest doses tested. U50488 and spiradoline appeared to decrease the initial response to quinpirole, followed by an increase in turning in the later time periods. Taken together, these data indicate that kappa agonists produce differing effects on D1 versus D2 agonist-induced behaviors; the data also suggest that endogenous dynorphin may act to dampen striatal output through the D1 receptor-regulated striatonigral pathway.

483.5

THE ROLE OF DOPAMINE IN THE MAINTENANCE AND BREAKDOWN OF D1/D2 SYNERGISM. G.J. LaHoste, M. Andreini* and J.F. Marshall. Depts. of Psychobiology and Physical Medicine & Rehabilitation, Univ. of California, Irvine, CA 92717.

Concomitant stimulation of both D1 and D2 subtypes of dopamine (DA) receptor is normally required for the expression of behavior, a phenomenon referred to as D1/D2 synergism. Certain treatments, however, such as destruction of DAergic neurons and repeated treatment with reserpine induce a breakdown in synergism such that independent stimulation of either receptor subtype elicits behavior. To identify the factors involved in the breakdown of synergism, we injected rats with vehicle or reserpine (1 mg/kg) at 0, 24 and 48 hours. Two hours after the last injection, reserpine- but not vehicle-treated rats showed stereotyped motor behavior in response to quinpirole (3 mg/kg) while D1 receptors were antagonized (SCH 23390, 0.1 mg/kg), indicative of a breakdown in synergism. Rats treated with the same reserpine regimen and given L-DOPA/carbidopa (100/25 mg/kg) every 6 hours from 0 to 48 hours also showed a breakdown in synergism. Rats treated with SCH 23390 and eticlopride (0.5 mg/kg each) every 6 hours from 0 to 48 hours also showed a breakdown in synergism when tested 24 hours after the last neuroleptic injection. The results suggest that treatments that profoundly interfere with the ability of DA to interact with its receptors over the course of 48 hours result in a breakdown in synergism. The inability of L-DOPA/carbidopa to reverse this effect may be due to the failure of the intermittent injection schedule to normalize synaptic DA content.

483.6

SYSTEMIC HALOPERIDOL DECREASES THE HYPERKINETIC PAW TREADING INDUCED BY VENTROMEDIAL STRIATOPALLIDAL LESIONS IN THE RAT. H.C. CROMWELL AND K.C. BERRIDGE. THE DEPARTMENT OF PSYCHOLOGY, THE UNIVERSITY OF MICHIGAN. ANN ARBOR MI. 48109.

Haloperidol is used to decrease the severity of several different hyperkinetic syndromes in humans with damage in the basal ganglia. Previous work has discovered a hyperkinetic syndrome in the rat which is induced by ventromedial striatopallidal lesions and triggered by oral sensory stimulation (Berridge et al.,1988). The hyperkinesia appears as exaggerated and perseverative forepaw treading and is never seen in intact rats.

To better characterize the neuropharmacological nature of the syndrome, systemic injections of haloperidol were given to rats expressing the paw treading syndrome. Quinolinic (QUIN) acid (60NM in .5ul) was injected bilaterally into the ventromedial striatopallidal complex. Behavioral testing began 48 hrs. post surgery. Rats were given injections (ip.) of either haloperidol (1mg\kg) or diazepam (5mg\kg) 30 minutes prior to the taste infusion. Most rats were tested with both haloperidol and with valium on separate days. Tests of motor abilities (wire mesh hangtime, righting reflex latency) were completed to record general motor effects of the drug injections.

Results showed that haloperidol was effective in ameliorating the hyperkinesia only in the rats that showed mild to moderate treading post-lesion and not in rats expressing severe treading post-lesion. Diazepam did not decrease the hyperkinesia and in fact, increased it in most cases. Diazepam but not haloperidol impaired performance on motor ability tests. These results implicate a role for dopamine in the expression of the hyperkinetic treading syndrome in the rat.

483.7

SIMPLE AND CHOICE REACTION TIME PERFORMANCE FOLLOWING UNILATERAL IBOTENIC ACID LESIONS OF MEDIAL OR LATERAL STRIATUM IN THE RAT.

D. O. Pretsell and T. W. Robbins, (SPON:Brain Research Association), Dept. Exptl. Psychology, University of Cambridge, Cambridge, U.K.,

Rats were trained to perform a visual spatial discrimination, where stimulus luminance provided information regarding the required direction of response. The visual stimuli were presented either in advance of a temporally unpredictable auditory imperative stimulus (simple reaction time condition) or simultaneously with the imperative stimulus (choice reaction time condition). Rats were given unilateral lesions of either the lateral or medial striatum using 1 microlitre of 0.06M ibotenic acid. Following the lateral lesion an ipsilateral response bias and lengthened contralateral reaction times were seen in both conditions. In the group with the medial lesions no response bias was observed. No consistent effect on reaction time was seen following the medial lesion although there was an indication of contralateral reaction time lengthening in the choice condition only.

These results suggest a functional dissociation of the medial and lateral striatum in attentional processes involved in the control of action and are relevant to studies of the effects of basal ganglia disorders on reaction time performance.

483.8

INITIATION IMPAIRMENT AFTER GLOBUS PALLIDUS LESION DEPEND ON THE REACTION TIME TASK. E. Trouche*, M. Alamy*, E. Legallet* and F. Viallet. Lab. Functional Neurosciences, LNF 3, CNRS, 13402 MARSEILLE CEDEX 9, France.

The authors of previous studies using reaction time (RT) procedure have described the effects of globus pallidus (GP) lesion on the performance of an arm movement in response to a visual stimulus. No change in the RT was reported in any of these studies as the result of GP lesion. These results suggested that the GP was not involved in motor initiation. This conclusion was not in agreement however with some electrophysiological data showing the existence of GP cells which discharge before the onset of the movement. In our study, we investigated the effects of GP lesion on both the simple reaction time (RTs) and the choice reaction time (RTc) in a pointing task with variable movement amplitude. Two monkeys were trained to perform the two RT paradigms. After unilateral neurochemical GP lesion, no change in the RTs was observed, whereas a significant increase in the RTc occurred in the contralateral limb. These results indicate that the GP may involved in motor initiation in RTc task particularly when there is uncertainty as to the amplitude of the forthcoming movement.

483.9

TREMULOUS ORAL MOVEMENTS AND FEEDING DEFICITS IN RATS WITH VENTROLATERAL STRIATAL DOPAMINE DEPLETIONS: POSSIBLE RELATION TO PARKINSONIAN SYMPTOMS. J. D. Salamone, G. Jicha, and S. Rogers. Dept. of Psychology, Univ. of Connecticut, Storrs, CT 06269-1020.

Local depletion of dopamine (DA) produced by injections of 6-hydroxydopamine in the ventrolateral striatum (VLS) caused a substantial decrease in food intake, from which the animals recovered. In behavioral observation sessions, rats with VLS DA depletions showed no reduction in time spent feeding, but did have substantial impairments in the rate of feeding and forepaw use during feeding. DA depletions in nucleus accumbens, anteroventromedial (AVMS) or dorsolateral striatum (DLS) did not produce signifcant feeding deficits. Rats with VLS DA depletions showed no deficits in locomotor activity or rearing. Tremulous chewing movements were produced by VLS DA depletion, but not AVMS or DLS depletions. Systemic administration of haloperidol (0.4 mg/kg) increased tremulous chewing responses in DA-depleted and control rats. Thus tremulous chewing responses can result from reduced functional activity of VLS DA, and these responses share some characteristics with human Parkinsonian symptoms. These data support the notion that the neostriatum is functionally heterogeneous and that the VLS is particularly important for oral and forelimb motor control.

483.10

BEHAVIOURAL AND ANATOMICAL ANALYSIS OF RATS WITH QUINOLINIC ACID-INDUCED STRIATAL LESIONS

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It is not understood how the cognitive impairment in patients with Huntington's Disease (HD) is related to the striatal degeneration that is the neuropathological hallmark of the disease. In order to explore this issue, we have examined the behavioural consequences of quinolinate-(QUIN) induced striatal lesions in rats. Animals with bilateral striatal QUIN lesions were impaired performance in the place task version of the Morris Water Maze and the delayed alternation task when compared to controls. However, the QUIN group was no different from the control group on some unlearned tests (eg, food manipulation, swimming posture, tongue extension, general motor ability). Post-mortem analysis using the Bioquant IV programme showed significant striatal atrophy but no differences in five cortical areas, in relation to controls.These findings demonstrate that rats with striatal lesions induced by quinolinic acid are impaired in the performance of tasks requiring visuospatial skills, long-term memory and cognitive flexibility. These results are consistent with the notion that cognitive impairment in HD may represent disordered function in the striatal-pallidal-thalamic-cortical feedback loop.

483.11

THE EFFECTS ON TRAINED ARM MOVEMENTS PRODUCED BY INJECTIONS OF MUSCIMOL OR BICUCULLINE INTO THE GLOBUS PALLIDUS. M. Inase* and M.E. Anderson. Depts of Physiol. and Biophys. and Rehab. Med. and Reg. Primate Res. Center, Univ. of Wash., Seattle WA 98195.

Disruption of nigrocollicular inhibition results in saccadic jerks during fixation and saccades with shorter reaction time (RT), higher peak velocity (PKV) but smaller amplitude (MAMP), especially to precued target locations (Hikosaka and Wurtz, 1985). We examined the effect on arm movements produced by changing the output of the globus pallidus (GPi). Monkeys were trained to make arm movements to targets that were illuminated coincident with a GO tone or were lit briefly as a precue.

Injection of muscimol into GPi produced a flexor drift of the contralateral arm from the start position. When the initial position was maintained until the GO tone, the RT was normal, but the MAMP and PKV to some targets were decreased and the amplitude/peak velocity scaling relation was disrupted. Injection of bicuculline into GPi did not cause a flexor drift, but it produced involuntary movements, primarily proximally. RT's were not changed, but the MAMP and PKV also were reduced to targets in some directions.

Positional instability of the eye or arm follows injections of muscimol into appropriate basal ganglia nuclei. However, changes in arm movements produced by changes in GPi output were more complex than predicted from oculomotor studies.

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483.12

D1 AND D2 DOPAMINE RECEPTOR SUBTYPES: MODULATION OF THE SPEED AND SUCCESS OF REACTION TIME PERFORMANCE IN THE RAT. R.E. Wilcox, P.K. Randall and R.D. Mayfield*. Dept. of Pharmacol., Univ. of Texas at Austin, Austin, TX 78712.

The purpose of this investigation was to determine whether D1 and D2 dopamine receptor subtypes have different roles in modulating the speed and success of rodent reaction time performance. Animals were shaped to release a lever in response to an auditory/visual stimulus in order to avoid mild footshock. Shaping was continued until the animals were responding reliably with response latencies of 180-220 msec. The effects of apomorphine (0.15, .30, .60, 1.2, 2.4 and 4.8 mg/kg) on reaction time were determined in animals pretreated with the selective D1 antagonist, SCH 23390 (0, 50 and 100 µg/kg), or the selective D2 antagonist, spiperone (0, 1 and 10 µg/kg). Apomorphine alone resulted in a dose dependent decrease in successful avoidance with an ED_{50} of 3.9 mg/kg. Response latencies were unaffected by apomorphine treatment. SCH 23390 pretreatment resulted in a significant impairment of both successful avoidance and response latency. Increasing doses of apomorphine effectively attenuated SCH 23390's effects on successful avoidance, and to a lesser extent blocked the SCH 23390-induced increase in response latency. Spiperone pretreatment attenuated the effects of apomorphine on successful avoidance, resulting in a 6-fold shift to the right in the apomorphine dose-response curve. These results suggest that D1 receptors play an important role in modulating the speed of reaction time responses and that D2 receptors predominantly modulate successful response initiation.

483.13

ORIENTATION TO EDGES AND ITS MODULATION BY STRIATAL DOPAMINE IN RATS. R. Sullivan', A. Fraser', C. Coulter' and H. Szechtman. Dept. Biomedical Sciences, McMaster University, Hamilton, Ontario, Canada L8N 3Z5.

Male Sprague-Dawley rats received either control operations (N=14) or unilateral 6-hydroxydopamine lesions of the left or right substantia nigra (N=22 per group). At eight weeks post-lesion, all rats were tested (undrugged) in a large open field (160 cm square and elevated 100 cm) for ten minutes. From videotapes, a measure of net orientation was obtained for time spent at the edge (locomoting or not). Following testing, brain regions were assayed for levels of DA and metabolites by HPLC. Left and right lesioned rats differed significantly in their orientation to edges ($p<0.001$), such that for each, the intact striatum was contralateral to the edge. When controls were subdivided according to their left/right asymmetry in striatal DA turnover, they too oriented differently ($p<0.015$), with the striatum of greater DA activity being contralateral to the edge. DA activity in other regions was unrelated to edge behavior, emphasizing the importance of striatal DA in directing behavior. Because attraction to, or exploration of edges appear to be regulated at behavioral and neurochemical levels, this suggests that edges are significant stimuli in the organization of behavior. Supported by NSERC.

483.14

INVOLVEMENT OF GLUTAMATE RECEPTORS IN THE ZONA INCERTA IN AMPHETAMINE-INDUCED STEREOTYPY BUT NOT LOCOMOTOR ACTIVITY. D.E. Supko*, N.J. Uretsky, L.J. Wallace. College of Pharmacy, The Ohio State University, Columbus, OH 43210

A pathway projecting from the nucleus accumbens (NA) to the subpallidum and on to the pedunculopontine nucleus (PPN) has a role in locomotor activity (LMA), including amphetamine (AMPH)-induced hypermotility. It is reported that the descending neurons projecting from the subpallidum to the PPN synapse first at the level of the zona incerta (ZI) and glutamate neurotransmission in the ZI may be involved in motor activity. We have found that activation of non-NMDA receptors by direct injection of AMPA or kainic acid into the ZI stimulates locomotion, which is attenuated by the specific antagonist DNQX. Considering this, we investigated the role of non-NMDA receptors in the ZI in AMPH-induced LMA. Injection of DNQX (4 nmol) into the ZI had no effect on the locomotor response to low dose AMPH (0.5 & 1.0 mg/kg, SC). DNQX, however, increased by 3 fold the locomotor response to high dose AMPH (10 mg/kg, SC) and attenuated stereotypy induced by both AMPH and apomorphine. The augmented locomotor response was likely due to an inhibition of AMPH-induced stereotypy which competes with LMA. Ibotenic acid lesions of the ZI produced effects similar to DNQX administration. Thus, non-NMDA glutamate receptors in the ZI appear to be involved in mediating the effects of AMPH resulting from striatal activation but not NA activation.

483.15

DOPAMINERGIC INPUT TO THE SUBTHALAMIC NUCLEUS REGULATES LOCOMOTOR ACTIVITY IN THE PARKINSONIAN RAT. G. Flores*, D. Martínez-Fong and J. Aceves. Dept. of Physiology. CINVESTAV-IPN. Mexico.

Here we studied the role of the DA innervation of the subthalamic nucleus on locomotor activity (loc act.) 6-OHDA was injected bilaterally into the pars compacta. Shams: saline. Injected site was assessed hitologically. DA content was measured in striatum (str), accumbens (acc) and subthalamic nucleus (STN). Two groups of rats could be distinguished, one with decreased (from 21 ± 2 to 7 ± 2 counts/10 min), and another with increased (from 23 ± 2 to 69 ± 6) loc act. No change (before: 23 ± 2; after: 24 ± 2) in loc act was seen in shams. Injected sites in hypokinetic rats were rostrally, and in hyperkinetics ones they were caudally located in pars compacta. DA (ng/mg prot.) contents were (mean ± SEM; n = 6):

nucleus	hypokin.	hyperkin.	sham
Str	3.7±1.0	4.6±0.8	100±4.8
acc	27±15	16±5	82±3.0
STN	0.6±0.1	3.7±0.1	3.7±0.2

Hypokinesia was associated with loss of DA input to subthalamus. Data suggest that STN is normally under a tonic inhibitory DA action via a nigrosubthalamic pathway. Loss of this action, disinhibits the STN, producing hypokinesia (or akinesia).

483.16

THE MOTOR EFFECTS OF UNILATERAL AND BILATERAL STIMULATION OF THE DEEP MESENCEPHALIC NUCLEUS AND SUBSTANTIA NIGRA IN THE RAT. D. ASDOURIAN. DEPT. OF PSYCH., WAYNE STATE UNIV., DETROIT, MI 48202.

Unilateral stimulation of either the deep mesencephalic nucleus (DpMe) or substantia nigra reticulata (SNr) in anesthetized animals (60 mg/kg pentobarbital) drives activity in the trapezius pars cervicalis and rectus capitis dorsalis major. Both muscles typically respond bilaterally to unilateral stimulation of DpMe or Snr. Each experimental session consisted of two phases. During the first phase stimulation was delivered unilaterally to DpMe or SNr as single square waves having a duration of 1.0 msec and intensities ranging between 0.5 and 2.0 mA. Muscle response amplitudes (measured electrophysiologically) were maximal at a stimulation frequency of 1/4 sec and declined steadily as the frequency was increased to 1/3 sec, 1/2 sec, 1/sec, and 15/sec. At frequencies 1/sec and 15/sec response amplitudes sometimes dropped to zero. During the second phase of the experiment, stimulation frequency was kept constant at 1/3 sec with stimulation duration and intensity as described above, however, stimulation was delivered bilaterally to DpMe or SNr with delays between stimulation of one side of the brain and the other. So, for example, stimulation of the left DpMe was followed, with a delay of 5.0 msec, by stimulation of the right DpMe. The delays used were 5.0, 10.0, 15.0, and 20.0 msec. Muscle activity was recorded bilaterally in response to both the first and the second stimulus. The major effect of this procedure was that the bilateral responses elicited by the second (delayed) stimulus were augmented at a delay of 5 msec, but were inhibited at delays of 10, 15, and 20 msec. The inhibition became deeper as the delay was increased. Future work should investigate the pathways and nuclei over which the two types of inhibition seen in the two phases of this experiment are mediated.

483.17

IN VIVO SUBREGIONAL EFFECTS OF AMPHETAMINE AND CORTICAL STIMULATION IN THE RAT CAUDATE PUTAMEN. G.E. GLYNN and B.K. YAMAMOTO. Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44263.

A topographic projection from the cortex to the striatum exists in the rat. The effects of dopamine on the electrophysiology of this corticostriatal projection are controversial. The purpose of this study was therefore to determine the relative electro-physiological importance of these projections and the regional role that dopamine plays in modulating this striatal input.

An 8 electrode array of graph-epoxy voltammetric electrodes, each simultaneously measuring multiple unit activity (MUA) and dopamine, was placed at two depths in the striatum. 8 cortical sites were repeatedly stimulated. Amphetamine was administered and its effects on dopamine and MUA monitored. Overall there was a 103% greater dopamine concentration increase in dorsolateral as compared to dorsomedial regions, a 52% greater increase in ventrolateral as compared to ventromedial and a 56% greater increase in dorsal as compared to ventral regions. A rostrocaudal gradient was also evident. A distinct regional pattern in the striatal electrophysiological response to each cortical stimulation site was evident. Data will be presented on the effects of dopamine concentration changes on this electrophysiology response within 14 subregions of the striatum.

CONTROL OF POSTURE AND MOVEMENT III

484.1

EVIDENCE FOR CHANGES IN LENGTH-TENSION CHARACTERISTICS IN SPASTIC MUSCLE. J.P.A. Dewald, A.C. Shen*, S.L. Delp*, D.C. Lin, and W.Z. Rymer. Sensory Motor Performance Program, Rehabilitation Institute of Chicago, Chicago, IL 60611.

In an effort to characterize length-tension relations in spastic muscle, torques produced over a range of elbow angles in the impaired versus unimpaired upper extremity were compared in hemiparetic stroke subjects. In the first part of this study, a biceps RMS EMG matching routine was used to ensure a constant biceps activation level at each elbow angle. EMGs from the remaining elbow flexors were recorded to check for variations in their activation, which could affect torque. The upper limb was placed in 90° abduction at the shoulder, and the elbow angle was varied from 120° to 15° flexion, lengthening the biceps approximately 7 cm. Torques were measured using a 3 d.o.f. loadcell. Four of five subjects showed marked changes in elbow torque-angle curves. The joint angle at which torque peaked shifted 20-30° to more extended elbow positions in the hemiparetic arm. In addition, the torque-angle curves were less steep for extended elbow positions in the impaired limb. Since moment arms in both arms are presumed identical in each subject, changes in the length-tension relation in spastic muscle are implied.

To further substanciate the above findings, electrical stimulation of the biceps muscle was used in the second part of this study. This allows for a more controlled activation of elbow flexors provided one stimulates both arms using the same current intensity. Surface stimulation of the biceps was performed using 3 ms pulses delivered at a frequency of 20 Hz for 500 ms during which torque was measured, followed by a 2 min. rest period. Four of four subjects showed shifts in the angle of peak torque to more extended elbow angles and thus concur with the findings observed during voluntary activation. In both parts of the study normal subjects did not show a shift in the angle of peak torque or a change in shape of the torque-angle curves when comparing their upper limbs.

The fact that torque-angle changes were observed in both voluntary and electrical stimulation paradigms points to possible changes in the length-tension property in the impaired limb. This may be caused by increased compliance of the series elastic element, increased tendon length, changes in fiber length, or other adaptations of the muscle-tendon complex. This work is supported by NIH grant NS-19331.

484.2

INTERSPIKE INTERVALS OF MASSETER SINGLE MOTOR UNITS DURING INCREMENTED BITE FORCES ON MOLARS, CUSPIDS AND INCISORS. B. Bishop, T-K. Mao, W. D. McCall, Jr. Depts. of Physiology & Oral Med. SUNY at Buffalo, NY 14214.

Is more masseter (MA) activity required to generate a given force when biting on incisors than when biting on molars? To answer this question we built splints for each of 4 subjects (Ss) to keep jaw separation at 10 mm and MA length constant whether the bite-transducer was between molars, cuspids or incisors. Fine wires in the right MA detected unit activity. Ss generated clenches of 15, 30 or 60 N while viewing the transducer output. From continuous recordings, relationships between firing rates and forces were determined for each bite location and clench strength. Interspike intervals (ISIs) were shorter for any given force when clenching on incisors than on molars. During a clench at each bite position, changes in ISIs and small, concomitant force fluctuations were seen. Rate coding was more pronounced when biting on incisors than on molars. Increasing bite force lead to recruitment of new units and a decreased mean ISI in every unit. We conclude that to maintain the same force at the incisors as at the molars more MA activity is required.

484.3

EFFECTS OF DORSAL COLUMN LESIONS ON MOTOR CONTROL AND LONG-LATENCY RESPONSES IN THE INDEX FINGER OF MACAQUES D.S. Glendinning and C.J. Vierck, Dept. of Neuroscience, Univ. of Florida, Gainesville, FL. 32610

These experiments were designed to test the hypotheses that afferents carried in the dorsal columns (DCs) 1) are needed to regulate the forces of finger movements, and 2) form the afferent limb of long-latency stretch reflexes. Three stumptailed-macaques were trained to hold the index finger stationary (within a ± 5° range) for 3 seconds. In one condition ("move and hold"), they flexed or extended the finger 20°, at the metacarpophalangeal joint, to reach the target zone. In a second condition ("hold"), the finger was positioned in the target zone at the start of the trial. Resistive loads were applied to the finger through a torque motor. The loads were either constant or varied from 0.3 - 1.0 Nm. The monkeys had to resist these torques to maintain the finger position. To elicit stretch reflexes, 500 ms. torque pulses were generated by the torque motor while the finger was holding against a small force. Electromyographic (EMG) activity was measured with surface electrodes placed over the forearm flexors and extensors, and over the first dorsal interosseous muscle. EMG records were rectified, integrated and averaged . The patterns of muscular activity were compared for each condition. Following the lesions, the monkeys were able to hold the finger against many of the resistive loads in the "hold" conditions, but performance deteriorated in the "move and hold" condition. Long-latency responses varied and were related to the severity of the behavioral deficit. These results suggest that afferents in the DCs are not necessary for force regulation of the fingers during postural holding, but are required to regulate muscular activity during active movements. Supported by MH09734 and NSS17474.

484.4

EFFECTS OF ALTERING VISUAL AND PROPRIOCEPTIVE INFORMATION DURING VISUOMOTOR TRACKING. S.H.Brown, J.D.Cooke, T.Ohtsuki*, Depts. of Physical Therapy and Physiology, University of Western Ontario, London, Ontario, Canada N6A 5C1

Gauthier et al (1988) have suggested that co-ordination of eye and arm movements relies heavily on limb proprioceptive information. We have studied the linkage between visual and limb afferent signals by disrupting their normal temporal and spatial relations during visuomotor tracking tasks.

Subjects performed sinusoidal movements about the elbow (frequency 0.1 - 1.5 Hz) in a pursuit tracking task or in time with an auditory cue. Visual feedback was altered by introducing a delay (0 - 400 ms) between actual and displayed arm positions. Proprioceptive information was altered by vibrating the biceps and/or triceps tendon. Horizontal eye movements were monitored by EOGs or an infrared detection system.

Under all conditions, saccadic and smooth pursuit eye movements remained time linked to the displayed arm position. Visual delay and/or vibration had no apparent effect on the time course of either type of eye movement. In contrast, both visual delay and vibration caused disruption in the arm movements with low frequency oscillations (0.4 - 1.0 Hz) superimposed on the tracking frequency. The severity of this disruption varied inversely with the tracking frequency and was greater during pursuit tracking than when movements were made in time with the auditory cue. The combination of visual delay and tendon vibration produced the most severe movement disruption.

Eye and arm movements can thus be temporally dissociated during visuomotor tracking. Eye movements do not rely on limb proprioception whereas limb movements are dependent on both visual and proprioceptive information. Supported by NSERC (Canada) and the Ontario Ministry of Health.

484.5

USING THE c-fos IMMUNOHISTOCHEMICAL METHOD TO LOCALIZE BRAINSTEM CELLS ACTIVATED DURING LOCOMOTION X.Dai, J.R.Douglas, B.R.Noga, J.I.Nagy and L.M.Jordan, Dept.of Physiology, University of Manitoba, Winnipeg, M.B., Canada R3E 0W3

Locomotion can be induced by electrical stimulation of the mesencephalic locomotor region (MLR) in the decerebrate cat. The MLR has been shown to project to the medial aspect of the pons and medulla (Steeves & Jordan, Brain Res., 307:263-276, 1984) where it is thought to produce locomotion by activation of cells located within the medial reticular formation (Noga et al., J. Neurosci., in press). Using the c-fos immunohistochemical method to label the nuclei of activated cells, we have previously investigated the distribution of labelled interneurons in the spinal cord following stimulation of the MLR (Dai et al., Soc. Neurosci. Abstr., 16:368.4). Here, we report on the distribution of labelled cells found within the brainstem of cats induced to walk by stimulation of the MLR.

Experiments were performed on cats decerebrated at the precollicular-postmamillary level. Data was obtained from cats induced to walk on a treadmill (n=2), from cats exhibiting fictive locomotion with stimulation of the MLR (n=2) or without stimulation of the MLR (n=2). Prominent labelling was found in the: a) magnocellular tegmental field; b) locus caeruleus; c) marginal nucleus of the brachium conjunctivum; d) cuneiform nucleus; e) ventrolateral reticular formation; and, f) the vestibular nuclei. Non-paralysed animals which were not subjected to the locomotor task (n=2) showed labelling of cells in the same areas but fewer cells were labelled compared to animals subjected to the MLR-evoked treadmill locomotion. In fictive locomotion preparations, larger numbers of labelled cells were found in cats that were induced to walk with stimulation of the MLR compared to animals which showed either spontaneous locomotion (n=1), locomotion produced by forelimb swinging (n=1) or in animals which served as non-locomoting controls (n=3). Supported by Medical Research Council of Canada and Rick Hansen Man in Motion Legacy Fund.

484.6

SPINAL INTERNEURONS SYNAPTICALLY ACTIVATED BY STIMULI APPLIED TO THE MESENCEPHALIC LOCOMOTOR REGION. L.M. Jordan and B.R. Noga, Department of Physiology, University of Manitoba, Winnipeg, Manitoba, Canada, R3E 0W3

Few lumbar spinal interneurons have been described which show activation by stimuli applied to the mesencephalic locomotor region (MLR). To date, this category includes only mid-lumbar group II muscle spindle afferent-activated interneurons (Shefchyk et al., Exp. Brain Res. 80:290, 1990) and contralaterally-projecting laminae VIII interneurons (Jankowska and Noga, Brain Res. 535:327, 1990). Regions of the spinal gray matter showing activity-dependent labelling by c-fos and transneuronal WGA-HRP due to MLR-evoked locomotion (Dai et al. Soc. Neurosci. Abstr., 16:889, 1990; Noga et al., SN Abstr., 13:826, 1987) or negative field potentials evoked by stimulation of the MLR (Fortier et al., SN Abstr.14:264, 1988) have now been identified and these areas have been searched in order to identify rhythmically active interneurons which receive short-latency input from the MLR.

Experiments were conducted on paralysed precollicular-postmammillary decerebrate cats with stimulation of the MLR (30-220 μA, 1.0 ms, 10-20 Hz) used to elicit fictive locomotion. Stimulating electrodes were placed at low thoracic levels to exclude ascending tract and propriospinal neurons from the sampled population. In mid-lumbar (L4-5) spinal segments, rhythmically active and MLR-activated spinal interneurons were found in laminae VIII and medial laminae VII. In caudal segments (L6-7), rhythmically active and MLR-activated neurons were localized throughout laminae VII and IX (laminae VIII not examined). The average latencies for activation ranged from 4.4-8.1 ms. In general, latencies of activation were shorter for neurons in mid-lumbar than caudal lumbar segments and for ipsilateral as opposed to contralateral MLR stimulation sites. Most cells tested showed polysynaptic activation by afferents of ipsilateral sural, superficial peroneal or saphenous nerves and occasionally produced late, long-lasting discharges in response to cutaneous nerve or MLR stimulation. Responses from various muscle nerve afferents were also observed. These results show synaptic activation of spinal neurons with latencies required to mediate short-latency MLR-evoked postsynaptic potentials in lumbar motoneurons (Shefchyk et al. J. Neurophysiol., 53:1345, 1985). Supported by Medical Research Council of Canada.

484.7

TRANSPORT KINEMATICS TO DOWELS OF DIFFERENT SHAPES. P.L. Weir[1] and C.L. MacKenzie[2], Dept. of Kinesiology, [1]University of Windsor, Canada, N9B 3P4 and [2]University of Waterloo, Canada, N2L 3G1.

Weir and MacKenzie (1990) examined the effects of task intent on the kinematics of prehension when reaching for a dowel that had been instrumented for quantifying grasping forces. While the grasping forces were found to be appropriately scaled for different tasks (lifting versus placing), no effect of task intent was found on the relative timing characteristics of the free motion phase. Marteniuk et al. (1987) had earlier reported that such a manipulation of task intent did influence the kinematics of the free motion phase. It was hypothesized that the lack of effect in Weir and MacKenzie (1990) could have been the result of using a dowel with square gripping plates, since Marteniuk et al. (1987) had used cylindrical dowels.

The purpose of the present study was to compare the effects of task intent on the kinematics of the free motion phase when grasping for different shaped dowels. The two dowels had identical cylindrical bases, but one dowel (the 'force' dowel) had flat, square gripping plates, while the other dowel (the 'cylindrical' dowel) had a curved gripping surface. Trials were blocked for the two dowels (weighing 155 grams), and the orders of presentation of the dowels and tasks (lift versus place) were counterbalanced across subjects.

Spatio-temporal analyses of a marker placed on the wrist (transport component) revealed that the decelerative portion of the free motion phase was longer when reaching to grasp the cylindrical dowel than the force dowel. In addition reaching for the cylindrical dowel was significantly influenced by task intent as evidenced by a differential lengthening of the deceleration portion for the lift task. No such effect was found for the force dowel. Once the subjects had acquired a stable grasp, dowel shape did not influence the transporting of the dowel to the target location. These findings suggest that dowel shape does influence the free motion phase of prehension, and may obscure task effects if the dowel has a large contacting surface. (Supported by NSERC)

484.8

MOVEMENT VARIABILITY ASSOCIATED WITH DIFFERENT SHAPED DOWELS. C.L. MacKenzie[1] and P.L. Weir[2]. Dept. of Kinesiology, [1]University of Waterloo, Canada, N2L 3G1 and [2]University of Windsor, Canada, N9B 3P4.

When reaching to grasp a dowel, the transporting of the arm is influenced by the shape of the grasping surface (Weir and MacKenzie, 1991). A longer percentage of time is spent in deceleration during the free motion phase when reaching to a dowel with a flat, square gripping surface as compared to a dowel with a curved gripping surface. The purpose of the present study was to examine the reasons for this difference. The two dowels were equated for weight (155 grams) and had cylindrical bases. The one dowel had square, flat gripping plates and was instrumented to quantify grasping forces, while the second dowel had a cylindrical top and a curved grasping surface. The amount of surface area available for grasping was equated between the two dowels.

Ink was applied to the pads of the thumb and index finger so that the amount of surface area used when contacting each dowel could be assessed. The area used was substantially smaller for the curved gripping surface than the flat. Given that a difference in contacting area was found, we hypothesized that this might reflect the terminal accuracy of the transport and grasp components. A difference in terminal accuracy may account for the lengthening of the deceleration phase observed by Weir and MacKenzie (1991), as it corresponds to the 'precision effect' reported by MacKenzie et al. (1987) and Marteniuk et al. (1987, 1990). Indeed, the variability of the wrist and index finger markers at dowel contact was greater for the flat gripping surface. In addition the variability in the wrist marker measured over the free motion phase was greater when moving to grasp the flat gripping plates. This increased variability occurred later in the free motion phase when the arm and hand were quite close to the dowel. These data suggest an influence of dowel shape on variability measures associated with both the arm and hand, reflecting the tight coupling between the two components. (Supported by NSERC)

484.9

Do Common Postural Synergies Regulate Upright Stance During External and Voluntary Perturbations? II. Biomechanical Patterns. E.M. Earl and J.S. Frank. Department of Kinesiology, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1.

Electromyographic (emg) analyses have been used to demonstrate that a common muscle synergy may serve to regulate stance posture in the presence of either voluntary or external perturbations (Frank and Earl, 1991). Emg analyses reflect the CNS drive which effects a compensatory adjustment, but kinematic and kinetic analyses are required to predict the body movements which stabilize stance. The purpose of this study was to conduct a biomechanical comparison of the compensatory strategies used to cope with external and voluntary perturbations. Subjects were instructed to maintain handle position against a 20 N background load, acting in the anterior direction. Voluntary perturbations were created when subjects pulled the handle against the load. External perturbations were generated by imposing a step increase in the handle force (100 N/300 ms). Ground reaction force data and kinematic data were combined in an inverse dynamic solution to calculate net moment and power profiles for the ankle, knee and hip joints. Net joint moments reflect the sum of the muscle forces acting about a joint; joint power indicates the energy absorption and generation which occurs at a joint.

Invariant compensatory actions, as illustrated by the net joint moments, were used to cope with both types of perturbations: predominant ankle plantarflexor moments were accompanied by smaller knee flexor and hip extensor moments. Joint power profiles revealed energy absorption at the ankle; between and within subject differences in energy absorption and generation were observed at the knee and hip joints. Anterior displacements of the center of pressure observed for the external perturbations exceeded the magnitude of those observed during the voluntary disturbances. Center of mass excursions differed depending on the source of the perturbation. (This research supported by NSERC Grant #OGP0022785).

484.10

Do Common Postural Synergies Regulate Upright Stance During External and Voluntary Perturbations? I. EMG Patterns. J.S. Frank and E.M. Earl. Department of Kinesiology, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1.

Upright stance is perturbed by external forces, as well as reactive forces arising from our own voluntary movement. We examined whether common postural synergies might subserve the regulation of upright stance under these two conditions when the direction of destabilization was the same. Subjects were instructed to grasp and maintain the position of a handle against a 20 N background load. Voluntary perturbations were created when subjects pulled or pushed the handle against the resisting background load. External perturbations were generated by imposing a step increase in the handle force (100 N, 300 ms) either forward or backward. The latency and magnitude (first 75 ms) of activation of 6 postural and 2 focal muscles were recorded: medial gastrocnemius, tibialis anterior, biceps femoris, rectus femoris, erector spinae, rectus abdominus, triceps brachii, and biceps brachii.

Voluntary displacements of the handle were preceded by activation of a sequence of postural muscles with a mean onset latency of -83 ms (focal muscle onset = 0 ms). External displacements of the handle triggered a stabilizing postural reaction with a mean onset latency of 85 ms. During forward destabilization, both postural adjustments accompanying voluntary movement and triggered postural reactions were characterized by predominant activation of posterior muscles. The first muscle activated consistently was the medial gastrocnemius; the order of activation of biceps femoris and erector spinae varied both between and within subjects. The pattern of muscle activation accompanying backward destabilization revealed greater variability. These findings provide some evidence for the use of common muscle synergies for the regulation of upright stance during forward destabilization imposed by external and voluntary perturbations. (This research supported by NSERC #OGP0022785)

484.11

CHARACTERISTICS OF FOOT TRAJECTORY WHILE GOING OVER OBSTACLES DURING LOCOMOTION. A.E. Patla and S. Rietdyk*. Department of Kinesiology, University of Waterloo, Waterloo, Ontario, Canada, N2L 3G1

Foot trajectory during the swing phase of normal unobstructed locomotion is primarily dictated by passive pendular dynamics of the limb and is characterised by minimal ground clearance (~1 cm) at very high velocity (~ 4 m/s). The focus of this study is to characterise the changes in foot trajectory when obstacles of different heights and widths are encountered at the normal minimal clearance position in the step cycle. Kinematics, ground reaction force data, and EMG signals from four ipsilateral limb muscles were collected and analysed while subjects (N=7) avoided obstacles placed in their path and maintain their step length.

The results revealed that the trajectory is modulated for height changes but not the width, provided the width of the obstacle does not force subjects to alter their step length. The average clearance of the toe over the obstacle was ~10 cm, with four subjects increasing the clearance for higher obstacles. The horizontal toe velocity while going over obstacles was reduced more than the hip velocity as a function of obstacle height, while the hip position was further back and closer to the contralateral limb. Subjects landed with an increase in vertical velocity, while reducing the horizontal velocity. These modifications serve to ensure stability and safety of the subject. The scaling of the trajectory for different heights was not achieved through simple scaling of muscle activity or ground reaction forces. This study provides insight into the critical information required for obstacle avoidance during navigation in a cluttered environment. (Supported by a grant from NSERC, Canada)

484.12

EFFECTS OF COMPLEXITY AND PRACTICE ON MOTOR PROGRAMMING. Hanneke van Mier (1,2), Wouter Hulstijn (2) 1= Dep. of Neuropsychology, School of Medicine, Washington Univ., St. Louis, MO 63110, 2= Nijmegen Institute for Cognition Research and Information Technology (NICI), Univ. of Nijmegen, The Netherlands.

A large number of reaction-time studies have shown that the initiation of more complex movements requires more advance programming than simpler movements, thereby increasing initiation time. In handwriting experiments, however, increases in initiation time as a function of the number of strokes tend to be very small or nonexistent. This may be caused by the well-practised nature of letter writing and the level of advance programming.

To investigate this, an experiment was done in which subjects had to copy, as quickly as possible, stimuli consisting of three categories. Not only letters but also figures and patterns were used, consisting of familiar figures and novel nonsense (unfamiliar) patterns both of which were rarely or never drawn before. These stimuli were presented on a computer screen; writing and drawing movements were recorded by means of an XY-tablet.

Initiation time was found to increase linearly with the number of strokes -- which was varied from four to ten -- but the effect was much larger for figures and patterns than for letters and rapidly decreased with practice (successive presentations). In order to try to eliminate a difference in initiation time on account of perceptual processing, the same stimuli were presented, but had to be written and drawn in another style, which differed only in motor complexity (the number of strokes had to be doubled by requiring the subject to draw each line twice).

Drawing double lines increased initiation time with increasing number of strokes significantly for the figures and patterns. For letters, the increase was irrespective of the number of strokes. These results suggest that the planning of a movement sequence occurs at several levels and that the level is highly influenced by the amount of motor practice.

484.13

MODULATION OF STRETCH REFLEXES BY SURFACE ELECTRICAL STIMULATION IN SPINAL CORD INJURED AND NEUROLOGICALLY INTACT SUBJECTS. B. Flaherty[1,2], C.J. Robinson[1,3], G.C. Agarwal[2] [1]VA Rehabilitation R&D Center, Hines, IL; [2]Bioengr. & EECS Depts., Univ. of Illinois at Chicago, Chicago, IL; [3]Neurology Dept., Loyola Univ., Maywood, IL

The effect of sub-maximal surface electrical stimulation on the stretch reflexes of spinal cord injured (SCI) (n=2) and neurologically intact (NI) (n=2) was studied by simultaneously stimulating the soleus muscle and applying rapid 20° dorsiflexion perturbations to the ankle. The stimulator was turned off for 100 msec at the onset of the perturbation so the M-waves would not interfere with evoked response. Stimulation was monophasic with a frequency of 20 Hz and a pulse width of 400 μsec, with current adjusted so that about 2 Nm of plantarflexion torque was developed in three of the four subjects. The rectified and averaged surface soleus EMG was used to measure the evoked reflex responses. Seven stimulated and seven relaxed trials were presented in random order interspersed with six non-perturbed trials. The perturbation was provided by position controlled torque motor driven footplate so that all the perturbations would be nearly identical (see Flaherty et al, Soc. Neurosci. Abstr. '90).

The EMG response consisted of a short latency (40 - 70 ms after start of perturbation) burst and longer latency (70 - 100 ms) burst of activity. In two subjects (one SCI, one NI) the short latency response was markedly decreased with stimulation, in the other NI subject it was unchanged. One SCI subject, who could not generate any torque with stimulation, showed no change in EMG response. Two subjects (one SCI, one NI) showed an increase in long latency response with stimulation while in the other NI subject it was unchanged.

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484.14

NONLINEAR DYNAMIC (CHAOTIC) DIMENSIONS OF MESENCEPHALIC LOCOMOTOR REGION (MLR) NEURONS DURING LOCOMOTION. K.L.Turner*, R.D.Skinner and E. Garcia-Rill. Department of Anatomy, University of Arkansas for Medical Sciences, Little Rock, AR 72205.

Locomotion is a rhythmic biological process which has parameter dependent transition points (gait changes). The locomotion generating system, then, should have characteristics of a nonlinear (chaotic) system which include an attractor with fractal dimension. The attractor is a phase space construct of the dynamics of a system as derived from a time series of data. The dimension of a system, then, is the number of parameters necessary to describe its attractor. An estimate of this dimension for MLR neurons was obtained using the Grassberger-Procaccia method. Interspike intervals were obtained from locomotor related (burster-each step and tonic-locomotor episode) neurons in the cat pedunculopontine nucleus, a part of the MLR, during spontaneous fictive locomotion using methods previously reported (Neurosci. 27:639, 1988). Two burster and two tonic neurons have been analyzed to date. The fractal dimensions were 3.6 and 3.7 for the burster cells and 5.1 and 5.5 for the tonic cells. The difference in the dimensions of the attractors of these two types of neurons suggests that during locomotion the firing pattern of the burster neurons is more coherent than that of the tonic. This is indicated by the lower fractal number of estimated parameters needed for a description of their behavior. Thus, tonic neurons display greater variability and may have a greater influence on the transition points of the locomotor pattern, which may be related to a greater variety of synaptic inputs and/or to intrinsic membrane properties.

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484.15

QUADRUPEDAL GALLOP: THE UNSTUDIED GAIT. J.L. Smith, S.H. Chung, J.A. Buford, and R.F. Zernicke, UCLA Kinesiology, Los Angeles, CA 90024-1568.

Galloping at moderately high treadmill speeds (3-4 m/s) is difficult to elicit in the cat and has not been well assessed. We trained cats to gallop at these speeds and quantified hindlimb dynamics (for methods see Smith and Zernicke, *TINS* 10: 123-128, 1987) and EMG of selected muscles (see Buford and Smith, *J. Neurophysiol.* 64: 756-766, 1990). We focus here on limb dynamics during the swing phase (69%) of the step cycle and action requirements for bifunctional posterior thigh muscles, such as semitendinosus (ST), that extend the hip and flex the knee as well as anterior thigh muscles, such as rectus femoris (RF) and anterior sartorius (AS), that flex the hip and extend the knee.

At the knee joint in early swing, an extensor muscle torque is needed to counterbalance a large flexor torque created by leg angular acceleration (LAA). But, during the walk and trot, active muscles provide a flexor torque and there is no need for an extensor muscle torque because LAA is relatively small. At the hip joint in early swing, LAA creates an extensor torque. In early swing, therefore, muscles that flex the hip and extend the knee must counter LAA; both requirements can be satisfied by activation of the RF or AS.

Later in swing, passive muscle forces appear responsible for decelerating forward motion of the limb associated with LAA, because muscles with hip extensor and/or knee flexor functions are not active before hip extension and knee flexion begin. During the walk and trot, active muscle torque from posterior thigh muscles (*e.g.*, ST) decelerates forward limb motion prior to paw contact, but during the gallop, ST activity occurs just at or after knee flexion begins and is associated with rapid knee flexion and hip extension prior to paw contact. Our results suggest that swing phase limb dynamics during the gallop, in contrast to the walk and trot, require different demands of active bifunctional muscles. These changes have not been considered by those who emphasize only the need to change interlimb coordination to control the gallop. Research supported by NIH NS 19864.

484.16

CONTEXT, PHASE, AND LOCATION DEPENDENT RESPONSES TO HIND PAW STIMULATION DURING THE STEP CYCLE. J.A. Buford and J.L. Smith. Neuromotor Control Lab, Dept. Kinesiology, UCLA, L.A., CA 90024-1568.

Our previous studies comparing backward (BWD) and forward (FWD) walking revealed differences in hindlimb kinematics, especially at the hip and knee joints (Buford et al. 1990). Yet underlying muscle synergies were similar, with flexors active in swing and extensors in stance (Buford and Smith 1990). Is sensory input modulated differently for BWD walking even though motor output is grossly similar?

Others have demonstrated phase- and location-dependent modulation in cutaneous reflexes (Forssberg 1979, Drew and Rossignol 1986), especially for stumbling corrective reactions. What about context-dependent modulation? A ventral stimulus in early swing would be an obstacle for BWD walking but not for FWD. Do responses to such stimuli differ for BWD and FWD walking? We recorded EMG from hindlimb muscles of cats trained to walk BWD and FWD on a motorized treadmill (0.4-0.6 m/s). Stimuli (1 ms pulse; ~1 mA) were applied at various phases of the step cycle through subcutaneous electrodes implanted at the dorsal and ventral aspects of the paw. EMG, stimulus records, and ciné data were recorded.

We focus here on semitendinosus (ST), a muscle that bursts at the start and end of FWD swing but is active throughout swing for BWD. For dorsal stimuli, responses for BWD and FWD walking were similar. ST did not respond to stimuli delivered in the extensor period. For stimuli delivered in the flexor period (Flx), ST displayed two waves of facilitation, one from ~10-25 ms and a second from ~30-50 ms post-stimulus. Perhaps ST responses programmed to avoid dorsal obstacles for FWD swing are retained for non-obstructing dorsal stimuli in BWD swing.

For ventral stimulation, responses were context-dependent: for stimuli delivered in early Flx, the first wave was inhibitory for BWD walking but excitatory for FWD. ST inhibition in early BWD swing may help avoid a ventral obstacle. Thus, responses to hind paw stimulation were influenced by the direction of walking. We attribute this context dependency to different modulation of sensory input despite similar motor ouput. NIH NS19864 & the Foundation for Physical Therapy.

484.17

HINDLIMB STANCE KINETICS DURING BACKWARD WALKING: UNEXPECTED FINDINGS. K.L. Perell*, R.J. Gregor, M.M. Ryan*, J.A. Buford, and J.L. Smith, Dept. of Kinesiology, UCLA, Los Angeles, CA 90024-1586.

Based on results from hindlimb kinematics and EMG (see Buford *et al., J. Neurophysiol.* 64: 745-755, 1990; Buford and Smith, *J. Neurophysiol.* 64: 756-766, 1990) extensor moments were predicted for the hip and knee during backward (BWD) stance. Combined with hip flexion and knee extension, extensor moments would lead to power (P = Moment x angular velocity) generation at the knee and absorption at the hip. Movement kinematics and EMG, however, are insufficient to test such predictions, and forces and torques that cause such motion must be determined (see Smith and Zernicke, *TINS* 10: 123-128, 1987). As a result, kinetic analyses were done on 2 cats trained to walk BWD through a plexiglass enclosed walking containing 2 concealed force platforms capable of measuring center of pressure (COP) and ground reaction force (GRF). For apparatus decription see Fowler *et al.* (*Neurosci. Abst.*, 1987). We focus here on the response of the muscle synergies to the external demands created by the GRF and posture during BWD stance.

During the BWD stance phase, the GRF is directed vertically upward and anterior to the knee joint. This creates a need for ankle plantarflexor, knee flexor, and hip extensor muscle moments. Power analyses show generation at the ankle and absorption at the knee and hip. The knee flexor moment was unexpected because the vastus lateralis (VL) is active as the knee extends during stance. The lateral gastrocnemius (LG) activity, however, increases as VL activity declines during stance. With the VL shortening and decreasing in activation, the LG and its bifunctional synergists may have the mechanical advantage necessary to produce the knee flexor muscle moment. Our results suggest that the bifunctional capability of the LG is exploited by the nervous system to control the unique demands of BWD walking. Research supported by NIH NS 19864.

484.18

A COMPARISON OF IPSILATERAL AND CONTRALATERAL CUTANEOUS REFLEX RESPONSES DURING HUMAN RUNNING. A.A.M. Tax*, J. Duysens, C.C.A.M. Gielen, M. Trippel* and V. Dietz* Dept of Medical Physics & Biophysics, Univ of Nijmegen, 6500 HB Nijmegen, The Netherlands and Dept of Clin. Neurology & Neurophysiology, Univ of Freiburg, D-7800 Freiburg i. Br., Germany.

By electrically stimulating the human sural nerve at the ankle one can obtain both ipsilateral and contralateral low threshold reflex responses with a latency of about 80 ms in the Biceps Femoris (BF) and the Tibialis Anterior (TA). The modulation of these reflexes during the step cycle is studied in volunteers running on a treadmill at a speed of 8 km/h.

Both ipsi- and contralaterally, BF shows large responses during stance, while near the end of swing the responses of both BF and TA are reduced on either side. Because the size of these reflex responses does not tightly covary with the background activity during the step cycle a premotoneuronal gating mechanism is proposed. In addition, the similarity of the modulation of the ipsi- and contralateral reflex responses shows that the gating of cutaneous reflexes in these muscles depends on the phase of the corresponding leg in the step cycle and not on the phase of the stimulated leg.

484.19

PERIPHERAL CONTROL OF ELEVATOR MUSCLE ACTIVITY DURING SIMULATED CHEWING IN MAN.
F.A.M. Ottenhoff*, A. van der Bilt*, H.W. van der Glas and F. Bosman. Dept. of Oral Pathophysiology, University of Utrecht, Padualaan 14, 3584 CH, Utrecht, The Netherlands.

The Additional Muscle Activity (AMA), which is required to overcome the resistance of the food during chewing, was investigated. Subjects made rhythmic open-close movements at their natural chewing frequency, while food resistance was simulated by a computer controlled external force. Sequences of cycles with a force were randomly alternated with sequences with a larger, a smaller or without force. Jaw gape, the applied force and the surface EMG of the masseter and temporal muscles were recorded. The AMA adapted to a larger, a smaller or no force within 30 ms after the force started to differ from the force in the previous cycles. However, when the force was applied for the first time after a sequence without force, the AMA started 130 ms after the onset of the force. Thus, the central nervous system is able to generate jaw elevator muscle activity within 30 ms when a force is expected, irrespective of the amplitude of the force actually applied. A mandibular load (food resistance) during a chewing cycle may enable the use of short, fast pathways in the following chewing cycle.

484.20

SELECTIVE ACTIVATION OF HUMAN SOLEUS OR GASTROCNEMIUS IN REFLEX RESPONSES DURING WALKING AND RUNNING. J.Duysens, A.A.M.Tax*, M.Trippel* and V.Dietz.*
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During the stance phase of running of the cat, stimulation of skin, innervated by the sural nerve, elicits responses in gastrocnemius (MG) with a latency of 25 ms, while such responses are absent in soleus (SOL, Duysens and Loeb,1980). In man, some evidence for a differential recruitment of these muscles was obtained in stretch responses, but not for sural stimuli and not during locomotion (Nardone et al.,1990).

Therefore, the phase-dependent modulation of MG and SOL responses to sural nerve stimulation was studied using a 20 ms train of 5 electrical pulses, applied to the sural (or tibial) nerve at the ankle, in 14 volunteers walking or running on a treadmill. Although both the spontaneous activity and the reflex responses were usually similar for both muscles, instances were identified in which separate control was evident. During walking (4 km/h), activity in SOL started earlier in the stance phase than GM activity. Correspondingly, the amplitude of the reflex responses was larger in SOL than in GM in early stance, both ipsi- and contralateral to the side of stimulation. In some cases, the same stimulus could elicit contralaterally a suppression of GM in synchrony with a facilitation of SOL (latency between 72 to 105 ms). During running (8 km/h or more), responses were seen selectively in GM instead (82 to 158 ms latency), without concomitant responses in SOL, during the first extension phase at the end of swing. The independence from the level of background activity suggests premotoneuronal gating.
(Supported by SFB 325)

484.21

ARE THERE COMMON PROCESSING CONSTRAINTS FOR VISUOMOTOR AND PERCEPTUAL MENTAL ROTATIONS? G. Pellizzer*, J.T. Massey, H. Bains*, and A.P. Georgopoulos. Bard Laboratories of Neurophysiology, Department of Neuroscience, The Johns Hopkins Univ. School of Medicine, Baltimore, MD 21205.

The hypothesis of a mental rotation of a movement vector when a movement has to be made at an angle from a stimulus direction is supported by the results of behavioral (Georgopoulos & Massey, Exp. Brain Res. 65: 361, 1987) and neural studies (Georgopoulos et al., Science 243: 234, 1989). Does this process share common constraints with the mental rotation of visual images (Shepard & Cooper, Mental Images and their Transformations, 1982)? To answer this question, we took advantage of the fact that mental rotation rates (reaction time vs. angle) may vary considerably among subjects: If common constraints underlie the spatial transformations involved in these tasks, then a correlation should be observed between the rates of mentally rotating visual images and those of mentally rotating a movement vector. We found that, indeed, these rates were significantly correlated ($p < 0.001$, N = 39 naive human subjects); in contrast, mental motor rotation rates did not correlate with rates (reaction time vs. number of movements in a memorized list) observed in a context-recall visuomotor memory scanning task (Georgopoulos & Lurito, Exp. Brain Res. 83: 453, 1991) which does not involve mental rotation. These results suggest that perceptual and visuomotor mental rotation tasks possess common processing constraints. (Supported by NIH, ONR and HSFP.)

484.22

PLANAR SEGMENTATION OF FORCE TRAJECTORY IN 3-D ISOMETRIC CONDITIONS. J.T. Lurito, G. Pellizzer*, J.T. Massey, and A.P. Georgopoulos. Bard Labs., Dept. of Neuroscience, The Johns Hopkins Univ., Sch. of Med., Baltimore, MD 21205.

Soechting & Terzuolo have shown (Neuroscience 23: 39, 1987) that the plane of motion of the wrist in free space may change abruptly between segments of the trajectory and be almost constant within each segment; this change of plane occurs usually at the points of inflection of the trajectory and may be a consequence of a segmentation at the level of motor programming. It was also shown that, for a given plane, the phase relations among upper- and fore-arm angular elevation are kept constant, which suggests that they may be part of an internal coordinate system for planning of movement trajectory. It can then be worth to analyze the plane of force trajectory in the absence of overt joint motion. In our experiment, subjects exerted forces with an unrestrained arm and the hand pronated on a 3-D isometric handle which controled the position of a visual cursor in a stereographic display (Massey et al., J. Neurosci. Methods 26: 123, 1988). They were asked to trace lemniscates (figures "8") in frontal or sagittal planes. We found that the planar orientation of the force trajectory often changed by more than 20 degrees between the two loops of the lemniscate, which by definition are divided by a point of inflection. These results are similar to those of Soechting and Terzuolo above and suggest that a common constraint might underlie planning of motor trajectory in space, whether or not it involves motion about joints. (Supported by NSF and ONR.)

484.23

MODELING OF POSTURAL ADJUSTMENTS DURING MULTI-ARTICULAR PULLS MADE BY STANDING HUMANS. W.A. Lee and C.F. Michaels*, Physical Therapy and Northwestern University Institute for Neuroscience, Northwestern University, Chicago IL 60611 and Psychology, Lake Forest College, IL 60045.

The control mechanisms by which standing subjects organize postural adjustments when they exert abrupt, bilaterally symmetrical near-maximal pulls on a handle in the sagittal plane are not well understood. We will present a kinematic and kinetic analysis of the kinematic and kinetic degrees of freedom of the task which helps explain empirically observed invariances and variations in body motion (postural adjustments). The analysis shows that the task has two kinematic degrees of freedom which can be expressed as the anterior-posterior (ap) and vertical (v) coordinates of the center of mass (CMap and CMv). The task also has three kinetic degrees of freedom (two Static; one Inertial); two of these are related to CMAP parameters. Six well-practiced subjects used qualitatively invariant proportions of Static and Inertial Components of pulling force (computed from force platform, load cell and kinematic data) and demonstrated invariant CMap phase planes during near-maximal pulls (~95% maximum). In contrast, CMV and joint angle motion varied substantially. The invariance in CMap motion appears to reflect the strong kinetic constraints on CMap revealed by the biomechanical analysis; variability in body motion is associated with the less constrained movement of CMv. We will present a preliminary dynamic model of how CMAP motion and horizontal pulling force are related. The model consists of an inverted pendulum acted upon by the force of gravity, a force due to a torque about the axis of rotation generated by a virtual spring between the pendulum and the support surface and a force due to an elastic cord connected to the pendulum mass. Simulated CMap phase planes compare well with the empirical data for near-maximal pulls. The model suggests that subjects may generate pulling movements by controlling dynamic parameters (e.g., stiffness; resting length) rather than torque parameters (timing, height and durations of impulses). We will present data from well-practiced subjects to determine if the model also predicts body motions during sub-maximal pulls. We predict that scaling the "resting length" of the elastic cord to peak pulling force will generate changes in CMap trajectories which match those which subjects actually produce.

484.24

PLANNING AND EXECUTION OF LIMB MOVEMENT IN CAT LOCOMOTION. L. Shen and R.E. Poppele. Dept. of Physiology, Univ. of Minnesota, Minneapolis, MN 55455

The cat hindlimb is a redundant mechanical system for producing planar limb movement in locomotion. The object of this study was to understand issues related to the control of this system. Assuming controls are realized at joint level, the problem then is to relate movements as specified in limb coordinates (limb length and orientation) and ground coordinates (foot position relative to ground) to movements in joint coordinates (limb segment orientation).

Cat hindlimb movements were recorded in unrestrained over ground locomotion for a variety of surface conditions and speeds (0.5-2.0 m/s). An analysis of movement kinematics revealed that: 1) the orientation of limb segments at touchdown relates linearly to whole limb orientation; and 2) trajectory of hind paw can be regulated by adjusting the timing of movements of limb segments. We propose therefore that different variables are used to control the trajectory of hind paw in the step cycle and the orientation of limb at touchdown, namely the timing across movement of segments and the segment orientation. For limb movements in locomotion, this control scheme provides a simple and adequate solution to the two most difficult problems in control of such redundant systems, namely trajectory planning and inverse kinematics. An interesting consequence of this control scheme is that regulation of the main features of hind paw trajectory is realized without planning the details of trajectory.

Supported by NIH grant NS 21143.

484.25

SEQUENTIAL MOVEMENTS IN TYPING. J.F.Soechting and M.Flanders. Dept. of Physiology, University of Minnesota, Minneapolis, MN 55455.

Last year we described the hand and finger movements of subjects who typed a single letter with one hand, while typing words in which the rest of the letters were typed with the other hand. For each letter (and subject) we found a characteristic pattern of movement (flexion-extension and rotation in the horizontal plane) that was independent of the movement of the other hand. Typically, this pattern comprised coordinated movements of all four fingers, beginning as much as 250 ms before the keystroke and lasting at least 500 ms. We have now extended this study to a description of the hand motion while subjects typed pairs of letters with one hand, embedded in words in which the rest of the letters were typed with the other hand. Up to the keystroke of the first letter, the kinematics of the movement do not depend on any subsequent movements; however, immediately after the first keystroke, the trajectory of hand motion for pairs of letters begins to diverge from the reference trajectory for a single letter. We have also found evidence that the planning of movement sequences in typing involves "look-ahead" processing, in the sense that the trajectory immediately after the first keystroke is altered even when there are as many as three letters typed with the opposite hand before the second keystroke of the pair. Therefore, the execution of skilled movements in typing exhibits serial aspects (the second movement of one hand does not begin until the first keystroke has been executed) as well as parallel aspects ("look-ahead" processing).

484.26

ARM POSITIONS WHEN POINTING IN A LARGE WORKSPACE. J. Hore, S. Watts* and T. Vilis. Dept. of Physiology, University of Western Ontario, London, Ont. Canada.

We and others have previously reported that when subjects point or grasp in a restricted workspace (e.g. 50 deg) the positions the arm adopts appear to follow an analog of Listing's law. This law states that with the head stationary the eye when fixating only assumes those positions that can be achieved by a single rotation about an axis that lies in a plane with zero torsion. Thus when positions of the arm were described in terms of a rotation from a reference position, the axes of these rotations lay in a plane-like surface. However further study of pointing movements over a larger (90 deg) range makes it clear that the axes surface is not flat but is consistently twisted like that associated with rotations around 2 axes of a Fick gimbal. The axes surface had a similar twisted shape whether subjects were pointing at targets or moving between them and whether subjects were pointing with or without vision.

Thus, although as previously reported, the arm shows major constraints in position when pointing, it does not accurately follow Listing's law. Instead the positions it adopts are similar to those of the eye in space in combined head-eye movements (Vilis, Glenn, Tweed this meeting).

485.1

THE MUSCLE SYNCYTIUM OF ASCARIS AND CONTRACTILE WAVE PROPAGATION. S. Solórzano*, J. Serrato*, C. Argüello* and A. Rivera. Dept. of Physiology, CINVESTAV-IPN, México City, DF, México.

The neuromuscular system of Ascaris suum consists of longitudinally oriented muscle cells that give off processes to contact the nerve cords. The prolongations from many cells intertwine at the surface of the cords to form the muscle syncytium or sarcopile. At this level the muscle cells exhibit myogenic activity, form neuromuscular junctions and are coupled by gap junctions. The possible role of gap junctions in contractile wave propagation is not yet known. Our data from scanning microscopy of the syncytium show the arms approaching the nerve cord in bundles and each bundle corresponds to arms from one or two adyacent rows of cells. Intracellular recordings show that the electrical coupling between cells is more efficient in narrow circular bands perpendicular to the longitudinal axis than along the length of the animal. We suggest that synchronization of electrical activity and contractile wave propagation is due in part to sequential activation of well coupled functional units of contraction (cell rows) that are perpendicular to the longitudinal axis. Thus, longitudinally oriented cells may present circular patterns of contraction as a consequence of the gap junction coupling between them.

Supported in part by NS-26127 (NIH), D111-902024 and 55306 (CONACyT, México).

485.2

A LASER CONFOCAL MICROSCOPIC STUDY OF THORACIC INTERSEGMENTAL INTERNEURONS IN THE MOTH, *MANDUCA SEXTA*. K. A. Mesce and K. A. Klukas*. Dept. of Entomology and Graduate Program in Neuroscience, University of Minnesota, St. Paul, MN 55108.

A population of intersegmental interneurons has been identified whose cell bodies are located in the adult pterothoracic ganglion and whose axons descend posteriorly through the unfused abdominal ganglia. Particular neurons within this population are important in controlling adult-specific ecdysis behavior and regulating selective neuronal death.

A variety of retrogradely transported neuronal tracers have been used to determine the number of neurons present, their morphologies and projection patterns. Smaller molecular weight tracers, such as Cobalt-Lysine and Biocytin, were found to label a number of putative 2nd order interneurons. These neurons were not filled, however, with higher molecular weight tracers such as Dextran Biotinylated Lysine and Dextran Tetramethyl Rhodamine, suggesting the occurence of trans-neuronal dye transfer through gap junctions.

To visualize small fluorescently labeled interneurons (somata less than 10 um in dia.) above background ganglionic autofluorescence, we used various dyes of cyanin derivation, some of which are not yet commercially available. Neuronal fluorescence was much increased above background by using a Bio-Rad Krypton/Argon laser to excite these fluorophores which had peak excitations at 647nm (red), far removed from excitation wavelengths in the blue and yellow ranges where autofluorescence is most apparent. We are currently examining whether these small intersegmental interneurons are generated *via* post-embryonic neurogenesis.

485.3

THE CONTRIBUTION OF VISUAL INTERNEURONS TO DIRECTION-SELECTIVE RESPONSES OF A FLIGHT MUSCLE. J.J. Milde, II. Zoologisches Institut, University of Cologne, Weyertal 119, D-5000 Koeln 41, F.R.G.

In the sphinx moth *Manduca sexta*, appropriate visual stimuli evoke compensatory body and limb movements. Intracellular stainings and recordings demonstrate that numerous brain interneurons responding to optomotor stimulation are present in the optic lobes (medulla, lobula and lobula plate) as well as in the posterior midbrain or the protocerebral bridge. To find out whether a recorded interneuron is really participating in behavior or not requires a simultaneous analysis of neuronal and motor responses.

In this study, the tonic unit of the mesothoracic third axillary muscle [Rheuben MB, Kammer AE (1987) J exp Biol 131:373], which has been shown to respond to visual stimuli even in non-flying moths [Dombrowski UJ (1991) Dissertation, University Cologne] is used as monitor for the motor output. The contribution of an interneuron to the motor activity is further evaluated by current injections. For example, a lobula plate neuron, which corresponds well with the muscle activity if the direction-selective responses are compared, can be shown by hyperpolarization to be obviously not involved in the generation of the muscle activity.

This approach is an important step towards an identification of neuronal elements underlying visually guided behavior.

485.4

SEGMENT-SPECIFIC FATES OF PROLEG MUSCLES AND MOTONEURONS DURING METAMORPHOSIS IN *MANDUCA SEXTA*. D.J. Sandstrom and J.C. Weeks. Graduate Group in Neurobiology, Univ. of Ca., Berkeley, CA 94720, and Inst. of Neuroscience, Univ. of Oregon, Eugene, OR 97403.

The abdominal prolegs of *Manduca* are larval locomotory appendages that are lost at pupation. In the proleg-bearing segments, A3-A6, the APR motoneurons (MNs) innervate the larval proleg retractor muscle, APRM. Apparent homologs of the APRs and APRMs are also present in the non-proleg-bearing segments, A1 and A2. To better interpret other experiments investigating metamorphic changes in synaptic inputs to the APRs [*J. Comp. Neurol.* (1991) **308**:311], we have examined these MNs and muscles in more detail. In larvae, each APRM is innervated by a pair of APRs, which appear identical by electrophysiological and anatomical criteria. Typically, the anterior fibers of an APRM are innervated by one APR and the posterior fibers by the other APR, with middle fibers being dually innervated. Synaptic potentials from the two APRs have similar amplitudes and shapes, and exhibit similar activity-dependent plasticity. During the larval-pupal transformation, the APRMs degenerate in all segments except A2 and A3, whereas the APRs die one day after pupation in all segments except A2, A3 and A4. The dendritic arbors of APRs in the different segments regress to a similar extent regardless of whether the APR, or its target muscle, is fated to die. The surviving APRs and APRMs persist through the pupal stage, with the APRs showing dendritic regrowth. During the first two days of adult life the APRs and APRMs degenerate, presumably after participating in adult emergence behaviors. Thus, the degeneration of the APRs and APRM is segment-specific, while the dendritic regression of the APRs is not.

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485.5

CALCIUM-DEPENDENT PLATEAU POTENTIALS IN THE LEG MOTONEURONS OF MANDUCA. R.B.Levine and J.M.Ramirez, Div. of Neurobiology, Univ. of Arizona, Tucson, AZ 85721

Larval leg motoneurons (MNs) in *Manduca* persist to innervate the new adult legs. We are interested in the intrinsic properties of these MNs that may be important for locomotor activity. Using whole-cell patch-clamp techniques in primary cell culture it was shown that the leg MNs express two types of putative Ca^{++} currents: the dominant current has a low-threshold (-40 mV) and is slowly inactivating, the smaller one has a higher threshold and is resistant to inactivation (Levine and Hayashi, S.N.Abs. 1990). To examine whether these Ca^{++} currents reflect properties expressed *in situ*, intracellular recordings were obtained from leg MNs in intact ganglia. Following perfusion with saline containing TTX and TEA, plateau-like potentials could be evoked with short depolarizing current pulses and could be terminated prematurely with short hyperpolarizing pulses. Two components of the plateau-like potentials were distinguished:(1) transient, large amplitude potentials evoked at negative DC offsets and suppressed at more depolarizing offsets, (2) smaller, persistent potentials activated from depolarizing offsets. The plateau-like potentials occurred only in the presence of TEA and disappeared following perfusion with normal saline. They were TTX-insensitive, enhanced by replacing Ca^{++} with Ba^{++}, and suppressed by Co^{++} and Ni^{++}. The significance of these potentials for normal function is unclear, but they may augment rhythmic synaptic input during locomotion, perhaps after being revealed by neuromodulatory substances.

485.6

REORGANIZATION OF SENSORY REGULATION OF LOCUST FLIGHT AFTER PARTIAL DEAFFERENTATION. A.Büschges, R.Driesang, J.M.Ramirez and K.G.Pearson. Dept. of Physiology, University of Alberta, Edmonton, Canada.

Previous investigations have shown that the flight motor pattern of the locust relies heavily on activity in the hindwing tegulae but not on activity in the forewing tegulae. Removal of the hindwing tegulae results in a decrease of the wingbeat frequency by about 20% and an increase in the interval between the hindwing depressor and elevator activity by about 50%. However, these changes are not permanent since the flight motor pattern returns to normal during a period of about two weeks after the operation. This recovery is due to a functional substitution of the hindwing tegulae by the forewing tegulae. The following physiological changes in the connections of the forewing tegulae were found to be associated with recovery: 1) the probability of the forewing tegulae connecting to flight interneurons was significantly increased (e.g for interneuron 566 it increased from 0.58 (n=12) to 1.0 (n=16)), and 2) the average amplitude of the compound EPSP evoked in elevator interneurons by electrical stimulation of the forewing tegula afferents was significantly increased. This increase in amplitude was due to previously unconnected forewing tegula afferents developing synaptic contacts onto the elevator interneurons during the period of recovery. These physiological changes were paralleled by sprouting of the forewing tegula afferents in the metathoracic ganglion and by sprouting of the dendritic processes of flight interneurons.

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485.7

THE PROBLEM OF RELIABILITY IN INSECT NEUROMUSCULAR CONTROL.
Jim H. Belanger and Ian Orchard. Department of Zoology, University of Toronto, Toronto, Ontario, Canada M5S 1A1.

The force which a muscle is capable of generating is proportional to its cross-sectional area, while the mass which it must accelerate is proportional to the volume of the organism. This means that, compared to vertebrates, insect muscles are relatively 'oversized'. It also implies that, in order to achieve a given level of control over its output (muscle tension), the insect neuromuscular system must exercise a greater degree of control over its input (motoneuronal signalling). We wanted to discover: 1. How reliably does a given insect neuromuscular system transduce a given motoneuronal input?; 2. What roles (if any) are played in maintaining reliability by neuromodulatory substances?

We approached this question using an *in vitro* preparation we have developed based on the locust (*Locusta migratoria*) ovipositor opener muscle. This preparation allows simultaneous monitoring of motoneuronal input, muscle electrical activity, and muscle force generation while the muscles are being driven spontaneously by a central pattern generator located in the terminal abdominal ganglion (K.J. Thompson, *J. Exp. Biol.* ***122***:387, 1986). In spontaneously active preparations, EMG activity is significantly correlated with neuronal input, but neither of these is correlated with the force produced by the muscle. When the spontaneous input is removed (by removing the ganglion), electrical stimulation of the opener nerve reliably predicts both the EMG and the force produced by the muscle, but only if single impulses are given. If trains of pulses are given, the summed EMGs are not significantly correlated with muscle force, even when the same population of axons is recruited with every stimulus. We are currently examining the possibility that proctolin acts to reduce this variability between axonal input and muscle output.

485.8

MOTOR PATTERNS IN COCKROACH LEGS DURING WALKING ON A SLIPPERY SURFACE.
F. Delcomyn. Department of Entomology and Neuroscience Program, University of Illinois, Urbana, IL 61801.

Intact cockroaches (*Periplaneta americana*) show a stereotypic pattern of activity in leg muscles during free walking. After amputation of a leg, free-walking insects show a pattern of motor activity that differs from normal mainly in the shifted timing of bursts in the leg in front of the amputated one during slow walking (Delcomyn, J. Exp. Biol. 156, 483-517). When suspended intact insects walk on a slippery surface, the pattern of motor activity is like that shown by intact insects walking freely. Amputation of a leg in suspended insects produces a motor pattern somewhat similar to that seen in free-walking amputee insects. However, this pattern has a reduced shift of timing of bursts in the leg in front of the amputated one, and a strong shift in timing of bursts in the leg behind it during slow walking. The differences between the motor patterns seen in free-walking and suspended amputee insects are likely due to the mechanical uncoupling of the legs during suspended walking. It is inferred that sensory feedback from leg mechanoreceptors is normally used by the leg of origin as well as by adjacent legs to coordinate motor activity.
Supported by a grant from the Whitehall Foundation.

485.9

NEURAL ACTIVITIES OF THE MUSHROOM BODIES OF THE COCKROACH DURING LOCOMOTORY BEHAVIOR.
M. Mizunami and N. J. Strausfeld. Arizona Research Laboratories, Division of Neurobiology, University of Arizona, Tucson, Arizona 85721.

In insects, two discrete sensory pathways supply premotor descending neurons, one via deutocerebral interneurons, the other via higher centers (mushroom bodies, MBs) in the protocerebrum. In 1960, Huber (Z. Vergl. Physiol. 44: 609-632), using electrical stimulation, concluded that MBs control complex behavioral repertoires. We have refined experimental techniques to re-examine this hypothesis.

Neural activities from MB neurons of the cockroach, *Periplaneta americana*, were recorded while animals were walking freely, using wire recording and staining techniques which reveal intact neurons at the vicinity of the electrodes (Mizunami & Strausfeld, Soc. Neurosci. Abstr. p759, 1990). Tactile stimulations were used to induce locomotory behaviors. Units from MBs were classified into (1) sensory units responding to mechanical stimulation of the body, (2) arousal units which exhibit long-lasting bursts after tactile stimulation, (3) locomotory units. Units recorded at MB input regions (the calyces) of MBs were exclusively sensory, whereas output regions (pedunculi and lobes) were characterized by arousal and locomotory units. Focal electric stimulations at the pedunculi and lobes induced complex locomotory sequences.

Although MBs are clearly indicated in spatial memory processing (see accompanying abstract; Weibrecht et al, 1991), and although lesions of MBs perturb this property, bilateral lesions of MBs do not perturb spontaneous motor actions. This suggests that motor activity per se can be independent of the participation of MBs and that MBs may specifically govern acquisitive motor repertoires. **Supported by NIH grant F05 TW 04390.**

485.10

THE MUSHROOM BODY OF COCKROACH BRAIN PARTICIPATES IN SPATIAL MEMORY PROCESSING.
J. M. Weibrecht, M. Mizunami and N. J. Strausfeld. Arizona Research Laboratories, Division of Neurobiology, University of Arizona, Tucson, Arizona 85721.

Social insects, such as bees and ants, can use complex spatial cues for orientation and navigation in environmental space. Here we present evidence suggesting that (1) cockroaches have a well developed spatial memory and (2) mushroom bodies (MBs) participate in the processing of spatial memory.

The ability of cockroaches to use spatial memory to identify a covert target was tested using the "Tenessee Williams" paradigm: cockroaches were placed in an arena, the floor of which was noxiously hot (50-55•C) except for a small invisible cool area (target). The wall of the arena was decorated with either visual patterns or olfactory cues having specific geometrical relationships with respect to the target. After five trials, cockroaches reached the target significantly faster with such cues than in their absence.

Effects of MB lesions on the ability to reach the target using spatial cues were tested. Small pieces of aluminum foil (width: 150-200 μm, length: 350 μm) were implanted into a selected area using relevant surface features. Subsequent histology determined the location and extent of lesions. In tests using visible targets, cockroaches with bilaterally lesioned MBs reached the target as fast as unoperated cockroaches (5 trials). When the target was invisible, however, the time taken for MB-lesioned cockroaches to reach the target was significantly longer than in unoperated cockroaches (5 trials). Unilateral MB lesions, or lesions of neuropils adjacent to MBs, did not significantly affect spatial learning, suggesting that MBs are indeed involved in spatial memory processing. **Supported by the Howard Hughes Undergraduate Biology Research Program and NIH grant F05 TW 04390.**

485.11

STRUCTURE OF OUTPUT NEURONS OF THE MUSHROOM BODIES IN HONEYBEE BRAINS.
B.A. Hurley*, W. Gronenberg, and N.J. Strausfeld, Arizona Research Laboratories, Division of Neurobiology, University of Arizona, Tucson, AZ, 85721 USA

Mushroom bodies (MBs) are protocerebral neuropils implicated in learning and memory [Erber J, Masuhr T & Menzel R (1980) Physiol Entomol 5:343-358; Mizunami M & Strausfeld NJ (1990) Soc Neuro Sci Abstr 16:759]. Each MB comprises a pair of calyces supplying a pedunculus branching into an α and ß lobe. Palisades of K-cells in the calyces receive a distributed afferent representation of olfactory, visual, and mechanosensory centers. This arrangement has been interpreted as reflecting multimodal sensory integration at K-cells [Mobbs PG (1982) Phil Trans Roy Soc Lond B. 298:309-354] whose axons go on to supply the pedunculi and lobes beneath. However, little is known concerning the pathways and targets of pedunculi and α-ß lobe outputs. The structure, arrangement, and targets of neurons leaving the α and ß lobes has now been analyzed using a modified Golgi technique and cobalt iontophoresis. Serially sectioned neurons were reconstructed using camera lucida at high power. In each α lobe, output neurons ("extrinsic neurons"; ENs) have elaborate dendritic arborizations characteristically interacting with specific configurations of the many thousands of parallel K-cell axons. Most EN axons extend around the α lobe and then terminate in specific premotor nuclei of the deutocerebrum. However, a substantial population of ENs project back to the calyces, comprising a "feedback" loop. Such reafferent supply may be important for memory acquisition, permitting information processed by the lobes to cycle back to their afferent supply (K-cells) for further processing [Schürmann FW (1974) Exp Brain Res 19:406-432]. **Supported by the Center for Insect Science (NSF DIR 82-20082) and NSF BNS 9011012**

485.12

THE MODULATION OF WING PHASE DURING TURNING RESPONSES OF DROSOPHILA.
M.H. Dickinson. Dept. of Organismal Biol. and Anatomy, Univ. of Chicago, Chicago, IL 60637.

The turning response of Drosophila to visual stimuli consists of several behavioral components including abdominal steering, leg position, and alteration of wing-beat amplitude. Here, I report an additional mechanism: the modulation of wing phase.

At the end of the downstroke flies rapidly rotate their wings before initiation of the upstroke. This rapid rotation is termed the ventral reversal. The timing of the ventral reversal can be precisely measured using pairs of photo-diodes (Götz, 1987). During optic stimulation in the open loop condition, flies actively modulate the timing of the ventral reversal such that the wing on the outside of the turn is in advance of the wing on the inside of the turn. The amplitude of the wing phase modulation is on the order of 5°. The phase response is much greater during stimulation with a single vertical black stripe (figure), than with oscillation of a large field pattern (ground). Wing phase modulation is greatest during oscillation frequencies near 1 Hz.

This work was kindly supported by the Max Planck Institute for Biological Kybernetics.

Götz(1987) J. Exp. Biol. 128,35

485.13

A NEURAL MODEL FOR OPTOMOTOR CONTROL AND THE LANDING REACTION IN FLIES. H. Öğmen. Dept. of Elect. Eng., University of Houston, Houston, TX 77204.

We present a neural model for the general optomotor control problem and test its predictions on a well-documented paradigm, viz. the landing reaction of the fly. The front-end of the model consists of elementary motion detectors (EMDs) (Öğmen & Gagné, *Neural Networks*, 3:487-505, 1990). EMDs are directionally selective and exhibit temporally adapting responses. The outputs of EMDs are pooled by wide-field directionally selective cells. The pooling takes into consideration the retinotopic position of EMD receptive field centers, their preferred direction axis, and binocularity. The pooling is an opponent process in that EMDs with opposite preferred directions undergo a shunting competition. Moreover, this opponent competition has sensitization and habituation properties. In addition to visual inputs, the wide-field integration cells receive other sensorial inputs such as mechanoreceptor outputs from legs and antenna. These sensorial inputs undergo habituation at their originating site before they modulate the pooling process. Finally, the opponent agonist antagonist outputs from the integration stage affect muscles controlling visually guided behaviors. The model will be compared with alternative models. It will be shown that there is a good agreement between model predictions and data from the landing reaction of flies.

485.14

NEURONAL CONTROL OF OPTOKINETIC NYSTAGMUS IN FLIES Cole Gilbert, Wulfila Gronenberg, and Nicholas J. Strausfeld. Center for Insect Science and Arizona Research Labs, Div. Neurobiology, Univ. of Arizona, Tucson AZ 85721

In insects, panoramic or small-field visual stimuli elicit optomotor head and body movements which stabilize the visual surround or the image of tracked objects on the retina. In tethered flies we optoelectronically quantified head yaw movements elicited by moving contrast gratings or small objects. These visually-induced saccadic head movements are suited to stabilize retinal images moving at velocities exceeding 3000°/s. Visual stimuli of the same spatio-temporal characteristics also elicit excitatory responses in 1) simultaneously extracellularly recorded motor neurons innervating the head/neck muscles which generate the observed behavior; 2) intracellularly recorded and dye-filled visual projection neurons, and 3) premotor descending neurons (DNs) which target the neck-muscle motor neurons. Recordings made with biocytin-filled electrodes often reveal dye-coupling between visual interneurons and DNs as well as between DNs and neck motor neurons. In the dorsolateral deutocerebrum of the brain these DNs are postsynaptic to 1) several wide-field motion-sensitive lobula plate neurons and 2) many motion- or flicker-sensitive small-field retinotopic elements originating in the lobula and lobula plate. Anatomical and physiological evidence suggests that each motor neuron integrates input from several DNs which, in turn, combine small- and wide-field visual input. We conclude that dorsal DNs integrate information about panoramic motion and movement of small objects and distribute this information to sets of neck muscle motor neurons which, in turn, control the muscles that produce the appropriate optokinetic head movements. **Supported by NIH Grant R01 EYO 7151-01, NSF Grant Dir 82-20082, and DFG Grant Gr 933 /2-1**

HYPOTHALAMUS II

486.1

DIVERGENT AXON COLLATERALS ORIGINATING IN THE RAT VENTROMEDIAL NUCLEUS OF THE HYPOTHALAMUS. S. Truitt, C. Ulibarri, and T.R. Akesson, Department of VCAPP, College of Veterinary Medicine, Washington State University, Pullman, WA 99164.

Cells of the ventrolateral part of the ventromedial nucleus of the rat (VMHvl) are perhaps the best characterized of neurons that influence female sexual behavior. Lesioning the VMH prevents and estrogen implants stimulate lordosis behavior. In addition to having their own estrogen receptors, neurons of the VMHvl also project to other steroid target nuclei, such as the medial preoptic nucleus (MPN), the medial nucleus of the amygdala (MeA), and the midbrain periaqueductal gray (PAG). One way in which a response to estrogen could be amplified is if an estrogen-receptive neuron sent collaterals to other nuclei involved in a particular function. This possibility prompted us to test for the presence of collateral projections by neurons of the VMHvl.

Female Long-Evans rats were iontophoretically injected in the PAG with a 3% aqueous solution of Fluoro-Gold using a 20 µm ID tip and 10 µa of positive current for 20 minutes. An incremental pressure injection (0.01-0.1 µl steps with 0.5-2 min pause to make a total of 0.05-0.5 µl) of a suspension of red and green latex spheres (LumaFluor, NY) were used to label the MPN and MeA respectively.

Ascending projection neurons (to MPN and MeA) were more numerous in the VMHvl than in central and dorsomedial parts of the nucleus but descending projection neurons (to PAG) were more uniformly distributed throughout the VMH. The vast majority of labeled cells in the VMH had only a single projection to either the MPN, MeA, or the PAG. A small but evenly distributed population of VMHvl neurons projected to both the MPN and MeA. In contrast, neurons which projected to the PAG were only occasionally found to also project to the MPN or MeA. Supported by HD22869.

486.2

A SUBPOPULATION OF NEURONS IN THE PARAVENTRICULAR NUCLEUS THAT PROJECT TO LOWER LUMBAR SPINAL CORD CONCENTRATE ^{3}H-ESTRADIOL IN THE MALE RAT. C.K. Wagner[1], L.G. Clemens[1] and C.L. Sisk[2]. Neuroscience Program and Departments of Zoology[1] & Psychology[2], Michigan State University, East Lansing, MI 48824

The paraventricular nucleus (PVN) of the hypothalamus sends projections to the lower lumbar spinal cord (*Brain Res.* 539:254, 1991). This level of the spinal cord is sexually dimorphic and contains the androgen concentrating motoneurons of the spinal nucleus of the bulbocavernosus and dorsolateral nucleus that innervate the sexually dimorphic perineal muscles involved in penile reflexes. PVN is known to play a role in the modulation of these reflexes. The purpose of the present study was to determine if neurons in PVN, that project to this region of the spinal cord, also concentrate steroids.

Sections through the PVN from male rats that had received injections of the retrograde tracer, Fluorogold, into the lower lumbar spinal cord, were prepared for steroid autoradiographic labelling using ^{3}H-estradiol. The results demonstrate that many neurons in the PVN of the male concentrate estrogen. The majority of these neurons are found in the anterior and lateral parvocellular subnuclei. Neurons that project to lower lumbar spinal cord are found primarily in the dorsal and lateral parvocellular subnuclei. A subpopulation of the neurons in the lateral parvocellular subnucleus that project to lower lumbar spinal cord also concentrate estrogen.

These findings suggest that androgen concentrating motoneurons of the lower lumbar spinal cord may receive steroid sensitive afferent input from the PVN.

Supported by HD26483 and HD06760.

486.3

HORMONE-SENSITIVE SEXUALLY DIMORPHIC NEURONS IN THE ANTEROVENTRAL PERIVENTRICULAR NUCLEUS PROJECT TO THE ARCUATE NUCLEUS OF THE HYPOTHALAMUS. B.S. Reinoso and R.B. Simerly. Division of Neuroscience, Oregon Regional Primate Research Center, Beaverton, OR 97006.

The anteroventral periventricular nucleus of the hypothalamus (AVPv) is a nodal point in the neural circuitry that controls ovulation in rodents (See Simerly and Swanson, J. Comp. Neurol. 270:209 for review). We previously determined that this important nucleus contains 3-4 times more dopamine cells, and approximately 4 times as many cells that contain prodynorphin (PDYN) mRNA in female rats, relative to male animals. Conversely, there are 2-3 times as many cells that express the opioid peptide precursor proenkephalin (PENK) in the AVPv of male rats compared with females; treatment of newborn female animals with testosterone reverses each of these neurochemical sexual dimorphisms. Moreover, sex steroid hormones appear to downregulate the expression of tyrosine hydroxylase in both male and female rats, and upregulate PDYN gene expression in female, but not male, rats. Thus, the maintenance of PENK cells, and the reduction in the number of hormone-sensitive dopaminergic and PDYN cells, in the AVPv may represent important developmental influences of perinatal androgens on the sexual differentiation of gonadotropin secretion. In the present project we used *in situ* hybridization in combination with retrograde transport and immunohistochemical methods to demonstrate that subpopulations of these sexually dimorphic neurons express estrogen receptors and project to the arcuate nucleus of the hypothalamus. These findings suggest a possible neural substrate whereby hormone-sensitive neurons in the AVPv provide sexually differentiated hormonal information to neurons in the arcuate nucleus, and may thereby influence the release of luteinizing hormone from the anterior pituitary.

Supported by NIH Grant NS26723.

486.4

ASYMMETRICAL VARIATIONS OF THE CHOLINERGIC SYSTEM OF THE PREOPTIC-HYPOTHALAMIC ANTERIOR AREA (PO-HAA) DURING THE OESTRUS CYCLE OF THE RAT. M.A. Sánchez*, J.C. López*, L. Huerto*, M.E. Cruz*, R. Tapia and R. Domínguez*. Instituto de Fisiología Celular, UNAM; Instituto Mexicano de Psiquiatría and Escuela Nacional de Estudios Profesionales Zaragoza, UNAM; Mexico.

It has been proposed that the PO-HAA participates asymmetrically in the ovulation process and a major role for its cholinergic system has been suggested. In this work, we studied the asymmetrical variations in muscarinic receptors and in choline acetyltransferase activity in both sides of the PO-HAA during the oestrus cycle of the rat. We observed that the activity of the enzyme varied along the oestrus cycle (Left: Oestrus (O)=32.6, Dioestrus 1 (D1)=24.3, Dioestrus 2 (D2)=30.1, Proestrus (P)=27.2; Right: O=39.1, D1=24.5, D2=20.5, P=26.6; nmol/min/mg protein). One way-ANOVA revealed that only the right side of the PO-HAA showed a significant variation during the cycle. Labeled n-methyl scopolamine binding constants also varied during the cycle, although only the Kd showed a marked asymmetry during the two dioestrus days (Left: D1=1.29, D2=0.95; Right: D1=0.4, D2=0.48; nM).

These data support the idea that the cholinergic system is involved in the asymmetric function of the PO-HAA in the ovulation process.

486.5

PRESENCE OF CALBINDIN (CB) AND LACK OF PARVALBUMIN (PV) IN PROGESTERONE RECEPTOR-CONTAINING (PR) NEURONS OF THE PRIMATE HYPOTHALAMUS T.L. Horvath,* F. Naftolin, C. Leranth. Dept. of Obstetrics & Gynecology, Yale University, School of Medicine, New Haven, CT 06510

In primates progesterone (P) decreases the frequency of luteinizing hormone (LH) pulses, but LHRH neurons do not contain PR. This suggests that P acts indirectly on the LHRH system. In the primate hypothalamus a large number of neurons contain PR and all of these cells are GABAergic (Leranth et al. '91). A large population of GABA neurons contain calcium-binding protein. In order to further characterize the PR-containing GABA cells we searched for their calcium-binding proteins. Experimental: Three out of 4 adult female ovariectomized vervet monkeys (*Cercopithecus aethiops*) received estradiol valerate. Thirty six hours later monkeys were sacrificed. Double immunostaining was performed for CB or PV and PR on hypothalamic sections. CB and PA was labeled by a Ni-DAB reaction, while PR with a DAB reaction. Results and Conclusion: In the ventromedial nucleus 41% of the PR cells were immunopositive for CB. The majority (78%) of the CB cells did not contain PR, and 59% of the PR cells did not exhibit immunoreactivity for CB. In the arcuate nucleus none of the PR cells were immunoreactive for CB. The hypothalamic PV cells, seen only in estrogen treated animals, did not contain PR. These results indicate that hypothalamic calcium-binding protein-containing GABA neurons can have different functions. These may be defined by their response to estrogen treatment and/or their PR-content. (Supported by NIH grants HD 23830, C.L. and HD 13587, F.N.)

486.6

TOPOGRAPHY OF HYPOTHALAMIC PROJECTIONS TO THE PERIAQUEDUCTAL GREY (PAG) IN THE RAT: A PHA-L STUDY. J.G. Veening*, L.M.G. Geeraedts*, G.J. Ter Horst**, P.G.M. Luiten** and T.A.P. Roeling*. Dept of Anatomy and Embryology, University of Nijmegen; **University of Groningen; The Netherlands. (SPON: European Neuroscience Association)

Descending projections have been studied in about 40 rats, with small iontophoretic injections of the anterograde tracer PHA-L in different parts of the hypothalamus. Projections from the paraventricular nucleus (PVN), dorsomedial nucleus (DMH), rostromedial and caudolateral parts of the ventromedial nucleus (VMH), intermediate hypothalamic area (IHA), ventrolateral and lateral hypothalamic area (LH) and the zona incerta (ZI), have been studied in detail. In addition, the structure of the PAG itself was investigated from its rostral, mesencephalic, to its caudal, pontine extension.

From all origins, mentioned above, fibers are distributed over the PAG in an intricate pattern of overlapping longitudinal columns. Rostrally, most projections, except LH, tend to converge in the dorsal PAG. More caudally, most descending fibers are present in the ventrolateral PAG, except the VMH-projection to the dorsal, the ZI-projection to the lateral and the LH-projection to the ventral PAG, including the dorsal raphe nucleus. Most caudally, some projections disappear (VMH, IHA), while others are mainly directed at the locus coeruleus (PVN, DMH, LH), or the adjoining nucleus of Barrington (ZI). Except for the projections from VMH and ZI, the central PAG is generally more densely innervated than the peripheral parts of the PAG.

486.7

THE NEURAL SUBSTRATE OF HYPOTHALAMICALLY INDUCED BEHAVIOURS IN THE RAT.
T.A.P. Roeling*, A.M.M. Van Erp*[1], W.H.M. Bressers*[2], E. Meelis*[2], M.R. Kruk*[1] & J.G. Veening*. Dept. Anatomy & Embryology, Univ. of Nijmegen, [1]Ethopharmacology Group, Univ. of Leiden, [2]Inst. for Theoretical Biol., Univ. of Leiden, The Netherlands.

On the basis of electrical brain stimulation, the hypothalamus of the rat has been divided into behavioural subregions. The anatomical correlates underlying this subdivision were investigated using local chemical brain stimulation for detecting the involvement of cell bodies and neuroanatomical tracers (PHA-L & HRP) for efferent and afferent connections.

Behaviour: A detailed analysis has been made of grooming behaviour elicited by microinjections into the hypothalamic paraventricular nucleus and adjacent dorsal hypothalamic area with glutamate agonists. Increases in boutlength and frequency of bouts were observed. The structure of the elicited grooming was similar to control situation. A brain cannula system was developed for experiments in a social environment. Microinjections into the subfornical region of the hypothalamus with bicuculline methiodide elicited digging, gnawing, agonistic behaviour and flight.

Anatomy: The efferent projections of the "grooming area" have been carefully mapped. Main projection areas are: lateral septum, medial amygdala, paraventricular thalamic nucleus, central gray, ventral tegmentum, locus coeruleus and nu. solitary tract. In comparison with other hypothalamic sites, many identical projection areas have been found. Within some of these areas, eg. septum and central gray, different spatial distributions can be distinguished.

486.8

THE POSTOPERATIVE HYPERACTIVITY OBSERVED FOLLOWING ELECTROLYTIC LESIONS OF THE LATERAL HYPOTHALAMUS IS ATTENUATED BY mCPP, BUT NOT HALOPERIDOL. T.R. Stratford and D. Wirtshafter, Univ. Illinois at Chicago, Dept. of Psychology, Box 4348, Chicago, IL 60680

Intense locomotor activity is often observed in rats awakening from anesthesia following electrolytic lesions of the lateral hypothalamus (LH). The hyperactivity usually persists for 2-3 hours and the magnitude of this phenomenon is often quite impressive. The mechanism underlying the locomotor activity may involve the non-specific release of dopamine at the target structures of damaged mesotelencephalic and/or mesolimbic axons which traverse the lesion site. In order to test this hypothesis we anesthetized rats with ether, lesioned the LH, and observed this postoperative activity in both an open field and in photocell activity cages following injections of the dopamine antagonist haloperidol (1.0 or 2.0 mg/kg, sc). Neither dose of haloperidol blocked the activity, although at the higher dose some jumping was introduced and the activity was directed increasingly in an upwards direction. In contrast, injections of another activity suppressing drug, the 5-HT agonist mCPP (1.0 or 5.0 mg/kg, sc), dose-dependently attenuated the activity, effectively blocking it at the higher dose. Therefore, it appears that dopamine release is not involved in the expression of this locomotor activity which can, however, be completely suppressed by mCPP.

486.9

EFFECTS OF REPEATED ELECTRICAL STIMULATION OF THE DORSOMEDIAL OR PARAVENTRICULAR NUCLEI ON GASTRODUODENAL EROSION FORMATION. D. Novin and N.S. Morrow. Depts. of Psychology and Psychiatry, Brain Research Institute, UCLA, Los Angeles, CA 90024.

Changes in the myoelectric activity of the stomach and the duodenum were recorded from freely-moving rats during electrical stimulation of the dorsomedial (DMH) or paraventricular (PVN) nuclei of the hypothalamus. Under pentobarbital anesthesia, a unipolar electrode was positioned stereotaxically and silver-chlorided recording electrodes were sutured underneath the serosa of the stomach and duodenum. Following postoperative recovery, rats were food deprived (16 h) and the DMH or the PVN stimulated (25-100 ua, 50 Hz, .5 ms) for 1 h; 3 stimulation sessions were given. Myogram activity was recorded preceding, during and for 2 h after stimulation. Rats stimulated in the DMH had significantly more gastric erosions than rats stimulated in the PVN. Duodenal damage was observed only in the PVN stimulated animals. Stomach and duodenal electrical activity was correlated with erosion formation. These results provide further support that changes in contractility mediate gastroduodenal damage.
(Supported by NIMH Fellowship Grant 5T32MH17140 and UCLA University Research Grant SF86.)

486.10

CENTROMEDIAL AMYGDALOID LESIONS ATTENUATE GASTRIC EROSION FORMATION DURING ACTIVITY-STRESS. N.S. Morrow[1], C.V. Grijalva[1], D. Novin[1], and P.J. Geiselman[1]. Depts. of Psychiatry[1], Psychology[1], UCLA, Los Angeles, CA 90024, & Dept. Psychology[1], Louisiana State Univ., Baton Rouge, LA 70803.

To examine the role of the centromedial amygdala (CMA) in gastric erosion formation and running activity, rats with electrolytic CMA lesions and sham-operated controls were housed in Wahman activity cages. Following a 3 day baseline period (food and water, ad libitum), rats were restricted to eating for 1 h each day for 21 days. Running activity, body weight, body temperature, and food and water were monitored daily. Rats with CMA lesions exhibited significantly less gastric erosions compared to sham-operated controls or rats with damage outside the CMA. No other significant differences were observed between groups. The results support the view that the CMA is a crucial structure for the maintenance of gastric mucosal integrity. The results also suggest that the extensive gastric damage normally viewed in activity-stress rats is not solely responsible for their eventual inanition and ultimate demise.
(NIMH Fellowship 5T32MH17140, NSF BNS-8709982, and UCLA University Research Grants SF86, PZ-06)

486.11

VASOPRESSIN INNERVATION OF THE MEDIAL PREOPTIC AREA IN GERBILS ORIGINATES IN THE SUPRACHIASMATIC NUCLEUS. B.J. Crenshaw, and G.J. De Vries. Dept. of Psychology and Prog. of Neurosci. and Behav., Univ. of Mass., Amherst.

This study investigated the origin of vasopressin-immunoreactive (AVP-ir) fibers in the medial preoptic area (MPOA) in the gerbil hypothalamus. The MPOA contains a sexually dimorphic area (SDA) that has a subgroup of cells--the SDA pars compacta (SDApc)--which is absent in females in Nissl-stained preparations. We previously found that AVP-ir fibers were clustered in a sexually dimorphic manner in the region that houses the SDApc in males: the clusters were much larger in males than in females. We wanted to determine whether these AVP-ir fibers came from the AVP cell bodies in the bed nucleus of the stria terminalis (BST), whose efferents to the SDA are sexually dimorphic, or from the suprachiasmatic nucleus (SCN). Animals in one group received cuts dorsolateral to the SDA and ventromedial to the BST, which would transect efferents from the BST. A second group of animals received cuts ventral to the SDA and immediately dorsal to the SCN, which would transect efferents from the SCN. We found that cuts dorsal to the SCN eliminated AVP-ir fibers to the medial SDA (mSDA) and the SDApc, whereas cuts ventromedial to the BST left intact the AVP-ir innervation of the MPOA. These results suggest that the mSDA and the SDApc receive their AVP innervation from the SCN.

486.12

SUPRACHIASMATIC NUCLEUS OF NORMAL HUMAN BRAIN. K.Arima*, B.S.Kolachana, M.F.Casanova*, T.M.Hyde, J.E.Kleinman. Clinical Brain Disorders Branch, NIMH Neuroscience Center at St.Elizabeths, Washington, D.C. 20032.

Despite its modulatory role on circadian rhythms, little is known about the human suprachiasmatic nucleus (SCN). In this study we analyzed 14 normal brains using serial Nissl stained coronal sections to establish the topography and cellular characteristics of the human SCN.

The SCN measured about 1-1.8 mm in length along its rostrocaudal axis, and about 1 mm in diameter in the central coronal plane. At the caudal levels of SCN, the supraoptic nucleus appeared laterally. Nissl stain revealed two types of neurons; one with nuclei of 7-10 um in diameter and dark granular nucleoplasm, the other type with nuclei of 10-14 um, prominent nucleoli and clear nucleoplasm. Both types of neurons were occasionally invaginated into the underlying optic chiasm.

Qualitative and quantitative analysis of neurons in the left and right SCN and in the subfields will be discussed.

HUMAN COGNITION: LANGUAGE, OTHER

487.1

EFFECT OF NOCICEPTIVE STIMULI ON TIME ESTIMATION. B.E. Thorn, P.L. Hansell* and S. Keith.* Dept. Psychology, Univ. AL, Tuscaloosa, AL 35487. The effect of cold pressor & ischemic pain on Ss response to unexpected time estimation requests was examined. Time estimation has been proposed to be based on stimulus complexity, attentional demand, or arousal in an interval (Ornstein, 1969; Thomas et al., 1975). Pain should increase complexity, attentional demand *and* arousal, but Ss experiencing headache *underestimate* duration of an interval (Isler et al., 1987). We reasoned that a specific goal (SG) given to Ss in pain would mitigate downward distortion of time judgments, but Ss with an unspecified goal (UG) would show the distortion. Results from cold pressor confirmed the hypotheses: At 120 sec, time estimations of the UG groups (M =70.7 +43) were lower than the SG group (M =105.3 +46.4), t(25) =2.33, p<.05 or the non-pain control groups. Results from the ischemic pain showed that at 360 sec, time estimations of the *SG* group (M =260 +63.4) were lower than the *UG* group (M =357 +218.7) or the non-pain control groups. Results suggest that type of nociception &/or time frame of the test are factors in understanding the interaction of pain & goals on time estimation.

487.2

LEVELS OF BINDING IN PERCEPTION AND COGNITION. E.Pöppel, K.Schill*, N.v.Steinbüchel*. Institut für med.Psychologie, Universität München, 8000 München, Goethestr. 31, FRG

Each perceptual or cognitive act is characterized by the simultaneous activity of several neuronal modules. Specific mechanisms are necessary to bind the distributed activities together. Furthermore, successive activities have to be linked together as well to account for perceptual reality. Five different kinds of binding on different levels can be distinguished: 1. spatial binding of identical features within one sensory quality (like the establishment of connectedness in the visual modality); 2. object binding within one sensory modality for different qualities (such as surfaces with colors); 3. object binding for several modalities (such as the establishment of one object by visual and auditory information); 4. temporal binding for separate temporal events (as necessary in motion or speech perception); 5. semantic binding for the content of consciousness (such as the establishment of continuity of thought that might be lost in schizophrenia). The different binding processes are predicted to be implemented by different neuronal algorithms as is suggested by special defects. Supported by DFG and BMFT.

487.3

EVENT-RELATED BRAIN POTENTIALS ELICITED BY ADDITION PROBLEMS. P. J. Holcomb, J. Allen*, S. Ballerini*, D. Rosner*, & J. Anderson. Dept. of Psychology, Tufts University, Medford, MA 02155.

Numerous studies have reported a late negative ERP component (N400) that is larger to semantically incongruent or unexpected words in visual and spoken language tasks. While waves with a similar time course and morphology have been reported for stimuli in other cognitive domains it is unclear to what degree this and similar ERPs are specific to language comprehension processes. The current study compared ERPs to correct and incorrect answers in a mental addition experiment.

Six subjects (3 male) were run in two blocks (words and digits) of a task (120 trials/block) where 3 to 5 numbers had to be summed prior to the presentation of a target answer number. Fifty percent of answers were the correct sum of the previous addition problem and 50% were incorrect. Subjects indicated whether the answer was correct or incorrect by rapidly pressing one of two buttons. Artifact free ERPs were recorded from 13 scalp sites to each answer number.

All answers were characterized by a large positive going component between 300 and 600 ms in latency. However, incorrect answers were less positive than correct answers between 275 and 500 ms, with a notable negative going peak between 300 and 400 ms at some electrode sites. Moreover, incorrect word problems elicited a more N400-like negativity then did incorrect digit problems. The relevance of these findings to models of N400 and language processing will be discussed.

487.4

CROSS-MODAL SEMANTIC PRIMING USING DIFFERENT STIMULUS ONSET ASYNCHRONIES: AN EVENT-RELATED POTENTIAL STUDY. J. Anderson and P. J. Holcomb. Dept. of Psychology, Tufts University, Medford, MA 02155.

Previous electrophysiological and behavioral studies have found similar but not identical semantic priming effects within the auditory and visual modalities. This study compared priming effects between modalities in a cross-modal lexical decision task in order to investigate the possibility of common or independent language systems for auditory and visual modalities.

One group of 12 subjects was presented with visual primes and auditory targets and another 12 subjects with auditory primes and visual targets. The stimulus list consisted of 360 word pairs with equal proportions of semantically related, semantically unrelated, and word/pseudoword pairs. Three stimulus onset asynchronies (SOAs; 0, 200, and 800 msec) were randomly mixed across the experiments.

When the prime was visual and the target auditory, there were large N400 effects (i.e., greater negativity for unrelated targets) at all SOAs. However, when the prime was auditory and the target visual, there were N400 effects at the 200 and 800 SOAs, but not at the 0 SOA. Similarities in the semantic priming effects in the cross-modal conditions indicate that there are common underlying priming processes in the two modalities. However, differences in the time course of the effects and in the morphology of the components indicate that semantic priming in the two modalities may not be identical. The results have implications for models of language comprehension that assume similar or different systems for the two modalities.

487.5

ELECTROPHYSIOLOGICAL EVIDENCE FOR INDIVIDUAL DIFFERENCES IN SYNTACTIC PROFICIENCY DURING SENTENCE COMPREHENSION. S.A. Kotz, L. Osterhout* & P.J. Holcomb, Dept. of Psychology, Tufts University, Medford, MA 02155.

Evidence from previous ERP research has shown that syntactic anomalies (e.g. verb subcategorization and phrase structure errors) elicit a positive-going component (P600) quite distinct from the N400 found for semantic anomalies. Behavioral measures were used to assess the relative stability of previously established ERP components and possible variability due to individual differences. Subjects were ranked as high and low in syntactic proficiency based on their scores from a standardized grammar test. Subsequently, event-related potentials were measured during a sentence judgement task in which subjects were required to evaluate sentences that varied in length and syntactic difficulty.

Distinct P600 effects were found for the two types of syntactic anomalies. Subcategorization violations elicited a broadly distributed P600 in the low proficiency group, while the high proficiency group displayed no differences. The P600 elicited by phrase structure violations was larger at right hemisphere sites for the high proficiency group and posterior on the midline and at left hemisphere sites for the low proficiency group.

These results confirm the existence of distinct components for semantic and syntactic anomalies and indicate that the distribution of these components can be affected by the proficiency and individual processing strategies of the individual speaker.

487.6

BRAIN POTENTIALS EVOKED BY DIRECTIONAL CUES DIFFERENTIATE POOR AND GOOD READERS.
L. Anllo-Vento. Dept. of Psychology , Univ. of North Carolina at Greensboro, NC 27412.

The relationship between selective spatial attention and reading ability was studied with a paradigm that included both directional and non-directional cues. The children in the study were selected in kindergarten as being at risk of developing a reading disability, and were attending the 4th grade when tested.

The task included two successive stimuli: a central cue and a peripheral target. The cue, which was either directional (a left or right arrow) or non-directional (a circle), was followed 600 ms later by a target flash appearing randomly 8° in the left or right visual field. Children were asked to respond every time the target appeared in the cued visual hemifield.

Brain potentials differed between good and poor readers starting 120 ms after arrow onset, increased over time, and were most pronounced immediately before target presentation. Good readers exhibited distinct ERP patterns in response to left and right arrows, which were greatly reduced or absent in lower readers.

Cue-related potentials starting 320 ms after cue onset, but prior to target presentation, were correlated with reading ability, and helped explain subsequent response speed and accuracy.

Supported by NINCDS Grant RO1 NS19413-07

487.7

PERISYLVIAN ANOMALIES IN DYSLEXIA: AN MR STUDY. C.M. Leonard[a], G.W. Hynd*[b],K.K.S. Voeller*[c], A.W. Alexander*[d], H. G. Andersen*[d], and J. Mao*[e], Depts of Neuroscience[a], Psychiatry[c], and Radiology[e], Univ. Fl. Coll. of Medicine, Morris Center[d], Gainesville, Fl. 32610, and Dept. of Special Ed., Univ. of Ga., Athens, Ga. 30602[b].

Dyslexia, or specific reading disability, may be due to anomalous development of the language areas surrounding the Sylvian fissure. Although the planum temporale is normally larger on the left, Galaburda and others have found that it is symmetrical in dyslexics.

We have examined other aspects of perisylvian morphology in two groups of dyslexics: four children with an IQ/reading discrepancy and four adults diagnosed by family history and interview. We scanned 15 age matched controls for comparison. All subjects were right handed. The adults were scanned using a volumetric acquisition that produced a gapless series of 1 mm thick sagittal images. The dyslexic children and their age matched controls were scanned with a standard T1 sequence (5 mm sagittal images). Sulcal patterns were classified with Steinmetz' method (Brain Lang. 38:515-533, 1990) as either normal (present in 82% of Steinmetz' sample and 14 out of 15 of our controls) or anomalous (extra or missing sulci).

Anomalous sulci were unexpectedly frequent in the dyslexics. Five dyslexics had extra sulci on the left while three had missing sulci on the right. Only two had normal patterns on both sides. These results support a role for anomalous brain development in the etiology of dyslexia. We conclude that thin sections in the sagittal plane afford an excellent view of the anatomical regions critical for language and that MR imaging can be used to identify behaviorally significant neuroanatomic variants.

487.8

NEUROANATOMICAL AND NEUROPSYCHOLOGICAL CORRELATES OF ACQUIRED AGRAPHIA. S.W. Anderson, J. Saver*, H. Damasio, and D. Tranel. Div. of Behav. Neurology & Cognitive Neuroscience, U. Iowa College of Medicine, Iowa City, IA 52242.

The neuroanatomical networks which subserve writing may vary considerably from individual to individual due to factors such as differences in learning history, but should have certain constancies due to neural constraints. In this study, motor and linguistic aspects of writing were evaluated in subjects with focal damage in 3 regions of the left hemisphere detected by CT/MR: 1) dorsolateral frontal lobe sparing primary motor cortex (n=13), 2) parietal lobe without extension into frontal or temporal cortices (n=8), and 3) posterior temporal lobe (n=8). Writing was evaluated for grapheme formation, spatial arrangement, spelling, word selection, perseveration, and grammar. The presence, nature, and severity of agraphia varied between and within the 3 groups, although frontal damage caused relatively severe impairments of grammar and grapheme formation. Motor and linguistic impairments of writing did not covary consistently with constructional apraxia or aphasic symptoms. These findings reflect the multifaceted nature and anatomically distributed basis of writing.

487.9

PRESERVED ACCESS AND PROCESSING OF SOCIAL KNOWLEDGE IN ACQUIRED SOCIOPATHY CAUSED BY VENTROMEDIAL FRONTAL DAMAGE. J. Saver*, A.R. Damasio, R.D. Jones. Div. of Cognitive Neuroscience, University of Iowa, Iowa City IA 52242

Damage to ventromedial frontal cortices produces abnormalities of decision making, especially in the realm of social conduct. To test the hypothesis that EVR-318, a prototypical patient with bilateral ventromedial frontal injury and "acquired sociopathy", would fail to access and process social knowledge, we investigated his performance and that of 5 controls matched for education, gender, and age, on standardized tasks which measure the abilities to (1) generate response alternatives to social dilemmas; (2) consider the consequences of pursuing each response option; (3) conceptualize effective means to achieve given social objectives; (4) predict the outcome of a particular response; and (5) perform moral reasoning at an advanced developmental level. The results revealed, unequivocally, that EVR exhibited normal or superior performance, being comparable to or better than controls on all tasks. The capacity to access and manipulate verbally the implications of social stimuli is not impaired in EVR. This result offers further indirect support to the alternative hypothesis that the mechanism underlying disordered social conduct in patients with ventromedial frontal lesions resides in an inability to mark with a specific somatic state (negative or positive) a future outcome that is otherwise normally evoked.

487.10

LAUGHTER: SONIC STRUCTURE AND CONTAGION. R. R. Provine. Dept. of Psychology, Univ. of Maryland Baltimore County, Baltimore, MD 21228.

Laughter is an ancient mode of pre-linguistic vocal communication performed in parallel with modern speech. A Sonographic analysis showed laughter is characterized by stereotyped laugh-note duration ("ha," X = 75 ms) and internote interval ("ha-ha," X = 210-218 ms), and a decrescendo. The simplicity and temporal symmetry of laugh-note structure and internote interval was demonstrated by reversibility; recorded laughter played backward sounded laugh-like. Contagion is another significant feature of laughter; most subjects laughed in response to 18 s of recorded laughter. Although the contagious response is well-known and the basis of "laugh tracks" on broadcast comedy shows, the implications of this potent and extraordinary behavior for brain function are unappreciated.

It is proposed here that stereotypic laughter is produced by a motor pattern generator that, in the case of contagious laughter, is triggered by a laugh-specific acoustic feature detector. Although the existence of human auditory feature detectors is debated, detectors are more likely for simple, species-typical laughter than for complex and culturally variable speech. Laughter and its contagion have numerous implications for theories of speech perception, the evolution of speech and language, the neural basis of social behavior, and cognitive neuroscience.

487.11

CATECHOLAMINE DEPLETION AND COGNITIVE PERFORMANCE: EFFECT OF SLEEP DEPRIVATION. U. McCann, D. Penetar*, Y. Shaham*, D. Thorne*, J.C. Gillin*, and G. Belenky. Dept of Behavioral Biology, Walter Reed Army Institute of Research, Washington, DC 20307-5100

Disturbances in CNS catecholamine systems have been postulated to underlie cognitive deficits in patients with a variety of conditions, including subcortical dementia, schizophrenia, and major depression. The effect of catecholamine depletion on cognitive performance in 40 healthy adult males was tested by preventing synthesis of catecholamines with AMPT, an inhibitor of tyrosine hydroxylase. Subjects were randomized to sleep deprived (no sleep for 40.5 hrs) or rested (7 1/2 hrs/night) conditions. In both sleep conditions, subjects received oral AMPT (or lactose placebo) at a dose of 750 mg QID over two days, for a total of 7 doses. Cognitive performance was measured repeatedly during the two days of drug administration and the day following the last drug dose, using a computerized battery of seven cognitive tasks. AMPT treatment alone, like sleep deprivation alone, was associated with decrements in cognitive performance. Furthermore, the combination of sleep deprivation and AMPT treatment was associated with greater performance decrements than either treatment alone. These findings support the view that catecholamines play a role in normal cognitive processes. Further, they suggest that decrements in cognitive performance associated with sleep deprivation may be mediated by the catecholamine neurotransmitters. Sleep deprivation may be a useful tool for detecting subclinical cognitive dysfunction in patients with a suspected cognitive deficit.

487.12

EXPOSURE TO HYPOBARIC HYPOXIA: A METHOD FOR THE COMPARISON OF NEUROPSYCHOLOGIC TESTS IN HUMANS
H.R. Lieberman, B. Shukitt-Hale, L.E. Banderet* and J.F. Glenn
Military Performance and Neuroscience Division, U.S. Army Research Institute of Environmental Medicine, Natick, MA 01760.

A common problem in psychopharmacology and neuropsychology is the absence of a reliable method to compare the sensitivity of behavioral tasks employed in human studies. Most environmental, behavioral and psychopharmacologic manipulations do not consistently produce readily detectable, graded deficits in human performance and mood. However, human cognitive function is sensitive to changes in oxygen availability and exposure to hypoxia should produce a continuum of effects as altitude level and duration are increased. We evaluated the behavioral effects of hypoxia as a function of time of exposure and altitude level with various standardized tests of cognitive function and mood-state. Twenty-three males were tested in an altitude chamber during a 4.5 hour exposure to hypobaric hypoxia. All subjects were exposed to two levels of hypoxia (4200 m and 4700 m) and a near sea level (550 m) control condition. Prior to altitude exposure, extensive practice was given on all tests. Cognitive function was significantly impaired on most tests. Even on relatively simple performance tasks, such as simple and choice reaction time, as well as complex tests of cognition, such as the Tower of Hanoi, function was significantly reduced in a graded manner. Mood-state, which was assessed using several standardized tests, such as the Profile of Mood States, was also impaired in a graded manner. In general, the magnitude of the effects observed was a function of exposure duration and altitude level. The robust and consistent effects observed and their graded, relatively non-specific nature, suggests that graded exposure of to hypobaric hypoxia may be a useful method for comparing the sensitivity of various tests of human performance, cognition and mood.

487.13

MOTOR DYSFUNCTION IN EARLY ASYMPTOMATIC HIV INFECTION. R.A. Stern,* N.G. Singer,* S.G. Silva,* H.J. Rogers,* D.O. Perkins,* C.D. Hall,* C.M. van der Horst* and D.L. Evans. Depts. of Psychiatry, Medicine, and Neurology, Univ. of North Carolina Sch. of Med., Chapel Hill, NC, 27599.

AIDS frequently results in CNS dysfunction. It remains unclear, however, whether asymptomatic HIV seropositive individuals exhibit neurobehavioral deficits early in the course of infection, prior to other symptoms and signs. The goal of this study was to assess the impact of several potential co-factors on the early presentation of cognitive and/or motor disturbance in HIV. Subjects included 35 asymptomatic HIV seropositive gay men and 44 seronegative gay male controls. Strict exclusion criteria were used to preclude the confounding effects of zidovudine use, substance abuse, or CNS disorder, including mild head injury or learning disability. Neuropsychological evaluations were conducted as part of a larger, longitudinal study. Group differences in summary ratings and individual test scores were first assessed with analyses of variance. Education, SES, B12 level, CD4 count, and Hamilton Depression Rating Scale score were then controlled for, individually and together, using analyses of covariance. The level of significance was set at .05 for all tests. Without any covariates, significant group differences were found in a variety of areas, with the seropositive subjects performing worse than controls. When controlling for education, the only significant group differences were on measures of motor speed. These significant group differences remained after controlling for SES, CD4 count, B12 level, and depression. In gay men without confounding factors of substance abuse, past CNS disorder, or zidovudine use, mild motor slowing was the only neurobehavioral deficit associated with early HIV infection.

487.14

Absence of Neurobehavioral Effects of High Dose Aspartame in Phenylketonuric Heterozygotes (PKUH). L.M.J. de Sonneville, B. Lanz-Englert*, F.K. Trefz*, Ch. Benninger, P. Matthis* and H. Bickel*. University of Heidelberg, Heidelberg, Germany.

The effects of aspartame (APM) on neuropsychological and biochemical parameters were evaluated in 48 adult (21 male, 27 female) PKUH whose carrier status had been proven by DNA analysis. Subjects received APM (either 15 or 45 mg/kg/day) and placebo in capsules in a randomized, double-blind, placebo-controlled, crossover study for twelve weeks on each treatment. A computerized battery of neuropsychological tests, the de Sonneville Visual Attention Tasks (SVAT), was evaluated at weeks -2, -1, 6, 12, 18, and 24. Through a series of complex reaction time paradigms, the SVAT evaluates cognitive changes in the main stages of information processing (encoding, memory search, decision, and response organization), in sustained and focused attention, and in short term memory. Notwithstanding increases in plasma phenylalanine concentrations after the morning dose in the 45 mg/kg/day APM group, there were no significant differences in any of the SVAT measures when APM was compared to placebo. Moreover, evaluation of individual SVAT scores did not reveal any changes from baseline. In addition, there were no significant differences in adverse experiences, urinary organic acids, and electroencephalograms when APM was compared to placebo. These results reaffirm the safety of APM in PKUH and refute the speculation that APM affects cognitive performance.

LEARNING AND MEMORY—PHARMACOLOGY: ACETYLCHOLINE II

488.1

CENTRAL CHOLINERGIC BLOCKADE BY SCOPOLAMINE DISRUPTS CLASSICAL CONDITIONING OF THE HUMAN EYEBLINK RESPONSE . P.R. Solomon, M.E. Groccia-Ellison, K.R. Edwards, and M.E. Stanton, Southwestern Vermont Medical Center, Bennington, VT 05201 and Williams College, Williamstown, MA 01267

The cholinergic system has been implicated in both normal and disordered memory. We have previously demonstrated that the anticholinergic drug scopolamine disrupts a simple form of learning and memory (Classical or Pavlovian eyeblink EB conditioning) in rabbits. Studying simple forms of learning and memory such as EB conditioning may be particularly useful for understanding mechanisms of memory disorders because the identical learned response can be studied in both humans and animals (rabbits). Recently, we have demonstrated that this form of memory is also disrupted in patients with Alzheimer's Disease (AD). This may be of some significance because of the hypothesized link between acetylcholine and disordered memory in AD. We now report that scopolamine disrupts human EB conditioning in a manner similar to that seen in AD patients. Human volunteers between the ages of 18 and 50 received oral administration of either scopolamine (SCOP) hydrobromide (0.6 or 1.2 mg), a peripheral cholinergic blocker, glycopyrrolate (GLY, 0.2 mg) or saline (SAL). Subjects then underwent classical conditioning of the EB response. Subjects receiving the low dose of SCOP or GLY conditioned at about the same rate as SAL controls. Subjects who received the high dose of SCOP conditioned significantly slower.

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488.2

THE REVERSAL OF SCOPOLAMINE'S EFFECTS ON RESPONSE ERRORS BY MUSCARINIC AGONISTS IN RATS. V.L. Coffin, M.L. Libonati, and M.B. Latranyi*. Schering-Plough Research, Bloomfield, NJ, 07003

Rats were trained on a fixed-ratio (FR) discrimination procedure. Rats were required to complete one of two FRs on the center bar followed by a choice on the right lever if the ratio was low (FR 8) or on the left lever if the ratio was high (FR 16) for a food reward. Scopolamine produced dose-related decreases in response rates and increases in response errors (.003-.01 mg/kg sc). Arecoline and physostigmine reversed the effects of scopolamine on response errors and failed to reverse the decreased response rates. Doses of these agonists which reversed scopolamine, had no effect on this schedule when administered alone. Oxotremorine failed to reverse any of the effects of scopolamine on this schedule.

Methylscopolamine produced a similar decrease in response rates as scopolamine but with smaller changes in response errors over the same dose range. Muscarinic agonists did not reverse the effects of methylscopolamine on response errors.

In conclusion, scopolamine produces centrally mediated deficits on accuracy which can be selectively reversed by arecoline and physostigmine but not oxotremorine.

488.3

LACK OF AMNESIC EFFECT OF SCOPOLAMINE IN UNDER-REINFORCED PASSIVE AVOIDANCE. G. L. Quirarte*, S. Rivas-Arancibia* and R. A. Prado-Alcalá. Psych. Sch., Univ. Guadalajara and Physiol. Dept. Med. Sch., Natl. Univ. of México, México, D.F. 04510, México.

Experimental data indicate that in conditions of overtraining or over-reinforcement central nervous system cholinergic activity is not needed for memory consolidation. The aim of this work was to determine if the same holds true for consolidation of passive avoidance, trained with low levels of footshock (under-reinforcement). Rats were submitted to one-trial passive avoidance and were given one footshock during training (0.0, 0.3, 0.4, 0.5, 0.6, 0.7, or 0.8 mA). Half the groups was given one ip injection of scopolamine (8 mg/kg), 5 min after training and the other half was not injected. Scopolamine-induced amnesia was found only in the animals trained with 0.7 and 0.8 mA. These data suggest that muscarinic cholinergic activity is not involved in memory consolidation of under-reinforced tasks.

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488.4

BEHAVIORAL MICROANALYSIS OF SPATIAL DELAYED ALTERNATION PERFORMANCE: REHEARSAL THROUGH OVERT BEHAVIOR, AND EFFECTS OF SCOPOLAMINE AND CHLORDIAZEPOXIDE. P. Dudchenko and M. Sarter. Dept. Psychology, The Ohio State University, Columbus, OH 43210.

Rats were trained in an operant spatial delayed alternation task employing various delays ranging from 2 - 32 seconds. In addition to response accuracy, operations of the levers during retention intervals were recorded and analyzed. In untreated animals, the relative number of correct responses and correct rehearsal operations (operation of the forthcoming correct lever during retention intervals) varied with the length of the retention intervals, and these measures were correlated. The response rate for rehearsal operations increased with the length of the retention intervals. It is speculated that the delay-dependent increase in response rate reflects an effect of delayed reward that was also associated with an increasing tendency to alternate between levers. These effects may have contributed to the delay-dependency of correct responding. Scopolamine-hydrobromide (0.01, 0.03, 0.1, 0.3 mg/kg) and -methylbromide (0.1, 0.3 mg/kg) produced main effects on correct responding, but did not interfere with the relative number of correct rehearsal operations. As both scopolamine-derivatives did not affect the relative number of correct rehearsal operations but of correct responses, it is speculated that these treatments increased the probability that the onset of the panel light (that was assumed to indicate lever activation) triggered a repositioning response, yielding a delay-independent reduction in the number of correct responses. These results suggest that in tasks that potently allow the development of rehearsal operations, delay-dependent response accuracy does not represent a sufficient condition for conclusions on task demands on memory. Furthermore, it seems likely that drug-induced changes in performance in such tasks may be a result of alterations within peripheral functions.

488.5

MUSCARINIC ACTIVITY OF A NOVEL M_1 AGONIST, YM796 AND ITS OPTICAL ISOMER. F. Wanibuchi, T. Konishi*, K. Hidaka*, H. Akiho*, Y. Nakamura*, K. Takahashi*§, S. Tsukamoto* and S. Usuda* Central Res. Labs., § Applied Pharmacol. and Develop. Labs., Yamanouchi Pharmaceutical Co., Ltd., 21 Miyukigaoka, Tsukuba, Ibaraki 305, Japan.

(±)-YM796 exhibits M_1 agonistic activity (Eur.J. Pharmacol. 187(1990) 479-486). We herein report the further characterization of its (-)-isomer, YM796 ((-)-2,8-dimethyl-3-methylene-1-oxa-8-azaspiro[4.5]decane, (-)-YM796) and its (+)-isomer on biochemical and behavioral studies. YM796 (0.016 - 0.25 mg/kg, po) like the racemate reversed the cognitive impairment induced by nucleus basalis magnocellularis (NBM) lesion in rat passive avoidance tasks, whereas (+)-YM796 was ineffective in the experimental amnesia. In addition, YM796 ameliorated the memory deficit of NBM-lesioned rats in Morris water maze. YM796 was weaker than RS86 in the induction of tremor, hypothermia and contraction of ileum which are mainly mediated by M_2 and/or M_3 receptors. YM796 like the racemate inhibited [^{3}H]pirenzepine ([^{3}H]PZ) binding to rat cerebral cortical membranes in the micromollar range and stimulated phosphoinositide (PI) hydrolysis in rat hippocampal slices. The (+)-isomer have essencially no agonistic activity in PI hydrolysis and behavioral changes in spite of inhibiting [^{3}H]PZ binding. These results indicate that YM796 but not its (+)-isomer is a potent and selective muscarinic M_1 receptor agonist.

488.6

BEHAVIORAL AND MICRODIALYSIS STUDIES OF A NEW AChE INHIBITOR, 3-PYRIDINOL DIMETHYLCARBAMATE, IN THE RAT

L.J. Cote, M.E. Bach, A.B. Naini, R. Burke, T. Oo, M. Sano, S. Ginsburg*, J.L. Bach*† & R. Mayeux*. Dept. of Neurology, College of P&S, Columbia University, New York, NY 10032 and †Ciba-Geigy Co., Summit, NJ 07901

In a previous study we found that rats with unilateral nucleus basalis magnocellularis (nbm) lesions (quinolinic acid-120nmol in 1 ul) displayed a learning deficit on the repeated acquisition task. In each session of the repeated acquisition task, the rat is required to learn in 10 trials, which new 4 arms, of an 8-arm radial maze, are baited with food. This study compared the effectiveness of the novel acetylcholinesterase (AChE) inhibitor 3-pyridinol dimethylcarbamate ("Norpyridostigmine") to physostigmine at enhancing repeated acquisition performance in the lesion rat. Norpyridostigmine tosylate at 5, 10, or 20 mg/kg and physostigmine salicylate at 0.6, 0.9 or 0.12mg/kg were administered i.p., 15 min prior to behavioral testing. The performance of AChE inhibitor treated lesion rats was compared to untreated lesion, sham and control rats. Both AChE inhibitors enhanced learning in a dose response manner. Also, Norpyridostigmine tosylate (30mg/kg, i.p.) increased the release of acetylcholine (ACh) from the striatum by about 80% above baseline, in an awake, free roaming rat. Peak ACh level occurred 2 hours following administration. In conclusion, Norpyridostigmine enhances repeated acquisition performance and increases ACh levels in vivo.

Supported by the Charles S.Robertson Foundation and the Parkinson Disease Foundation

488.7

SCOPOLAMINE PRODUCED IMPAIRMENT OF REPEATED ACQUISITION PERFORMANCE BY THE NBM LESIONED RAT IS REVERSED BY THE NOVEL AChE INHIBITOR 3-PYRIDINOL DIMETHYLCARBAMATE M. Sano, M.E. Bach, A.B. Naini, L.J. Cote, S. Ginsburg*, J.L. Bach*† & R. Mayeux*. Dept. of Neurology, College of P&S, Columbia University, NY, NY 10032. and †Ciba-Geigy Co., Summit, NJ 07901.

Learning and memory impairments in Alzheimer's disease have been attributed to the disruption of the cholinergic system. We have demonstrated in rats that unilateral lesions (quinolinic acid-120 nmol in 1 ul) of the nucleus basalis magnocellularis (nbm) produced cholinergic cell loss and learning deficits in repeated acquisition performance. We hypothesized that the performance of the lesion rats would be more vulnerable to a disruption by scopolamine, a muscarinic antagonist, than sham and control rats. Scopolamine (0, 0.1, 0.2, & 0.4mg/kg, i.p.) administration began when the repeated acquisition task had been learned. In each session of this task, across 10 trials, the rat is required to learn which new set of four arms, of an 8-arm radial maze, are baited with food. Lesion, sham and control groups received scopolamine 20 minutes prior to testing. Across all doses, the lesion group was more impaired than the sham and control groups, who were similarly effected. Scopolamine impaired accuracy and increased reference and working memory errors in a dose-response manner with the former error type more effected. To determine if acetylcholinesterase (AChE) inhibitors could reverse the scopolamine impairment, 3-pyridinol dimethylcarbamate, "Norpyridostigmine" (5, 10 & 20mg/kg) and physostigmine(0.6, 0.9 & 0.12mg/kg) were administered i.p., 5 min following a scopolamine injection (0.4mg/kg). Both inhibitors consistently reversed the impairment. Initial studies suggest that Norpyridostigmine improves performance in a dose-response manner. These results support the hypothesis that cholinergic deficits produce memory impairments and suggest that AChE inhibition may serve as a viable treatment for cognitive/behavioral deficits.

488.8

USE OF RESPONSE STRATEGIES DURING RADIAL MAZE PERFORMANCE BY NBM LESIONED RATS. M.E. Bach and S.L. Teitelbaum*, Depts. of Neurol.& Rehab. Medicine, College of P&S, Columbia University, NY, NY 10032. In a previous study, we found that rats with unilateral nucleus basalis magnocellularis (nbm) lesions (quinolinic acid-120nmol in 1ul) displayed a repeated acquisition (RA) task learning deficit. They required 22 days to learn the task as compared to controls who needed 7 days. In the RA task, the rat is required to learn, across ten trials, which four arms of an 8 arm radial maze are baited with food; a new set of 4 baited arms is randomly selected for each session. During data collection, it was observed that lesioned rats followed response strategy rules more frequently than control rats. The observed strategies included: 1) response patterns: arm entries (AE) which follow a spatial pattern rule e.g. always enter arm at a right (90°) angle or always enter adjacent (45°) arm, 2) response sequences: repeated use of the same set of AE which are entered in the same sequential order and 3) entering arms in a predominantly clockwise (CW) or counterclockwise (CC) direction. To determine if lesioned rats (N=8) were more apt to use response strategy rules during the learning deficit period (sessions 11-20), we compared their AE choices to those of the controls (N=8). For response pattern analysis, the frequency of right angle, adjacent, nondescript (135°), across and reentry AE was assessed along with the prevalence of CW and CC moves. Response sequence analysis included: 1) examination of the first four arm choices from each trial for repetitions of a specific sequence of AE and 2) determination of which baited 4 arm sequence was used the most in each session. Using the Mann-Whitney U test we found that lesioned rats made significantly more right angle AE ($p<.02$) and also entered arms in a predominantly CW or CC direction ($p<.0001$). The two groups did not differ in any of the response sequence analyses. We propose that lesioned rats may employ response strategy rules because they make minimal demands on memory while still enabling the rat to solve the RA task.

488.9

EFFECT OF RX77368 AND SCOPOLAMINE ON 8-ARM RADIAL MAZE PERFORMANCE IN THE RAT. C.D.Watson*, M.J.Hewitt*, K.C.F.Fone*, G.W.Bennett*, & S.L.Dickinson[1]*. (SPON: Brain Research Association). Dept. of Physiol.& Pharmacol., Queen's Medical Centre, Nottingham NG7 2UH, UK. [1]Reckitt & Colman Psychopharmacol. Unit, University Walk, Bristol BS8 1TD, UK.

Thyrotrophin-releasing hormone (TRH) analogue RX77368 (dimethyl-Pro-TRH), is known to reverse an atropine-induced water maze deficit. We evaluated whether RX77368 could attenuate a scopolamine-induced deficit in the radial arm maze. Rats were cannulated i.c.v., placed on a 23hr food-deprivation schedule then tested on days 12-16, 19-23 and 26-30 post-surgery in a radial maze. Four groups of rats (n=8 each) were pretreated (-40min) with either saline (SAL; groups A and B; 2µl i.c.v.) or RX77368 (groups C and D; 2µg i.c.v.) followed 10min later with either SAL (A and C; 1ml/kg i.p.) or scopolamine (SCP; B and D; 0.3mg/kg i.p.). After the last trial, TRH levels in the septum, hypothalamus, nucleus accumbens, hippocampus and medial-parietal cortex were determined by radioimmunoassay. Performance of all groups significantly improved over time, as measured by unrepeated arm entries (UAE, $P<0.001$), total arms entered (TAE, $P<0.01$) and total maze time ($P<0.01$). UAE were significantly reduced by SCP alone ($P<0.05$, days 13-29), indicating a SCP-induced learning deficit. SCP alone also showed significantly fewer repeated arm entries (RAE) at the start of the trials ($P<0.05$) and significantly more at the end ($P<0.05$), suggesting SCP had non-cognitive effects. RX77368 significantly attenuated the SCP-induced deficit on days 13-21 but not on later days. RX77368 alone significantly increased UAE ($P<0.05$, days 12, 15) compared to SAL, suggesting that the TRH analogue either alone or in combination with SCP improved performance. Hypothalamic TRH levels (SAL; 169.2±18.2pg/mg protein) were significantly elevated in the SAL/SCP (580.5±106.6, $P<0.01$), RX77368/SAL (378.5±75.4, $P<0.05$) and RX77368/SCP (354±94, $P<0.05$) groups. Changes in other areas were less pronounced. Taken together these results suggest that SCP-induced radial maze performance deficit may result from a reduction in locomotion rather than solely a learning impairment and that RX77368 may improve performance by increasing arousal and exploration.

C.D.W. is a SERC CASE Student with Reckitt & Colman.

488.10

IMPROVEMENT OF MEMORY IN NORMAL AND IMPAIRED MICE BY BMY 21502: A COMPARISON WITH HYDERGINE® AND PHYSOSTIGMINE. H. Lal, K. C. Retz, and M.J. Forster. Dept. of Pharmacology,Texas College of Osteopathic Medicine, Fort Worth, TX 76107

BMY 21502, a substituted pyrrolidinone, was tested in three animal models for its potential to reverse age-related memory dysfunction. In two of the models, efficacy of BMY 21502 was compared with physostigmine and hydergine®. BMY 21502 treatment prior to training improved the ability of mice to learn a goal arm discrimination in a T-maze. In addition, the BMY 21502-treated mice showed improved recall one week following initial discrimination learning. Physostigmine and hydergine® also facilitated retention of discriminated escape in the young mice. Pre-treatment with BMY 21502 (from 0.64 to 10 mg/kg) was effective in reducing or eliminating amnesia induced by treatment with the anticholinergic, scopolamine. Hydergine was less effective than BMY 21502 in the drug-induced amnesia paradigm. In the third model, daily injections of BMY 21502 prior to testing improved acquisition of an active avoidance response in 4-month-old NZB/BINJ mice, a genotype noted for accelerated age-related decline of learning/memory capacity (Forster & Lal, *Br. Res. Bull.*, *25*:503-516, 1990). These results suggest that BMY 21502 may be effective in improving cognitive ability in both normal subjects and individuals with age-related memory impairment. (Supported by a research grant from Bristol Myers Squibb Company and NIH grant AG06182)

488.11

EFFECT OF ORAL ADMINISTRATION OF REVERSIBLE INHIBITORS ON BLOOD ACETYLCHOLINESTERASE IN HUMANS: E2020 AND TACRINE. **K.A.Sherman, Dept. Pharmacol., SIU Sch. Med., Springfield, IL 62794-9230**

Reversible, allosteric inhibitors of acetylcholinesterase (AChE), e.g. tacrine (THA) and E2020, a novel piperidine derivative developed for dementia treatment (Yamanishi et al. Soc.Neurosci.Abst. 14:59, 1988), slow phosphorylation of AChE by organophosphates, e.g. diisopropylfluorophosphate (DFP). A modest dose of E2020 (2 mg/kg, s.c.) produced marked and sustained DFP antagonism in rat cortex (>90% for 3 hr; >50% at 5 hr); AChE inhibition *ex vivo* at minimal tissue dilution (5-fold) was less (70% maximum; <10% by 5 hr) (Sherman, Soc.Neurosci.Abst. 16:137, 1990). The ability to protect AChE against irreversible inhibition by DFP *in vitro* thus provides a sensitive, new approach for quantifying the pharmacodynamics of these reversible inhibitors. In the present studies, we show that phosphorylation of AChE in red blood cells (RBC) is also antagonized after oral administration of E2020 or tacrine in humans. In 34 dementia patients, the effect of E2020 was selective; plasma ChE activity was not altered. E2020 produced dose-dependent antagonism of DFP which increased dramatically with duration of treatment up to 2 wk (1 mg daily) or 4 wk (2 mg). In pre-drug control samples, the conditions selected [25 µl of 4.5 µM DFP per 100 µl cells; 30 min at 37°C] resulted in 84.8 ± 0.8% inhibition of AChE activity subsequently measured at 262.5-fold dilution to prevent further action of DFP and to fully dissociate E2020. The effect of DFP was reduced by 30% or more (maximum 45%) in 43% of the RBC samples from patients treated with 2 mg/d E2020 for 1 to 3 mo. After ≥ 2 wk treatment, the degree of DFP antagonism in RBC was closely correlated to log concentration of E2020 in plasma. With chronic dosing, the relation of DFP antagonism to plasma [E2020] became progressively steeper; less than half as much drug level was required to achieve the same degree of DFP antagonism compared to the first week of E2020. A single oral dose of THA inhibited plasma ChE maximally at 1½ to 2 hr by 39 ± 2.5 % (range 29 to 55%) in 12 elderly controls. The effect in RBC was less and was variable ($\bar{x}$ = 18.7 ± 3.0%; range: 5.6 to 43%), but correlated well with peak plasma inhibition.

488.12

BIOCHEMICAL AND BEHAVIOURAL CHARACTERISATION OF HEPTYLPHYSOSTIGMINE (HPS): A POTENT ANTICHOLINESTERASE WITH LONG DURATION OF ACTION. **S.B.Freedman, G.R Dawson*, L.L. Iversen, S.D. Iversen*, P.L.Rugarli* and E.A.Harley*. Merck, Sharp and Dohme Research Laboratories, Terlings Park, Harlow, Essex CM20 2QR, England and Mediolanum Farmaceutici Research Laboratories, Milan, Italy.**

HPS was a potent inhibitor *in vitro* of both human acetylcholinesterase (ACHE)(IC_{50} 20nM) and horse serum butyrylcholinesterase (IC_{50} 5nM). In comparison to physostigmine (PHY), HPS had a slower association rate and dissociation rate. In an *ex vivo* assay in mouse brain HPS (ED_{50} 3.8mg/kg i.p.) had a significantly longer duration of action compared with PHY, tacrine and E-2020 with ACHE inhibition for up to 18 hours. In contrast, the inhibition with PHY (0.3 mg/kg) rapidly decayed over a 90 min period. HPS administered s.c. 80 min prior to testing fully reversed a scopolamine (0.2mg/kg) induced deficit in mouse and rat passive avoidance at doses of 3.0 and 8.0 mg/kg respectively. In the same rat passive avoidance experiment a dose of 0.6 mg/kg PHY (s.c 5 min pretreatment) only partially reversed the deficit. In a working memory test (DMPT), 4.0 mg/kg HPS partially reversed a scopolamine induced deficit whereas PHY was without effect. The longer duration of action and greater efficacy of HPS, compared with traditional ACHE inhibitors in rodent models of cognition, may mark a major development in drug treatment for dementia of the Alzheimer's type.

488.13

BUTYRYLCHOLINESTERASE PROTECTS AGAINST NERVE AGENT EXPOSURE IN RHESUS MONKEYS TRAINED ON A SERIAL PROBE RECOGNITION TASK. C.A.Castro, A.V.Finger, D.M.Maxwell, R.P. Solana, D.E.Lenz and C.A.Broomfield. USAMRICD, Aberdeen Proving Ground, MD 21010-5245.

Soman is a highly toxic nerve agent capable of producing death in humans and experimental animals within minutes of exposure. It has recently been demonstrated that exogenously administered enzymes such as equine butyrylcholinesterase (EBuChE) can prevent death and physical incapacitation in non-human primates exposed to multiple-lethal doses of soman. We report here that EBuChE can also prevent soman-induced behavioral incapacitation in rhesus monkeys as measured by performance on a Serial Probe Recogntion (SPR) task. Four monkeys were trained to perform a six-item SPR task at an 80% correct performance level. Next, all monkeys received 27500 U (503 nmol) of EBuChE followed 1.2-1.4 hr later by 220-260 nmol of soman (2 MLDs; a lethal dose in untreated animals). All monkeys survived the soman challenge and none showed any clinical signs of nerve agent intoxication. SPR performance was depressed after enzyme administration and 1 hr after soman challenge. By 8 hr after soman challenge, however, all monkeys performed the SPR task at pre-soman challenge levels. These findings indicate that EBuChE can protect against the behavioral as well as the lethal effects of nerve agent intoxication.

488.14

NICOTINE-INDUCED ATTENUATION OF SPATIAL MEMORY DEFICITS IN RATS WITH SEPTAL LESIONS. M. W. Decker and M. J. Majchrzak. Neuroscience Research, Pharmaceutical Products Division, Abbott Laboratories, Abbott Park, IL 60064.

The septohippocampal system is important for spatial memory. Septal lesions impair performance on a variety of spatial memory tasks in rats, and septal rats have been used as an animal model of Alzheimer's disease (AD). In this experiment, we found that pretraining administration of nicotine improved spatial discrimination learning in rats with septal lesions.

Rats with radiofrequency or sham lesions of the medial septum were required to learn which of two visible platforms in a pool of water provided a means of escape. The platforms were identical in appearance but could be distinquished on the basis of spatial location. Six trials were given for 5 consecutive days. On each of the first 4 days of training, the rats received an injection of (-)nicotine (0, 0.1, or 0.3 mg/kg, i.p.; 9-10 rats per group) 15 min. before training. Nicotine did not significantly alter the performance of sham-lesioned rats. However, nicotine markedly improved the performance of septal rats, such that on the 5th day of training, septal rats who had received a 0.3 mg/kg dose of nicotine performed as well (0.9 ± 0.3 errors) as control animals (0.7 ± 0.4 errors), whereas septal rats who had received only pretraining saline injections performed essentially at chance levels (3.1 ± 0.5 errors).

These findings suggest that nicotine can enhance spatial memory in rats in which septohippocampal function is disrupted. In that impaired septohippocampal function has been implicated in the memory deficits associated with AD, our findings further suggest that nicotinic receptor stimulation might be useful in the treatment of these cognitive deficits.

488.15

DIFFERENTIAL EFFECTS OF CHLORISONDAMINE AND MECAMYLAMINE ON NICOTINE INDUCED CHANGES IN LOCOMOTION AND LEARNING. M.J. Majchrzak, and M.W. Decker, Neuroscience Research, Pharmaceutical Products Division, Abbott Laboratories, Abbott Park, IL 60064.

Nicotinic agents have been implicated as modulators of learning and memory. Agonists tend to enhance, while antagonists tend to disrupt learning and memory. This study compared the ability of mecamylamine (MEC) and chlorisondamine (CHLOR), two central nicotinic antagonists, to disrupt nicotine's (NIC) agonist effect on locomotion and cognition. Post-training NIC administration (0.3 mg/kg max effect) enhanced 24 hour retention of an inhibitory avoidance task. Chronic CHLOR pretreatment (10 µg, icv), did not antagonize the enhanced retention produced by NIC. Pretraining administration of the central and peripheral antagonist MEC (1.0 mg/kg), did effectively block the enhancement produced by post-training NIC. Pretraining administration of the peripheral nicotinic antagonist hexamethonium (5.0 mg/kg), was not able to block the memory enhancing effect of nicotine. Animals from the previous experiments were then challenged with an acute dose of NIC (0, 0.1, 0.3 or 1.0 mg/kg, ip) and placed in activity monitors for 60 min. CHLOR blocked both the depressant and stimulatory effects of NIC. In a separate group of animals MEC (1.0 mg/kg, ip) was administered 10 minutes prior to the challenge with NIC. MEC produced a comparable blockade of NIC. CONCLUSION: CHLOR appears to be a very potent antagonist of NIC with respect to locomotor activity, yet does not disrupt the enhancement of cognition by NIC. However, MEC is able to disrupt cognition at doses that are as effective as CHLOR in blocking NIC's locomotor effects. Therefore, the ability of a nicotinic antagonist to block the locomotor effects of an agonist does not appear to predict its ability to disrupt the effects of an agonist in a cognitive task. The present findings suggest that these nicotinic antagonists may act at different sites, and could be useful tools in differentiating nicotinic receptor subtypes.

488.16

CLASSICAL NICOTINIC AGONISTS DIFFERENTIALLY AFFECT COGNITION, CORTICAL CEREBRAL BLOOD FLOW (CBF) AND DOPAMINE (DA) RELEASE. J.D. Brioni, D.G. Linville, E.D. Cadman, S. Williams *, M Buckley, D.J. Anderson and S.P. Arneric, Neuroscience Dept., Pharmaceutical Discovery, Abbott Laboratories and Dept. of Pharmacology, Southern IL University, Springfield.

Molecular biological evidence supports the existence of multiple neuronal nicotinic receptors in the brain. This study sought to determine whether potent classical nicotinic agonists differentially affect nicotinically-mediated events, possibly through subtype-mediated specificity. (-)Nicotine (NIC), (-)Cytisine (CYT), and (-) Lobeline (LOB) each potently displaced 3H-NIC and 3H-CYT binding from rat brain membranes (Ki= 0.1-1.1 nM). In contrast, NIC and CYT (EC50= 26.2 & 10.3 nM), but not LOB, enhanced the release of tritium from slices of rat striatum prelabeled with 3H-DA. Behaviorally, a consistent pattern was observed in the inhibitory avoidance task (IA) in mice. NIC and CYT (0.03-0.3 mg/kg, IP) but not LOB, induced a significant enhancement of retention. When the effects on cortical CBF were evaluated in urethane-anesthetized rats using laser-doppler flowmetry, NIC and LOB (less potently) enhanced basal forebrain-elicited increases in cortical CBF (350% increase). CYT had a biphasic effect with high doses inducing a 50% inhibition. CONCLUSIONS: 1) The memory facilitating effect of NIC and CYT correlate with the 3H-DA release effect; 2) Enhancement of cortical CBF mediated by the basal forebrain does not translate directly into enhancement of cognition in tasks such as IA.

488.17

NICOTINE-INDUCED FACILITATION OF RADIAL-ARM MAZE PERFORMANCE IN RATS. E.D. Levin, F.H. Brucato* H.S. Swartzwelder* and J.E. Rose. Departments of Psychiatry and Neurology, Duke University Medical Center, Durham, N.C. 27710

Abundant research has demonstrated the importance of muscarinic acetylcholine (ACh) receptors for cognitive function. Nicotinic ACh receptors have received less attention, but they are also appear to be important for cognitive function. We have found that like muscarinic blockade nicotinic blockade impairs choice accuracy in the radial-arm maze. Stimulation of nicotinic receptors with nicotine improves radial-arm maze choice accuracy after either acute subcutaneous injection of 0.2 mg/kg nicotine ($p<0.01$) or chronic subcutaneous infusion of 12-14 mg/kg/day for 3 weeks ($p<0.025$). Our most recent study has shown that facilitation of radial-arm maze choice accuracy also occurs after intraventricular infusion of 4 µg of nicotine. In a radial-arm maze exploration paradigm female Sprague-Dawley rats (N=13) averaged 3.95±0.48 (mean±sem) entries to repeat after control infusions and 4.87±0.31 after nicotine infusions ($p<0.05$). The dose of the nicotinic antagonist mecamylamine tried thus far (200 µg) did not significantly alter choice accuracy. Alzheimer's patients show decreased cortical nicotinic receptor binding. Additional nicotinic stimulation may help to reverse their cognitive impairment. (Supported by an Alzheimer's Association/Neil Bluhm Research Grant and NIDA grant 02665)

488.18

EFFECTS OF CHRONIC NICOTINE ADMINISTRATION ON THE COGNITIVE PERFORMANCE OF AGED RATS. G.W. Arendash and G.J. Sengstock, Department of Biology and Institute for Biomolecular Science, University of South Florida, Tampa, Florida 33620.

Aged rats have been shown to be impaired in the acquisition and/or retention of a wide variety of cognitive tasks. Despite reports showing that nicotine administration to *young adult* rats can affect cognitive performance, the effects of nicotine in *aged* rats have not yet been investigated. The purpose of this study was, firstly, to characterize the effects of aging on three dissimilar tasks and, secondly, to elucidate the effects of daily nicotine administration on the performance of *aged* rats in these tasks. To accomplish this, male Sprague-Dawley rats aged 2-3 months (young) and 22-24 months (aged) were tested in one of the following tasks: 17-arm radial maze, one-way active avoidance, or Lashley III maze performance. Aged rats received an i.p. injection of either nicotine (0.2 mg/kg) or saline vehicle 15 minutes prior to daily behavioral testing; young control rats received saline vehicle injections. Compared to young controls, aged vehicle-treated rats were significantly impaired in the general learning and reference (long-term) memory components of **17-arm maze performance**; working (short-term) memory was not affected. In contrast, nicotine pretreatment resulted in a significant improvement in both general learning and reference memory of aged rats in this task. An age-related impairment in the acquisition of **one-way active avoidance** pole jumping was also found. Nicotine pretreatment of similar aged rats was able to markedly improve overall learning and rate of learning compared to aged controls. In **Lashley III** maze testing, aged control rats made substantially more errors than young control rats; aged rats pretreated with nicotine made less errors than aged controls. These results indicate that chronic nicotine administration can improve the impaired learning/memory abilities of aged rats in several different tasks.

488.19

MEMORY IN THE MONKEY: AN AUTOMATED PARADIGM AND THE EFFECT OF NICOTINE. J.W. Ashford and H. Edwards*. Department of Psychiatry, University of California, Davis, VA Medical Center, Martinez, CA., 94549.

This study examined the formation of memory which is not disrupted by interference. The study used rhesus monkeys in a new automated testing paradigm. Monkeys learned to push a joystick lever to the left or the right in response to a video screen letter stimulus (7cm x 5cm) for a juice reward. Four animals learned to discriminate 12 letters: ABCD were given constant directional assignments (LRLR, respectively); EFGHIJKL were varied in their assignments for each day (4 for each direction). Letters were introduced in directionaly opposed pairs, 10 consecutive correct responses for one pair required to proceed to the learning of the next pair. If the animal learned all of the pairs in less than an hour, he was tested for the remainder of the hour on the sequence EFGHIJKL. One animal could learn the associations within 30 min, then score 99% correctly during the remaining 30 min (314 correct trials/320 total).

To test the effect of nicotine in this paradigm, doses up to 10ug/kg i.m. were administered 5 minutes before the task began. Nicotine improved task performance in all four animals: the optimal dose for 1 animal was 2.5ug/kg, and for the other 3 was 5ug/kg. Such automated tasks will facilitate analysis of memory in primates. (Supported by a donation from R.J. Reynolds Tobacco Co.)

488.20

NICOTINE REPLACEMENT AFTER 12-HR ABSTINENCE REDUCED MIRROR-TRACING TIME BUT DIFFERENTIALLY AFFECTED SUPRASPAN DIGIT LEARNING IN TYPE A AND B SUBJECTS. K.-T. Kao, S. Amick* and D. Moss*. Dept. of Psychol., Wabash College, Crawfordsville, IN 47933.

A supraspan (18) digit learning (SDL) and a mirror-tracing (MT) task were studied with five repetitions each. Computerized visual signal detection and Type A and B personality (TA & TB) were also assessed. Male college student smokers (N=18) and chewers (N=16) were randomly assigned to one of three treatment conditions after 12-hr abstinence: no replacement, low dose, and high dose (1/2, and 1) cigarette, or nicotine gums (2, and 4 mg), respectively. The two replacement groups were combined, as were the smokers and chewers, for analyses. Nicotine replacement had no effect on signal detection but reduced MT time during the initial trials without affecting MT error; nicotine also enhanced supraspan digit learning in TA subjects (8.05 ±.67, and 10.27 ±.45) but impaired it in TB subjects (13.63 ±.77, and 11.18 ±.84). The results suggest that studies of cognitive effects of nicotine should take into account their subjects' personality factors associated with coronary heart disease risks.

489.1

DORSOMEDIAL HYPOTHALAMIC LESIONS FAIL TO DISRUPT SELF-STIMULATION OF THE CAUDAL MFB.
M. Waraczynski, K. Conover, and P. Shizgal. Concordia Univ., Montreal, Quebec H3G 1M8

It has been proposed that the dorsomedial hypothalamic nucleus (DMH) gives rise to most or all of the substrate for the rewarding effect of stimulating the medial forebrain bundle (MFB) (Glimcher and Gallistel, Soc. Neurosci. Abstr., 1989, **15**, 33). To test this hypothesis, a caudal MFB stimulation electrode and an ipsilateral DMH lesioning electrode were implanted in male Long Evans rats. Rate-frequency curves were collected at each of 2-3 stimulation currents. For some subjects, the minimum effective stimulation current was also determined. Neither measure of rewarding effectiveness was altered by DMH damage in 6 rats. Some decrease in rewarding effectiveness was noted in the case of two rats in which the lesions extended beyond the DMH to invade the perifornical lateral hypothalamus and caudal MFB. These results challenge the notion that the DMH gives rise to all or most of the substrate for MFB reward. Nonetheless, they do not rule out the possibility that reward fibers in some part of the caudal MFB arise in the DMH.

489.2

ATTENUATION OF MFB REWARD BY ROSTRAL LH LESIONS DEPENDS ON STIMULATION SITE AND CURRENT.
B. Murray and P. Shizgal. Concordia Univ., Montreal, Quebec H3G 1M8

We have previously reported that electrolytic lesions of the antero-lateral portion of the lateral hypothalamus (LH) can reduce the rewarding impact of electrically stimulating more posterior MFB sites (Soc. Neurosci. Abstr., 1988, **14**, 1101); in one subject, the effect of the lesion varied with the stimulation current. To further investigate these phenomena, electrodes were implanted in male rats at 3 levels of the MFB: the anterior LH, middle LH, and anterior ventral tegmental area (VTA). Rate-frequency curves were collected using 3 currents at each middle LH and VTA site. Electrolytic lesions (anodal, 1.0 mA for 10 sec) were made through the anterior LH electrode. In 4 subjects, lesions of the anterior LH produced long-lasting increases of up to 78% in the frequency threshold for self-stimulation of the middle LH and/or VTA sites. The magnitude of these effects was highly dependent upon the stimulation site and current. The lesions failed to alter the frequency threshold in 5 other subjects. These data suggest a precise spatial relationship between reward-relevant neural elements in the anterior, middle, and posterior MFB.

489.3

THE EFFECT OF PERIPHERALLY ADMINISTERED BOMBESIN ON LH STIMULATION-INDUCED FEEDING AND SELF-STIMULATION.
T. Harris, C. Bielajew, and Z. Merali. School of Psychology, Univ. of Ottawa, Ottawa, Ontario, Canada.
Bombesin's purported role in satiety mechanisms prompted this investigation of its effect on lateral hypothalamic (LH) stimulation-induced feeding (SIF) and self-stimulation (SS); these behaviors can be elicited from the same site and may share some common neuronal elements. Using a Latin Square design, SIF and SS thresholds derived from the same electrode were assessed in five rats following four doses of bombesin: 0, 2, 4, and 8 µg/kg i.p. An additional dose of 16 ug/kg was tested in three of the animals. In SIF trials, a 20 sec on/20 sec off alternating cycle of pulse periods was delivered on an ascending logarithmic scale; the threshold was defined as the first period that failed to elicit feeding. SS period thresholds were interpolated from descending rate/period functions using a constant response criterion. For each behavior, thresholds were collected at six current values, ranging from 80 µA to 800 µA. Bombesin at any dose had no effect on SS thresholds but slightly lowered SIF period thresholds at the higher concentrations. These results suggest that unlike other satiety factors (e.g.CCK), periperal bombesin mechanisms are less involved in LH induced reward and feeding.
Supported by NSERC Grant #U0514 to C.B.

489.4

MORPHINE MICROINJECTION INTO THE VENTRAL TEGMENTAL AREA (VTA): EFFECTS OF REPEATED ADMINISTRATION ON LATERAL HYPOTHALAMIC BRAIN STIMULATION REWARD.
P. Bauco, Y. Wang* and R.A. Wise. Center for Studies in Behavioral Neurobiology, Department of Psychology, Concordia University, Montreal, Canada, H3G 1M8.

While some of the behavioral properties of opiates show tolerance following repeated administration, the locomotor effects of morphine injections into the VTA show sensitization or "reverse-tolerance." VTA morphine also facilitates the rewarding effects of lateral hypothalamic electrical stimulation; we assessed the effects of repeated injections to determine whether there is sensitization of this opiate action. Unilateral injections of VTA morphine (2.5µg/0.5µl) were given on alternate days for a total of 10 treatments. These treatments caused leftward shifts in the function relating response rate to stimulation frequency, lowering the "dose" of stimulation required to maintain responding at pre-treatment levels without altering the maximum rates of responding seen with high stimulation doses. There was no apparent sensitization of the ability of morphine to potentiate the rewarding effects of brain stimulation reward. If anything there was a decrease in effectiveness over the course of treatments. This decrease in effectiveness may reflect some degree of pharmacological tolerance or it may reflect local tissue changes caused by the repeated central injections.

489.5

VENTRAL TEGMENTAL NUCLEI OF GUDDEN: A "SEROTONERGIC" REWARDING SITE?
M. Sarkar, D.L. Rosene and C. Kornetsky. Boston University School of Medicine, Boston, MA 02118.

The medial forebrain bundle (MFB) at the level of the lateral hypothalamus (LH) is probably most commonly studied in brain-stimulation reward (BSR) experiments. Although the MFB is rich in dopaminergic (DA), cholinergic (ACh), adrenergic, and serotonergic (5-HT) fibers of passage, current work has mainly implicated the DA system for the rewarding effects of BSR, with ACh and 5-HT serving a complementary inhibitory role. Among the sources of 5-HT fibers in the MFB is a brainstem nucleus, the ventral tegmental nucleus of Gudden (VTg) which gives rise to ascending fibers to the median raphe nucleus, VTA, MFB and other structures. The majority of studies suggesting an inhibitory role for 5-HT in self-stimulation behavior used the MFB-LH as the stimulation site. To investigate the possible role of direct stimulation of 5-HT in BSR, bipolar electrodes were implanted in the VTg and the MFB-LH, respectively, in each of four male F-344 rats. BSR thresholds were determined at each site. The results suggest that VTg stimulation is as effective as MFB stimulation in learning and maintenance of reinforcing behavior. We conclude that VTg is a novel reinforcing site suggesting that 5-HT may have rewarding properties (Supported in part by NIDA grant DA02326 and Research Scientist Award DA00099 to CK).

489.6

THE EFFECTS OF REPEATED PHENCYCLIDINE (PCP) ON LATERAL HYPOTHALAMIC BRAIN STIMULATION REWARD (BSR) AND LOCOMOTION IN RATS.
W. A. Carlezon, Jr. and R. A. Wise, Center for Studies in Behavioral Neurobiology, Department of Psychology, Concordia Univ, Montreal, Quebec, Canada H3G 1M8.

It has been suggested that the rewarding effects of psychomotor stimulants are intimately associated with forward locomotion in rats. Phencyclidine (PCP), like most drugs that are rewarding in their own right, potentiates lateral hypothalamic brain stimulation reward (BSR). In rats trained to lever press for electrical stimulation of the medial forebrain bundle (MFB), this facilitation was demonstrated after acute systemic administration of PCP (2.5 and 5.0 mg/kg) by leftward shifts in the functions that related response rate to stimulation frequency; that is, a lower than normal level of stimulation was sufficient to maintain baseline responding in phencyclidine-treated animals. When animals were tested in the BSR paradigm once per week for 8 weeks, no progressive changes in the facilitating effects of these doses of PCP were observed. On the other hand, another group of animals administered PCP (5.0 mg/kg) in a chamber outfitted with photocells demonstrated a progressive increase in sensitivity to the locomotor-stimulating effects of the drug with repeated testing. Thus, these data suggest that the reward-facilitating and at least one of the psychomotor stimulant effects of this drug can be dissociated.

489.7

FAILURE OF CHRONIC ESTROGEN TO ALTER LATERAL HYPOTHALAMIC BRAIN STIMULATION REWARD. C.M. Pudiak & M.A. Bozarth, *Department of Psychology, University at Buffalo, Buffalo, NY 14260.*

The threshold tracking procedure was used to determine the effect of chronic estrogen replacement after ovariectomy on brain stimulation reward (BSR). Female, Long-Evans rats were implanted with monopolar electrodes aimed at the lateral hypothalamic level of the medial forebrain bundle. After 5 to 10 days recovery from surgery, rats were trained to lever press for monophasic cathodal stimulation pulses during 60-min daily test sessions. The threshold tracking procedure determined the minimum stimulation frequency that maintains a predetermined response rate (i.e., 30 presses/min).

Following ovariectomy, 20 μg of 17-β-Estradiol was subcutaneously injected for 4 days followed by 4 days of vehicle injections; this injection series was repeated for 3 cycles. Chronically administered estradiol had no effect on BSR thresholds. These data along with previous data examining the effect of acute estrogen (Pudiak & Bozarth, *Soc. Neurosci. Abstr.*, 1990) suggest that estrogen does not influence the rewarding impact of lateral hypothalamic BSR.

489.8

EFFECTS OF LATERAL PREOPTIC AREA KNIFE-CUTS ON POSTERIOR HYPOTHALAMIC SELF-STIMULATION REWARD J.R. Stellar and Hugh Albert*, Psychology Dept., Northeastern University, Boston, MA 02115

Previously we reported that coronal knife-cuts placed in mid-lateral hypothalamus could be effective in reducing posterior hypothalamic self-stimulation reward in the rate-frequency paradigm using multiple stimulation currents. This effect was particularly strong at low currents (Stellar et al. NS Abst 16:591, 1990).

This year we extended these findings to coronal knife-cuts placed farther anterior, in the lateral preoptic area, keeping electrode placement the same. Half-maximal rate-frequency reward thresholds were raised (reward decrease) about 0.1 - 0.4 log Hz and were related to the size of the knife cut. Cuts ranged from minimal ventral damage just above the optic chiasm to a medial forebrain bundle transection that ran the entire dorsal-ventral length of the lateral preoptic area. Reward thresholds were less dependent on current than previously reported, suggesting that electrode/knife-cut alignment was not as important with these placements.

(Supported by the Whitehall Foundation)

489.9

MODELLING DRUG SELF-ADMINISTRATION WITH SELF-ADMINISTRATION OF BRAIN STIMULATION (SABS): STABILITY OF SABS WITH PARTIAL REINFORCEMENT SCHEDULES. Marino Lepore*, Keith B.J. Franklin and Frances V. Abbott., McGill University, 1205 Dr Penfield Ave, Montreal, Que., Canada H3A 1B1

We have previously shown that rats trained on conventional self-stimulation will self-administer trains of brain stimulation that rise and fall in pulse frequency with a time course that mimics the rise and fall of drug concentrations in the brain. We now report that rats will spontaneously acquire such self-administration of brain stimulation, and that the pattern of behaviour is stable when "doses" of brain stimulation are delivered on a partial reinforcement schedule. As response requirement is increased from FR5 to FR15, the mean response rate increases while the mean frequency of stimulation (analogous to mean plasma drug concentration) decreases. This relationship is also found in drug self-administration. Another characteristic feature of drug self-administration is that the rate of self-administration decreases as the elimination half-life of the drug increases. This characteristic is also mimicked by SABS on a partial reinforcement schedule. Using single frequency modulated 16 sec trains of brain stimulation as reinforcers on an FI60 sec schedule we have shown that the rate of "absorption" contributes to the overall reinforcing effect of a stimulation train.

489.10

EFFECTS OF MOTIVATIONAL STATE ON OPERANT BEHAVIORS DIFFERENTIALLY SENSITIVE TO CHANGES IN REINFORCER VALUE. M. Vigorito, J.C. Carretta*, C.B. Kruse* and S.A. Radice*. Dept. of Psychology, Seton Hall University, South Orange, NJ 07079.

Rate-reducing effects of neuroleptic drugs on operant behaviors reinforced with food have been interpreted as resulting from the reduction in the reward value of the reinforcer. However, operant response rates are not always sensitive to changes in the value of the reinforcer. We previously found that in well-trained hungry rats VR rates were more sensitive to reductions in the value of a sucrose reward than VI rates; increases in the value of the reinforcer did not elevate response rates in either schedule. In the present experiment we replicated our earlier findings when rats were hungry and found that when the rats were not hungry sensitivity to reinforcer reductions was enhanced in both schedules. Moreover, an increase in the reinforcer value under the ad libitum condition elevated VI rates but not VR rates. We suggest that the pharmacological manipulation of operant behaviors that are differentially sensitive to changes in reinforcer value can be useful in understanding how drugs alter behaviors motivated by food reward.

489.11

SELF-ADMINISTRATION OF ELECTRICAL SELF-STIMULATION OF THE BRAIN.
E.O. McCaskill, R. Allen*, J.R. Stellar, Dept. of Psychology, Northeastern University, Boston, MA 02115.

Striking response differences have been observed between Intracranial Electrical Self-Stimulation (ICSS) and Intravenous Drug Self-Administration in rats. Recently these differences have been attributed to temporal parameters (Franklin et al. NS Abstracts:15:1092,1989). In our study, ICSS was delivered in a "cocaine-like" waveform in which pulse frequency changed each second over a 50-150 second period, rising quickly to a peak and then decaying slowly. Responding during a waveform triggered a new waveform which was added to the ongoing one.

As with cocaine, rats responded with periods of high lever press rates alternating with periods of abstinence. Decreasing ICSS current, or "dose", resulted in increased responding with some rats following the standard ICSS psychophysical trade-off between current and self-selected pulse frequency. Decreasing the duration of the waveform also resulted in increased responding.

(Supported by the Whitehall Foundation)

489.12

LOCUS COERULEUS STIMULATION-INDUCED PLACE PREFERENCES ARE PREVENTED BY PRETREATMENT WITH THE DOPAMINE ANTAGONIST PIMOZIDE. C.L. Duvauchelle, L.A. MacConell*, A. Eremia* and A. Ettenberg, Behavioral Pharmacology Lab, Dept. of Psychology, University of California, Santa Barbara, CA 93106.

Electrical stimulation of the norepinephrine cell bodies in the locus coeruleus (LC) has been shown to support self-stimulation behaviors in rats. The present study employed a Conditioned Place Paradigm to test the hypothesis that dopamine antagonism can attenuate the reinforcing properties of LC stimulation. Animals were pretreated with pimozide (0.0, 0.5, 1.0 mg/kg) prior to exposure to one of two distinctive environments paired with rewarding LC stimulation. While LC stimulation resulted in reliable preferences for the stimulation-paired environment, this effect was dose-dependently attenuated by pimozide pretreatment. Since pimozide alone does not result in place aversions, it was hypothesized that dopamine neurotransmission may be important for the brain stimulation reward resulting from activation of cell bodies even far removed from dopaminergic fiber systems.

489.13

AUTOTITRATION SELF-STIMULATION ANALYSIS OF INDIVIDUAL DIFFERENCES IN THE RAT'S RESPONSE TO AMPHETAMINE. D. B. Neill and R. Puett*, Dept. of Psychology, Emory University, Atlanta, GA 30322.

It has recently been reported that it is possible to predict the "vulnerability" of rats to self-administration of amphetamine by their locomotor response to a novel environment. Rats showing a high locomotor response ("high responders") will acquire self-administration at doses below those necessary for "low responders." We examined the reaction of "high responders" and "low responders" to amphetamine using medial forebrain bundle/lateral hypothalamic self-stimulation. Rats were selected on the basis of their 1 hr locomotor response to a novel environment (Digiscan activity device). Those in the top and bottom one thirds of the activity distribution were implanted with a bipolar electrode in the lateral hypothalamus. All were trained to self-stimulate using the autotitration procedure where intensity dropped every 5 responses but could be reset to the maximum by a response on a second lever. We found that "high responders" showed greater responding and faster reset times; the responses significantly correlated with the previously determined locomotor activity. However, there was no difference between the two groups in response to amphetamine sulfate over a dose range of 0.5-2.0 mg/kg. Thus the "vulnerability" to self-administer amphetamine does not seem related to the functioning of the medial forebrain bundle "reward system."

489.14

CHRONIC DESMETHYLIMIPRAMINE ADMINISTRATION ATTENUATES STRESSOR INDUCED ICSS ALTERATIONS FROM THE PREFRONTAL CORTEX. C. Wolfe and R. M. Zacharko. Department of Psychology, Carleton University, Ottawa, Ontario, K1S 5B6.

Uncontrollable stressors have been found to induce a decline in responding for previously rewarding intracranial self-stimulation (ICSS) from a number of mesocorticolimbic sites. The stressor provoked attenuation of the rewarding properties associated with electrical brain stimulation has been proposed to be reminiscent of the anhedonia evident in clinical depression. In the present study, ICSS was assessed from the prefrontal cortex in CD-1 mice immediately, 24-hrs and 168-hrs following exposure to uncontrollable footshock. Marked reductions in ICSS rates were observed in all mice immediately following stressor exposure. Animals in which the ICSS deficits were still evident 1-week post stressor were administered either saline or desmethylimipramine (DMI) twice per day for a 20 day period. Chronic DMI administration ameliorated the stressor induced deficits in ICSS from the prefrontal cortex. Potential explanations for the stressor provoked variations in ICSS and the effects of DMI are discussed.

489.15

EFFECT OF INTRACRANIAL SELF-STIMULATION ON SELECTED PHYSIOLOGIC PARAMETERS IN RATS M.L. Burgess*, J.M. Davis*, T.K. Borg* and J. Buggy. Depts. of Exercise Science, Pathology and Physiology, University of South Carolina, Columbia, SC 29208.

The purpose of this investigation was to characterize selected metabolic, cardiovascular and hormonal responses to reinforcing intracranial self-stimulation (ICSS) of the ventral tegmental area (VTA) in rats. Ten, male Sprague Dawley rats were stereotaxically implanted with bipolar electrodes aimed at the VTA, origin of the dopaminergic mesolimbic projection, and trained to lever-press for ICSS. They were then adapted to the experimental environment (a metabolic operant chamber) for 30 min/d, 5 d/wk for 12 wks during which they were connected to the electrode cable but did not lever-press. At the end of 12 wks, all rats were instrumented with indwelling arterial catheters and performed a 30 min lever-pressing session in the metabolic operant chamber. Oxygen consumption, heart rate, blood pressure and rectal temperature increased with the onset and continuation of ICSS over 30 min ($p<0.05$). Plasma norepinephrine, epinephrine and corticosterone increased significantly over resting values ($p<0.05$). The results suggest that ICSS elicites heightened sympathetic, metabolic and neuroendocrine activity. These responses provide important information for studies that use ICSS as a motivator of behaviors, such as physical exercise in rats. They also provide valuable information about the physiologic consequences that may be related to inappropriate reinforcing behaviors, such as addictive drug abuse.

489.16

CAN METAL SELF STIMULATION WIRES BE USED IN AN ELECTROMAGNETIC FIELD? C. J. Sherry[1]*, T. J. Walters[1]*, P. A. Mason[2], J. M. Ziriax[2]*, C.J. Gonzales[1]*, M. H. Berent[3]*.
[1]Systems Research Laboratories, [2]Operational Technologies, [3]Armstrong Laboratory, Brooks AFB, Tx 78235-5301.

Most investigators appear to believe that metal wires, typically used to stimulate the brain or record its electrical activity, cannot be used in animals that will be exposed to radio frequency radiation (RFR) since RFR could couple to the wires, causing current flow (stimulating the brain) or a local temperature increase (causing a les ion).

Following an approved protocol, under aseptic conditions, a twisted pair of insulated nicrome wires (dia. 250 μm) were implanted in the central gray within the brains of rats (AP -6.9, L 3.28 (30°),D 5.76 mm). Stimulating (or lesioning) this site causes a species specific withdrawal response (WR). After recovery, the electrical stimulation threshold was determined and the rats were exposed to 9.3 GHz pulsed RFR in the near- and far-fields. During the RFR exposure rats did not display the WR, but did display thermoregulatory behavior (spreading saliva on face/ears). The rats did display WR when stimulated during RFR exposure. The stimulation threshold did not change after exposure, which suggests that exposure did not cause a lesion; this was confirmed histologically.

BIOLOGICAL RHYTHMS AND SLEEP IX

490.1

MANDUCA NEURAL ANTIGENS REVEALED BY ANTIBODY TO *DROSOPHILA PERIOD* PROTEIN. Kathleen K. Siwicki and Jessica L. McGrew*. Biology Department, Swarthmore College, Swarthmore, PA 19081

The *period* (*per*) gene of *Drosophila melanogaster* regulates the periods of behavioral circadian rhythms. An antibody to the *per* protein was used previously to localize its expression to specific cellular sites in the fly nervous system, and to monitor a circadian rhythm in the protein (Siwicki et al., 1988, Neuron 1: 141-150; Zerr et al., 1990, J. Neurosci. 10: 2749-2762). We have used the same antibody to study *per*-related proteins in a larger insect, the hawkmoth *Manduca sexta*, with the long-term objective of investigating the effects of *per* on cellular physiology.

In *Manduca* larvae, anti-*per* staining was found in 2 pairs of large (40 μm) dorsomedial brain neurons. In adult moth nervous systems, a variety of cellular sites were labeled, including nuclei of many small cortical cell bodies, cytoplasmic granules in some large neurons, and glial nuclei in neuropil regions. Many aspects of this staining pattern are reminiscient of the distribution of the *per* protein in *Drosophila*. We are now extending these studies to developing adults, in hopes of finding structures that might be involved in the circadian gating of eclosion.

Also, in immunoblots with protein extracts of moth brains, 44 kD and 110 kD *per*-like antigens were detected. We are now looking for daily rhythms in the levels of these antigens as a possible indication of functional homology with *per*.

Supported by NSF grants BNS-9010691 and BNS-9057703, and the Swarthmore College Faculty Research Fund.

490.2

LABELLING OF CELLS IN THE CNS OF THE CRICKET TELEOGRYLLUS COMMODUS BY AN ANTIBODY TO THE DROSOPHILA PER-PROTEIN. H.W. Honegger, W. Leser, W. Loher* and K.K. Siwicki**. Inst. of Zoology, TU Munich, D- 8046 Garching, Germany, *Dept. of Entomology, Univ. of California, Berkeley, CA 94720, **Biology Dept. Swarthmore College, Swarthmore, PA 19081.

The cricket Teleogryllus commodus shows a clear circadian rhythm of its calling song. We have stained sections of the CNS of Teleogryllus with an antibody against the Drosophila period (per) protein, which has been used previously to label the sites of per expression in Drosophila and putative circadian pacemaker neurons in the eyes of Aplysia and Bulla. Singing male crickets were sacrificed at four time points of a 12h:12h light-dark cycle. The brain with the retrocerebral complex, the suboesophageal ganglion, and occasionally the ventral nerve cord were fixed in 4% paraformaldehyde. Frozen sections were stained with affinity-purified anti-per antibody and the avidin-biotin-HRP method.

The anti-per antibody consistently labelled two somata (approximately 20 μm in diameter) on each side of the lateral protocerebrum, with their axonal branches in all areas of the CNS (including the optic lobes, the mushroom bodies, and the central body). Two fibers project via each NCCII into the corpora cardiaca, the corpora allata and the hypocerebral ganglion. In the cell bodies and the corpora cardiaca, the intensive staining was independent of the phase of the light-dark cycle or the crickets' state of activity. But in the labelled projections throughout the CNS, staining was distinctly lower at "lights-on" than at other times of day, suggesting that they may be involved in daily rhytmic functions.

490.3

AN ANTIBODY AGAINST AN APLYSIA EYE-SPECIFIC 48K PROTEIN RELATED TO THE PERIOD-PROTEIN OF DROSOPHILA SPECIFICALLY STAINS THE APLYSIA RETINA AND OPTIC PROJECTIONS INTO THE BRAIN. S. Strack* and J.W. Jacklet, Dept. Biology and Neurobiology Research Center, SUNYA, Albany, NY 12222.

We have partially sequenced the 48K protein, the major Aplysia eye antigen recognized by a Drosophila *period*-protein antibody (Neuron 3:51-58). Preparative 2-D gel electrophoresis followed by V8 digestion and sequencing yielded a 20 residue and a 10 residue sequence. Sequences are not similar to the *period*-protein but the short sequence shows homology to cAMP-dependent protein kinases. By metabolic labeling, we have found that the 48K protein is synthesized exclusively in the retina and transported along retinal afferents. Subcellular localization experiments show that 48K is likely to be associated with the cytoskeleton or cell surface. An antibody raised against the long sequence recognizes a family of 47K-48K, Pi 5.9-6.1 spots on 2-D Westerns, and on Aplysia tissue sections stains fibrous structures in the retina and optic nerve. Retinal somata are not stained. The projections of retinal afferents into the cerebral ganglion stained, revealing extensive terminal fields of afferents in the "optic ganglion". Some stained fibers cross over to the contralateral half of the cerebral ganglion, or follow the tentacular and other nerves. Other fibers form tracts and terminal areas in the ganglion. Some continue to the pleural and pedal ganglia and beyond to the abdominal ganglion. These results agree with previous optic tract tracing studies using tritiated markers (J.Neurosc. 5:3214) and identify the putative primary brain synaptic areas of retinal afferents. Localization of anti-48K on the EM level is being done. The visual system of Bursatella, a close relative of Aplysia, is stained by anti-48K, but not the visual system of Bulla, a more distantly related ophistobranch. (Supported by NSF grant BNS 8819773 to JWJ)

490.4

TYROSINE PHOSPHORYLATION AFFECTS THE CIRCADIAN PACEMAKER IN THE EYE OF THE MARINE SNAIL, BULLA GOULDIANA. M.H. Roberts and J.A. Towles*. Department of Biology, Clarkson University., Potsdam, NY 13699.

Previously we have shown that inhibition of kinase activity lengthens the period of the circadian rhythm recorded from the isolated eye of Bulla gouldiana (Roberts, et al., 1989). We now report the results of additional inhibitor studies.

The circadian rhythm in optic nerve impulses is unaffected by chronic treatment with okadaic acid (0.5 uM), a specific inhibitor of 1 and 2A protein phosphatases in vivo (Haystead et al. 1989). This raises the possibility that serine/threonine phosphorylation/dephosphorylation is not involved in rhythm regulation. In order to investigate this possibility, we found that phenylarsine oxide, a tyrosine phosphatase inhibitor (Bernier et al. 1987), irreversibily blocks impulse activity (5–100 uM).

In spite of the interpretive difficulties caused by the irreversibility of the treatment, we have further explored the role of tyrosine phosphorylation with genistein, a reversible inhibitor of tyrosine kinase activity (Akiyama, et al., 1987). Chronic moderate doses of genistein (11 uM) eliminate impulse activity; upon drug removal impulses return as do light responses, although the circadian rhythm is absent. At lower doses (3.7 uM) period is lengthened while even lower doses (0.37 uM) have no effect on the rhythm. Genistein's actions are phase dependent. When applied from CT 2–6 phase delays (–3.0 hrs) are induced. From CT 22–2 the rhythm is damped and phase advanced (+1.5 hrs) or completely eliminated, although impulses and light responses remain. Taken together, these results suggest that tyrosine phosphorylation/dephosphorylation plays a role in circadian rhythm regulation in the Bulla eye. Supported by NS26272 to MHR.

490.5

POTASSIUM CHANNELS MEDIATE CIRCADIAN CONDUCTANCE CHANGES IN PACEMAKER CELLS OF *BULLA*

S. Michel and G.D. Block, Department of Biology, Gilmer Hall, University of Virginia, Charlottesville, VA 22901

The eyes of the marine snail *Bulla* express a circadian rhythm of compound action potentials (CAP) in vitro driven by endogenous changes in membrane potential of basal retinal neurons (BRN), which are believed to be the pacemaker cells. Intracellular recordings and conductance measurements reveal circadian and light-induced changes in membrane conductance of the BRN. We used the current clamp technique to measure the current-voltage relationship of the cell membrane. Before dawn (circadian time = CT 23) the conductance of BRNs decrease followed by depolarization to -55 mV. During the early subjective night (CT 13-15) the conductance increases again and the membrane potential returns to hyperpolarized values (-65 mV). Bath application of the K^+ channel blocker TEA (100mM) at CT 20 leads to a reversible decrease of conductance and a depolarization of the cell membrane by 10 mV. The current-voltage curve in TEA at CT 20 is similar to the one measured after dawn (CT 3) in normal seawater. When TEA is given at this time (CT 2) no additional decrease in conductance was observed. The results suggest that K^+ currents, which are activated at hyperpolarized membrane potentials, like the inward rectifier, are involved in the circadian changes of membrane conductance. K^+ blockers like TEA are able to simulate the conductance changes near dawn, but have little effect after dawn when the channels are supposedly already closed. We are currently investigating the effect of other K^+ channel blockers like Ba^{2+}, 4-Ap and Quinine to describe the pharmacology of this inward rectifier in order to further characterize and identify the type of channel, which probably is an essential part of the output pathway of the circadian pacemaker in *Bulla*. Supported by NS 15264 to GDB and DFG-Mi328/1-1 to SM

490.6

THE ROLE OF EXTRACELLULAR Ca^{2+} IN THE GENERATION OF THE CIRCADIAN RHYTHM IN *BULLA* EYE. S.B.S. Khalsa, M.R. Ralph & G.D. Block. Dept. of Biology, Univ. of Virginia, Charlottesville, VA 22901.

The eye of *Bulla* contains a population of electrically coupled retinal cells which functions as a competent circadian pacemaker, expressing a rhythm in compound action potential (CAP) activity which can be recorded from the optic nerve in artificial seawater (ASW). Previous studies have indicated that extracellular Ca^{2+} is involved in the entrainment of the pacemaker; in this study we assess its role in the generation of the circadian rhythm.

To assay the state of the pacemaker during low Ca^{2+} treatment, various long duration pulses of low Ca^{2+} ASW (no added Ca^{2+}, 10 mM EGTA) were applied, and the phase of the subsequent rhythm was measured. All pulses started at CT 6 during the first cycle and pulse durations to different preparations ranged from 4 to 72 hrs. Although no phase shifts were apparent following pulses that ended before subjective dawn (CT 24, pulse durations 4-16 hrs), phase shifts of about 4 hrs were observed for those pulses that ended after subjective dawn (durations 20-36 hrs). Furthermore, for longer pulses that included the next subjective dawn (durations > 40 hrs), phase shift magnitude increased to about 8 hrs.

These data suggest that the pacemaker was not stopped by the treatment, since the subsequent phase remained correlated to the phase of untreated control eyes, and was not solely determined by the timing of the treatment. Furthermore, although most CAP activity records showed no evidence of rhythmicity during low Ca^{2+} treatment, some did show clear circadian rhythms with a phase indistinguishable from that of controls. Together, these data suggest that extracellular Ca^{2+} is not an essential requirement for rhythm generation; the phase shifts observed to long pulses of low Ca^{2+} ASW may be a specific effect of the pulsatile treatment. *Support by NS15264.*

490.7

OPSIN-LIKE IMMUNOREACTIVITY IN THE PUTATIVE PACEMAKER NEURONS OF THE BULLA EYE. M.E. Geusz, R.G. Foster, M.D. Lawrence*, and G.D. Block. Dept. of Biology, University of Virginia, Charlottesville, VA 22901.

A circadian rhythm in the frequency of compound action potentials in the optic nerve of the mollusk Bulla gouldiana is believed to be generated by the basal retinal neurons (BRNs) of the eye. Light responses from the BRNs persist in eyes with the distal photoreceptor layer surgically removed, and the circadian rhythm can still be phase shifted with light. In addition, artificial seawater with low $[Ca^{2+}]$ and high $[Mg^{2+}]$ fails to block the light response. However, further evidence that the BRNs are intrinsically photosensitive has been lacking.

An antiserum generated against the cone opsin of chickens has now been found to preferentially label the BRNs and their processes, suggesting that the protein involved in photoreception is present in the BRNs. In one group, eyes were treated with the antibody following lens removal, fixation, cryoprotection, sectioning at -20°C, and treatment with normal goat serum (NGS). In a second group, desheathed retinas were treated with the antibody after fixation and treatment with NGS, but prior to dehydration and embedding in the resin Durcupan. The retinas and eye sections were processed according to the avidin-biotin-HRP procedure. In both groups, the BRNs were stained with the diaminobenzidine (DAB) peroxidase product. Control retinas and eye sections, incubated with NGS alone, lacked staining. The anti-cone opsin antibody failed to label the distal photoreceptors, suggesting possible differences between the photopigments of the BRNs and the distal photoreceptors. Supported by NS08806 to MEG and NS15264 to GDB.

490.8

IS THE OCULAR CIRCADIAN CLOCK LOCATED IN PHOTORECEPTORS? G.M. Cahill and J.C. Besharse, Department of Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, KS 66103.

An ocular circadian oscillator regulates retinal melatonin synthesis in cultured *Xenopus* eyecups, which include the retina, pigment epithelium (RPE), choroid and sclera. Several lines of evidence suggest that melatonin is produced in photoreceptors. We report here on tissue reduction experiments that suggest that photoreceptors are also capable of circadian rhythm generation. Isolated neural retinas, maintained in darkness in superfusion culture, produce circadian rhythms of melatonin for up to 3 cycles. The peak:trough relative amplitudes of these rhythms are normal, but melatonin levels are reduced 75-90%, suggesting that an oscillator is located in the neural retina, but that interaction with the RPE is necessary for maximal melatonin production. Eyecups without neural retina do not produce melatonin. We have developed methods that produce nearly complete lesions of the inner retina, but leave the photoreceptor layer largely intact. The vitreal surface of the retina within an eyecup is washed sequentially with detergent (1% Triton X-100 or SDS), deionized water, and culture medium. This results in lysis of inner retinal cells and splitting of the retina within the inner nuclear layer (INL). The inner retina can then be peeled away, leaving intact photoreceptors adhering to the RPE. These lesioned eyecups, some with >97% of INL cells destroyed, produce circadian rhythms of melatonin for 3 days. Although contributions from other cells can't be ruled out, these data suggest that photoreceptors can generate circadian melatonin rhythms.

490.9

TAIL PHOTORECEPTORS IN *LIMULUS* ENTRAIN THE VISUAL CIRCADIAN RHYTHM OF THE LATERAL COMPOUND EYES. George H. Renninger and W.J. Brad Hanna, Biophysics Group, University of Guelph, Guelph, Ontario N1G 2W1

Electroretinograms (ERGs) of the anterior photoreceptor organs [lateral compound eyes, median ocelli (1), and ventral eye (2)] of the horseshoe crab, *Limulus polyphemus*, exhibit a circadian rhythm in amplitude, driven by efferent optic nerve activity from the brain to the anterior photoreceptor organs during subjective night (1). Light pulses to these photoreceptor organs can shift the phase of the rhythm (1,3). Illumination of the tail alone can also shift the rhythm's phase (4).

We report here that periodic illumination of just the tail can entrain the rhythm in the ERG amplitude of the lateral eyes. The threshold light intensity for entrainment is consistent with that observed for light pulses to the tail to shift the phase of the rhythm (4). Illumination of the anterior photoreceptors alone can also entrain the rhythm.

While the tail and the anterior photoreceptor organs separately can entrain the lateral eye rhythm, there are differences in their influence on the rhythm: (i) the phase relation of the entrained rhythm to the entraining light depends on which photoreceptors (tail or anterior) are exposed to light; and (ii) the anterior photoreceptors produce larger phase shifts than the tail, when exposed to long light pulses (up to 12 h) of similar intensities.

(1) Barlow *et al.*, Science **197**: 86, '77; Barlow, J. Neurosci. **3**: 856, '83. (2) Kass & Renninger, Vis. Neurosci. **1**: 3, '88. (3) Horne & Renninger, J. Comp. Physiol. **A162**: 127, '88. (4) Hanna *et al.*, J. Comp. Physiol. **A162**: 133, '88.

490.10

TRANSCRIPTION AND TRANSLATION APPEAR CLOSELY COUPLED IN THE OCULAR CIRCADIAN SYSTEM OF *Aplysia*. C. Koumenis*, U. Raju, and A. Eskin. Dept. of Biochem. and Biophys. Sci., Univ. of Houston, Houston, TX. 77204

A model of the circadian system in the eye of *Aplysia* suggests that proteins are elements of the oscillator mechanism. Regulation of the synthesis of these proteins can occur at the transcriptional or translational level, or both. Translation inhibitors such as cycloheximide (CHX) have been shown to phase-shift the rhythm and also to block some of the effects of entraining agents.

Previously, we have shown that pulse treatments of isolated eyes with the reversible transcription inhibitor 5,6,1-b-d-dichlororibobenzimidazole (DRB) phase-shift the rhythm, while continuous treatments of DRB lengthen the free-running period of the rhythm. Camptothecin (a transcription inhibitor with a different site of action from DRB) also caused phase-shifts and period lengthening.

Is there a relationship between the effects of transcription and translation inhibitors on the rhythm? The onsets of the sensitive periods of the phase-response curves to CHX and DRB coincide. This similarity indicates that transcription and translation are closely coupled processes in the regulation of the rhythm. This conclusion is also supported by the rapid appearance of delay phase-shifts following DRB treatment.

To further investigate the roles of transcription and translation, we looked for proteins whose synthesis was affected by DRB. Overall protein synthesis as measured by leucine incorporation was not affected during a 2h DRB pulse. However, using 2D gel electrophoresis, we found that DRB altered the incorporation of ^{3}H-leucine into a small number of proteins. Some of these proteins may mediate the effects of DRB on the rhythm. A more detailed role for transcription and translation in the regulation of the oscillator will require the identification and study of these important proteins and their mRNAs.

490.11

CIRCADIAN BILATERAL EQUALIZATION OF CRAYFISH RETINAL SENSITIVITY. J.A. Barral*, E. Barrera-Calva*, O.A. Barrera Calva*, and B. Barrera-Mera. Proyecto y Maestría en Neurociencias. ENEP-Iztacala-U.N.A.M.; E.N.P. No.2-U.N.A.M. and Fisiología-Medicina-U.N.A.M.-A.P. 70-250, 04510 México, DF

As the crayfish processes the visual information of environmental movements and shapes, an instantaneous adjustment in photosensitivity of retina simultaneously occurs, as measured by the amplitude of electroretinogram (ERG). The last phenomenon is caused by an efferent clock-work program from the central nervous system. Anatomical, as well as enzimatic changes induce the neural modifications to become the main event revealing circadian photosensitivity. Aside from such a central regulation, however, a peripheral source of modulatory action arises from contralateral retinal photoreceptor. Its activity is explained by its own temporal course over the bilateral equalization of retinal photosensitivity, which suggests reciprocal left and right modulation of crayfish retina operating in a sustained manner. In order to determine its retinal origin, as well as its direct and indirect pathways through the protocerebral and the subesophageal ganglion respectively, we tested the effects of sustained light stimulation upon the contralateral eyestalk in all animals: intact, lesioned, with protocerebrum only, and splitbrain. A long latency time of 90 to 115 min to diminish ERG in the last animals but not in the rest suggests contralateral modulatory influences which travel throughout subesophageal ganglion.

490.12

THE CRAYFISH CAUDAL PHOTORECEPTOR IN VITRO, SHOWS A CIRCADIAN PATTERN IN THE RESPONSE TO LIGHT. M. L. Fanjul-Moles, J. A. Prieto-Sagredo* and A. Bernal-Moreno*. Lab. de Invertebrados, Fac. de Ciencias, U.N.A.M., Ap. Postal 70-371, México D.F., o4510, MEXICO.

In order to investigate a possible circadian rhythm in the caudal photoreceptor (CPR) of the crayfish, in vitro, spontaneous (SA) and light evoked activity (LEA) were recorded. The abdominal nerve cord was isolated from a group of 10 adult crayfish maintained previously in L:D 12:12. Henceworth the preparation was immersed in Van Harreveld solution at least 72 hours under constant temperature and darkness. The SA and LEA relayed by a suction electrode to a Grass P511 AC preamplifier were registred with an oscilloscope and captured in a PC computer to be analyzed. As a control, every hour during 72 hours the SA was recorded for 10 s. Afterwards, a 10 s light stimulus of 6500 lux was delivered to the CPRs and LEA was recorded. The results indicate a circadian pattern both in the SA and in the LEA. The rhytm showed a peak between 18 and 21 hours (external time) and a period value of approximately 21 hours.

This work was partially supported by PADEP-UNAM and CONACyT (Pos. Con. 39)

490.13

ASYMMETRICAL PHOTOPERIODS SYNCHRONIZE THE ERG CIRCADIAN RHYTHM IN CRAYFISH. E. Moreno-Sáenz and B. Fuentes-Pardo. Depto. de Fisiología, Facultad de Medicina, UNAM. A.P. 70250, México 04510, D.F.

The ERG circadian rhythm (CR) of the crayfish can be synchronized by both complete photoperiods (PPc) and skeleton photoperiods (PPs) (data from our laboratory). These facts led us to study the effect of asymmetrical skeleton photoperiods (PPa) on the characteristics of the ERG CR. The ERG from adult crayfish Procambarus digueti, of either sex, housed individually in a constant temperature chamber was recorded by conventional procedures for periods up to 8 days. The PPa used were equivalents to the following PPc: 8:16, 11:13, 12:12, 13:11, 16:8 and 20:4. Each PPa consisted of two light signals of 4 and 1 h duration, respectively, the first one applied at the beginning and the second one at the ending of each PP. After the animals were stimulated with PPa, they were left in free run. The results suggest that the ERG CR is synchronized to all the PPa adjusting its phase of maximal amplitude to the longer dark time. This means that, through a phase jump, the crayfish, as many other species, modifies its subjective night phase in order to increase the night duration when the time between the two light signals, determining the night length, is too short.
Supported in part by CONACyT (P228CCOX891642).

491.1

EFFECTS OF FEEDING SCHEDULES ON LABELLED 2-DEOXYGLUCOSE UPTAKE IN RAT BRAINS. G.J. Coleman, J. Redman[1] and M. Brown[1] Dept Psychology, La Trobe Univ. Bundoora 3083, Australia and Dept Psychology, Monash Univ. Clayton 3168 Australia[1].

The anatomical location of the postulated food-entraind oscillator in rats has not been identified. This study was designed to compare ^{14}C-labelled 2-DG uptake in the brains of rats exposed to either a feeding schedule or to random feeding.

Rats were maintained on either a random or fixed 2-hr daily meal schedule under an LD cycle for 59 days. Rats were then killed, their brains frozen then later sectioned, filmed and subjected to densito-metric analysis.

Random-fed rats showed substantial disruption of wheel-running activity while meal-scheduled rats showed the usual activity anticipatory to the meal. In the brain areas analysed, differences between treatments were only observed in the SCN and VMH where random-fed rats showed lower levels of 2-DG uptake than did meal-scheduled rats.

These data are preliminary, but suggest that there is no evidence yet for an anatomically distinct site for a food entrained oscillator.

491.2

SUPRACHIASMATIC NUCLEI AND PHOTOINDUCED TESTICULAR DEVELOPMENT IN SIBERIAN HAMSTERS. K.Kelly*, J.Dark, & I.Zucker. Dept. of Psychology, University of California, Berkeley, CA 94720.

Light influences the mammalian neuroendocrine system by altering the duration of pineal melatonin secretion. The suprachiasmatic nucleus (SCN) is a melatonin target tissue involved in the neural innervation of the pineal gland. The present study assessed whether changes in SCN activity beginning several days after animals received a single inductive light stimulus are essential for gonadal growth in photoregressed Siberian hamsters. A single long day at 19 days of age produces sustained testes growth over the course of the next two weeks in male animals maintained in short daylengths. At 35 days of age testes weights of light-treated hamsters were significantly greater than those of untreated animals (143±43 vs 31±4 mg). Hamsters that sustained complete ablation of the SCN 3 days after the light treatment had testes weights of 291±35 mg ($p<0.001$ relative to untreated controls); those that received SCN lesions only, had testes weights of 147±45 mg. These results indicate that changes in SCN neural activity are not necessary for photic stimulation of gonadal growth once the light stimulus has been perceived and neuroendocrine changes initiated. Endocrine sequelae of SCN damage, including hyperprolactinemia, may account for the greater than normal testes weights of light-stimulated hamsters with SCN damage.

491.3

CHANGES IN PROLACTIN AND CORTICOSTERONE ASSOCIATED WITH PUBERTY AND PHOTOPERIOD IN THE COLLARED LEMMING, DICROSTONYX GROENLANDICUS. B. Gower, T. R. Nagy* and M. H. Stetson*. School of Life & Health Sciences, Univ. of Delaware, Newark, DE 19716 (BG, MHS), and Dept. of Biology, Univ. of Utah, Salt Lake City, UT 84112 (TRN).

The effect of photoperiod on the onset of puberty and associated neuroendocrine changes was examined in male collared lemmings, Dicrostonyx groenlandicus. Animals were gestated in either a 16:8 or an 8:16 photoperiod. Those from the 16:8 photoperiod were switched to either a 20:4 or an 8:16 photoperiod at weaning, while those from the 8:16 photoperiod remained in 8:16 throughout the experiment. At 33, 38, 43, and 48 days of age, groups were sacrificed and individuals examined for body, testes, and seminal vesicle weights and the presence of epididymal sperm. Serum was collected and later analyzed for prolactin, corticosterone, and testosterone. Maturation occurred significantly earlier, by 5 d, in the 20:4 group than in the group switched to 8:16; the group raised in 8:16 was not different from either of the other 2 groups. Testes and seminal vesicle weights were similar in the 20:4 group and the group raised under 8:16, while those animals switched to 8:16 had significantly lower tissue weights. Serum prolactin was significantly depressed by short photoperiod (both 8:16 groups), as was serum corticosterone. These data suggest that, although significant, the effect of photoperiod on reproductive development is slight. Photoperiod does, however, influence the neuroendocrine system during the peripubertal period. The low levels of prolactin observed in the 8:16 animals suggest that this hormone is not important for reproductive development, but may play a role in the annual pelage cycle.

491.4

DO SIBERIAN HAMSTERS RESPOND TO CHANGING PHOTOPERIODS? M.L. Tubbiola, E.L. Meyer*, S. Matsumoto* and E.L. Bittman, Dept. of Zoology and Program in Neuroscience & Behavior, Univ. of Massachusetts, Amherst.

Photoperiodic cues received during gestation determine the reproductive and body weight responses of Siberian hamsters to intermediate daylengths after birth. When pregnant dams are maintained in 12L:12D and transferred with their litters to 14L:10D at birth or 15 days later, their offspring mature rapidly. Puberty is delayed in pups gestated in 16L:8D but moved to 14L:10D after birth. In nature, however, pups are exposed to increasing or decreasing photoperiods rather than constant 14L:10D. We tested the possibility that steadily changing postnatal daylengths would override gestational cues.

Beginning on postnatal day 15, pups gestated on 16L:8D or 12L:12D were exposed to constant 14L:10D (groups A and D, respectively), or to daylengths which decreased 8.57 min/day from 16L:8D to 14L:10D (groups C and F) or increased at the same rate from 12L:12D to 14L:10D (groups B and E). Body weight and testis dimensions were measured at laparotomy on day 30. Pups were then held on 14L:10D until sacrifice on day 35.

As expected, postnatal exposure to 14L:10D induced larger final body, epididymal, and testes weights in pups gestated in 16L:8D than those gestated in 12L:12D (groups A vs. D, $p<0.01$). Gestational daylength also determined responses to steadily increasing or decreasing photoperiod (groups B vs. E and C vs. F, $p<0.01$). In general, pups responded more to the absolute amount of light than to the direction of photoperiod change. Increasing daylengths were no more inductive than constant 14L:10D. Decreasing daylengths stimulated less testis growth, however, than constant 14L:10D in pups gestated in 12L:12D (groups D vs. F, $p<0.01$). The results suggest that photoperiodic threshold might be set close to gestational photoperiod and/or that a period of particular sensitivity to daylength may occur shortly after day 15. (Supported by NIMH RO1-44132.)

491.5

KNIFE CUTS DORSAL TO THE PARAVENTRICULAR NUCLEUS (PVN) PREVENT TESTICULAR REGRESSION BUT NOT THE EFFECTS OF SHORT DAYS ON PROLACTIN SECRETION IN HAMSTERS. A.A. NUNEZ, L.L. BADURA and C.L. SISK. Dept. of Psychology/Neuroscience Prog. Michigan State University, East Lansing, MI 48824 and Dept. of Physiology and Neurobiology, University of Connecticut, Storrs, CT 06268.

Male Syrian hamsters kept in a long-day photoperiod (16L:8D) received horizontal hypothalamic knife cuts just dorsal to the PVN or underwent sham stereotaxic surgery. Following surgery, animals with knife cuts and a subset of the sham-operated animals were transferred to a short-day photoperiod (6L:18D). The width of all the animals' right testes was measured 2 weeks and again 12 weeks after the hamsters were placed in short days. At the same sampling times, blood was drawn from all animals via cardiac puncture. Prolactin (PRL) concentration for each plasma sample was assessed via a RIA. At the time of the first sample, all groups had large gonads (mean=11.5mm) and PRL levels typical of animals kept in stimulatory photoperiods (mean=78.2ng/ml). At the 12-week sampling time, the sham-operated animals kept in 6L:18D had regressed gonads and low plasma PRL levels. As previously reported (Brain Res. Bull, 15:149, 1985), the knife cuts completly prevented gonadal regression. However, they failed to block the short-day induced reduction in PRL secretion (mean=8.3ng/ml). These results indicate that damage to the dorsal connections of the PVN blocks testicular regression without disrupting photoperiodic modulation of PRL. Supported in part by grants BNS8908576 to A.A.N. and HD21588 to C.L.S.

491.6

SEASONAL NEUROENDOCRINE RESPONSES IN MALE TURKISH HAMSTERS: MODULATION BY HYPOTHALAMIC PATHWAYS. L.L. Badura and B.D. Goldman. Dept. Physiology & Neurobiology, Univ. of Connecticut, Storrs, CT 06269.

The role of the hypothalamus in the modulation of neuroendocrine responses to photoperiod was investigated in Turkish hamsters via placement of horizontal knife cuts in the paraventricular nucleus (PVN) region. Adult male hamsters maintained under a long-day (16L:8D) photoperiod received knife cuts aimed either ventral to the PVN (VKC) or dorsal to this nucleus (DKC), or sham surgery (SHAM). Following surgery, approximately half of the animals in each group were transferred to a short-day (8L:16D) photoperiod. Blood samples and testicular measurements were taken at various times over a 16 week period. The testes were then removed and weighed, the brains prepared for histological evaluation of the extent of the lesion sites, and the serum assayed for prolactin (PRL) and follicle-stimulating hormone (FSH).

Similar to the effects of pinealectomy in this species, VKC lesions induced a significant decrease in testicular size, as well as accompanying declines in circulating PRL and FSH, under both photoperiod conditions. In contrast, DKC lesions induced a maintenance of testicular size and circulating FSH levels under both photoperiods that closely resembled the values obtained for SHAM animals in 16L. However, for animals in 8L, PRL levels were markedly reduced to values indistinguishable from SHAM animals. These data add further support for the importance of PVN projections in the modulation of pineal-dependent reproductive responses to photoperiod. Furthermore, they suggest that knife cuts dorsal to the PVN may prevent some photoperiodic responses by disrupting a neuroendocrine pathway responsible for inhibition of FSH release under short photoperiod conditions.

491.7

HYPOTHALAMIC INVOLVEMENT IN THE SEASONAL NEUROENDOCRINE RESPONSE IN SIBERIAN HAMSTERS. A.M. Snyder and L.L. Badura, University of Connecticut Health Center, Farmington, CT 06032 and Dept. Physiology and Neurobiology, University of Connecticut, Storrs, CT 06269.

Photoperiodic modulation of neuroendocrine activity in the Siberian hamster is dependent upon the integrity of the pineal gland. To further identify the functional neuroanatomy of the photoperiodic system in this species, female hamsters received horizontal knife cuts in the hypothalamus aimed between the suprachiasmatic (SCN) and paraventicular (PVN) nuclei. The animals were then transferred to short days and were blood sampled and scored for pelage condition at various times over a 14 week period. Uteri were then removed and weighed, and the brains prepared for histological evaluation of the lesion sites. PRL and FSH levels were determined via RIA.

The animals were divided into two groups based upon placement of the knife cuts: 1) cuts located ventral to the PVN leaving the SCN intact or 2) through the extent of the SCN. Similar to pinealectomized animals, animals in the SCN group maintained stimulated uteri and high levels of FSH and PRL as compared to control animals. These animals also retained the pigmented pelage coloration characteristic of long days. In contrast, animals in the PVN group maintained stimulated uteri and high FSH; however, they showed a marked reduction in PRL and a concurrent molt to the winter-type pelage. These results suggest that not all effects of photoperiod on neuroendocrine activity in this species are mediated directly by the pineal gland, and that the PVN region of the hypothalamus may contain an important pineal-independent neuroendocrine pathway that is responsible for the stimulatory modulation of PRL, but not FSH, release.

491.8

CONVERGENCE OF RETINAL AND OLFACTORY PROJECTIONS WITH LHRH NEURONS. H.M. Cooper*, G. Mick*, M. Magnin, F. Jourdan*. Vision and Motricité, I.N.S.E.R.M. U-94, 69500-Bron, FRANCE

The neuropeptide LHRH, secreted in a pulsatile manner in the basal hypothalamic region, stimulates the release of LH by the pituitary. Although the secretion of LH is known to be modulated by sensory information of both visual and chemosensory origin, the anatomical pathways involved in relaying this input to the LHRH system are unknown. LHRH containing neurons and fibers have been shown to be diffusely distributed in several diencephalic and telencephalic regions. Immunohistochemical demonstration of LHRH, combined with anterograde tracing methods, was used to examine the possibility of direct sensory input to the LHRH neuroendocrine population. Intraocular injections of tritiated amino acids or CT-HRP, in both primates and non-primates, showed that several structures containing LHRH positive cells and/or fibers also receive retinal projections: medial preoptic region, anterior and lateral hypothalamus, sub-paraventricular zone, medial septal nucleus, bed nucleus of the stria terminalis, medial amygdaloid nucleus, olfactory tubercle. Simultaneous injection of the anterograde tracer fluoro-ruby in the main and accessory olfactory bulbs showed converging visual and chemosensory projections in the BNST, lateral hypothalamus and basal telencephalon. Injections of retrograde tracers into the BNST and lateral hypothalamus confirmed that projection fibers mainly arise from the accessory olfactory bulb.

These convergent pathways could thus provide an anatomical basis for a monosynaptic relay of visual and/or chemosensory information to LHRH neurons. Work is currently in progress to confirm this hypothesis at the ultrastructural level. This sensory neuroendocrine convergence would be functionally analagous to the olfacto-retinalis system of teleosts, in which the LHRH neurons directly connect the retina with olfactory bulbs.

NEUROETHOLOGY: ARTHROPODS

492.1

ACTIVITY OF DESCENDING BRAIN NEURONS IN WALKING CRICKETS. H.Böhm* and K.Schildberger MPI f. Verhaltensphysiologie 8130 Seewiesen, Germany.

In order to study the neural mechanisms involved in the course control of crickets we recorded intracellularly from descending brain neurons (DEBs), while the animals walked on an air-supported sphere and oriented toward various stimuli like calling song, moving gratings or air streams directed to the cerci.

DEBs responding to only one of the presented stimuli often showed a selectivity for the direction of the stimulus and were in most cases not influenced by walking activity. DEBs responding to all tested stimuli showed in most cases strong habituation, no directional selectivity and a strong influence of walking on their activity.

Moreover, there seem to be single DEBs responsible for specific aspects of walking. The activity of one DEB was increased before walking started and was linearly correlated with the forward but not with the turning speed of the animal. Another DEB seems to be a command neuron initiating walking. Hyperpolarizing the neuron immediately stopped walking while depolarizing above a certain firing rate always elicited walking. Further enhancing the firing rate did not produce faster walking and there was no correlation of the neuron's activity and the animal's turning tendency.

492.2

THE BIASING EFFECT OF PRIOR MATE OR COMBAT EXPERIENCE ON COURTSHIP AND AGGRESSION IN THE CRICKET, *GRYLLUS BIMACULATUS*. S.A. Adamo and R.R. Hoy. Section of Neurobiology and Behavior, Cornell University, Ithaca, N.Y. 14853

In crickets, naive, sexually receptive males normally respond to tactile contact from conspecific males with aggressive behavior, but will court similarly presented females. A male cricket's response to conspecifics is not fixed, however, but varies depending on its previous behavior. 15 min. after an aggressive encounter, both winning and losing males exhibited increased aggressive behavior towards sexually receptive females ($p<0.05$, $G=3.85$, $n=29$ males). A losing male also required more time and more contact with a sexually receptive female before it would begin to court ($p<0.004$, Wilcoxon, $n=19$ males). Previous aggressive encounters did not inhibit courtship initiation in winning males. Losing males were also less likely to win their next aggressive encounter (15 min. later) even if they were paired with a smaller opponent ($p<0.01$, $G=8.65$, $n=35$ pairs). Losing males, unlike naive or winning males, fled from the tactile contact of other males ($P<0.01$, $G=8.8$, $n=35$).

Immediately after courtship, males tended to respond to male tactile contact with courtship song ($p<0.001$, $G=17.22$, $n=35$), at least initially. 15 min. after a bout of courtship, males showed an enhanced response to tactile contact with females, requiring less time to initiate courtship ($p<0.001$, Wilcoxon, $n=16$ males).

These results suggest that a cricket's prior behavioral performance can result in physiological changes lasting many minutes which alter its behavioral response to tactile contact from conspecifics. Current experiments are exploring the possible role of neuromodulators such as serotonin and octopamine in mediating this change.

492.3

NEURAL STUDY OF THE "PRECEDENCE EFFECT" IN NEGATIVE PHONOTAXIS OF THE CRICKET *TELEOGRYLLUS OCEANICUS*. R.A. Wyttenbach and R.R. Hoy. Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853.

Tethered flying crickets make steering movements away from pulses of ultrasound. These movements include forewing tilt, abdomen swing, and metathoracic leg swing, all of which are lateralized relative to the sound source. A bilateral pair of ascending prothoracic auditory interneurons (int-1) has been shown necessary and sufficient to elicit these responses: stimulation of an int-1 (electrically or by ipsilateral ultrasound) causes steering to the contralateral side, while hyperpolarization prevents steering in response to sound. To correctly localize a sound source in the presence of echoes, directional information must be taken only from the first-arriving wavefront (precedence effect) and subsequent wavefronts cannot be treated as coming from additional sources (echo suppression).

Using EMGs of metathoracic leg abductors as an indicator of turning, previous work in our lab showed that the precedence effect and echo suppression occur for delays of 4 to 75 ms between a pulse of ultrasound and a simulated echo. For shorter delays, a random turn is made, while longer delays give rise to separate turns in response to each pulse of ultrasound. Bilateral recordings from the two int-1s show no difference in spike number or frequency between the int-1 receiving the first pulse and the one receiving the echo. Therefore, the behavioral effects cannot be accounted for by patterns of activity of the two int-1s.

We have begun recording from another pair of prothoracic auditory interneurons, the omega cells. These are thought to be involved in localization of calling song, but also respond to ultrasound. The omega cells are mutually inhibitory, inhibit the contralateral int-1s, and are respond to a broad spectrum of frequencies. Furthermore, these cells have a long-term delayed intensity-dependent self-inhibition that increases their directionality. These effects have so far been studied only for the calling song frequencies, but may also occur in ultrasound. If so, this would be evidence that the same mechanisms are used to localize both calling song and ultrasound.

492.4

POSSIBLE ROLES FOR JUVENILE HORMONE BINDING PROTEINS IN THE REGULATION OF CRICKET PHONOTACTIC BEHAVIOR. J. Stout, D. Zacharias*, and G. Atkins. Dept. of Biology, Andrews Univ., Berrien Springs, MI 49104.

In response to models of the males calling songs, young (0-2 day-old) female crickets have higher auditory thresholds (80-90dB) for positive phonotaxis than do older (4-21 day-old) females (45-55 dB). Changes in the firing threshold of the L1 auditory interneuron parallel the changes with age in the phonotactic threshold of these females. In 1 day-old females, topical application and microinjection of juvenile hormone III (JHIII) demonstrate that JHIII decreases (25-40 dB) both behavioral and neuronal thresholds within several hours, and that the site of JHIII action on neurons and behavior is within the prothoracic ganglion. The results of the JHIII microinjection experiments strongly suggest that the soma of L1 is the locus for this hormonal effect.

Juvenile hormone binding proteins have been isolated from the hemolymph and, apparently for the first time in insects, from protein extracts of nerve tissue of crickets by labeling them with the tritiated photoaffinity analogs for JHIII (EFDA) or methoprene (MDK), separating them using SDS- or native PAGE and identifying them by autofluorography. JHIII binding proteins with a weight of about 23 and 38 kDa were isolated from the pro- and metathoracic ganglia. The 38 kDa protein was found in both ganglia, while the 23 kDa binding protein was much more apparent in extracts from the prothoracic ganglia.

If JHIII were to directly influence the response properties of auditory interneurons by regulation of the expression of an appropriate gene(s), then binding proteins should be present in the somata of these neurons. We are presently attempting to localize JHIII binding proteins in these somata.

492.5

EXPRESSION OF NICOTINIC ACETYLCHOLINE RECEPTORS IN THE AUDITORY NEURONS OF CRICKETS - A POSSIBLE ROLE IN THE REGULATION OF PHONOTAXIS. G. Atkins, V. Hayes*, J. Henley* and J. Stout. Dept. of Biol., Andrews Univ., Berrien Springs, MI 49104.

Two auditory interneurons (L1 and L3) in the prothoracic ganglion of the cricket *Acheta domestica*, are involved in phonotaxis and have been shown to be influenced by Juvenile hormone III (JHIII). L1's threshold to 5 kHz calling songs decreases as JHIII levels increase either naturally with increasing age, or in response to topically applied hormone. This change correlates with a similar decrease in phonotactic threshold with age and JHIII application. Transcription inhibitors block JHIII effects. L3's threshold does not change, but its decrementing response which is syllable period selective, changes in response to natural or artificially changing JHIII levels, and correlates with changes in phonotactic selectivity under similar conditions .

We are using *In situ* hybridization to identify transmitter receptor mRNA present in these cells with the goal of determining whether the regulation of response properties of auditory neurons might involve regulation of the production of their ligand-gated channels. Ganglia containing iontophoretically labeled neurons were sectioned (20 um). Sections containing the labeled somata were exposed to an oligonucleotide probe and later visualized using a phosphatase detection system. We have demonstrated the presence of alpha1-like nicotinic receptor mRNA (Marshall et al. 1990) using three different 27mer oligonucleotide probes. We are presently determining whether changing JHIII levels might influence the expression of mRNAs for nicotinic receptors in these neurons.

492.6

HIGH FREQUENCY ACOUSTIC SENSITIVITY IN *ACHETA DOMESTICUS:* ROLES IN COMMUNICATION AND PREDATOR AVOIDANCE. T.G. Nolen, C. Lam*, J. Wong*, L. Luayon* and J. Aracena*, Dept. of Biology, University of Miami, P.O. Box 249118, Coral Gables, FL 33124.

Male crickets employ acoustic signals to establish and defend their territories and to attract mates. In *Acheta domesticus* the RIVALRY SONG is crucial for a male's territorial success: Muting the dominant, or deafening the subordinate male reduces the dominant's chance of gaining or keeping a territory. [Nolen and Lam, (1990)].

Spectral analysis of *A. domesticus* songs reveal three pulse types: LF, with significant energy at 4-5 kHz, but little above 20kHz (e.g. the CALLING SONG); LF-HF, with a 4-5 kHz fundamental and a high frequency component 10-40kHz; and HF, nearly exclusively high frequency (10-40kHz). The COURTSHIP SONG contains both LF and LF-HF pulses (HF peak at ~17-21kHz). The RIVALRY SONG is bimorphic: RSa is mostly LF and LF-HF, whereas RSb, which is sung primarily by the dominant male, is mostly HF (short, low amplitude pulses, usually > 30kHz). Both the CALLING SONG and RSa are relatively loud, and should travel greater distances than either the COURTSHIP SONG or RSb. The pressure component, rather than the near field (wind) component of the RS song is critical for the dominant male's success: Cercal ablation had no effect on territorial success [p>0.2, Wilcoxon SR test], whereas muting (HF reduced more than 30dB) did.

HF pulses are aversive to *A. domesticus* in a different behavioral context: During flight, this cricket steers away from high frequency pulses (analogous to the bat avoidance behavior of other species of crickets [Nolen and Hoy, 1986]). Recordings from ascending auditory neurons reveal sensitivity to HF; this HF response demonstrates two-tone suppression by 4-5kHz. Thus, the HF network in *A. domesticus* is not as strongly activated by LF and LF-HF pulses (CALLING, COURTSHIP and RSa) as by HF pulses (RSb and bat sonar). *A. domesticus*, is sensitive to the HF pulses of bat sonar (as are other crickets) and to the rivalry song of the dominant male. We hypothesize that in both behavioral contexts, HF pulses could activate the same general aversion circuit, mediating the avoidance of a predator or the avoidance of aggressive, territorial males. [Supported by NSF]

492.7

HABITUATION OF ULTRASONIC ACOUSTIC STARTLE IN FLYING CRICKETS. R.R. Hoy and M. L. May. Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853.

The acoustic startle response (ASR) is a rapid, highly stereotyped motor reaction to a loud, transient sound. The survival value of the ASR is reflected in its wide phylogenetic distribution among animals that have a sense of hearing, ranging from insects through all vertebrates, including humans. In mammals, the ASR shows classical habituation. Using Thompson and Spencer's nine criteria for mammalian habituation, we show habituation of the ASR in crickets.

Tethered flying crickets (Teleogryllus oceanicus) steer away from ultrasound, by making lateralized movements of wings, legs, and abdomen. We investigated habituation in the ultrasound-induced metathoracic leg kick; eight of nine of Thompson and Spencer's critera were satisfied. Habituation of the ASR showed: (1) exponential decline of the response; (2) spontaneous recovery; (3) stimulus generalization; (4) dishabituation to a novel stimulus; (5) habituation of the dishabituating stimulus with repetition; (6) sronger and more rapid habituation with weak sounds than with more intense sounds; (7) stronger and more rapid habituation with repeated trials; and (8) stronger and more rapid habituation if the interstimulus interval is decreased.

By standard criteria, the ASR in crickets shows classical habituation. It is now possible to explore the neural basis of this relatively simple fixed act in a restricted neural network composed of identifiable neurons.

We acknowledge funding from NIH DC-00103-18, a Javits/Pepper Neuroscience Award.

492.8

ANATOMICAL AND FUNCTIONAL CHARACTERIZATION OF THE CENTRAL COMPLEX IN THE BRAIN OF THE LOCUST *SCHISTOCERCA GREGARIA*. U. Homberg. Fak. für Biologie, Universität Konstanz, FRG

The central complex (CC) comprises a group of neuropils in the center of the insect brain. Its subdivisions, including the protocerebral bridge and the upper and lower divisions of the central body constitute a system of horizontal layers, each beeing partitioned into a series of 8 or 16 vertical columns. This study investigates the anatomical organization of the CC and physiological properties of CC neurons in the locust *Schistocerca gregaria*.

Single-cell injections of Lucifer Yellow and neurotransmitter immunocytochemistry revealed two principal categories of CC-neurons. Tangential neurons innervate single layers of the CC. They connect the CC to visual pathways from the two compound eyes and to neurosecretory areas in the superior protocerebrum. Subtypes of tangential neurons are immunoreactive with antisera against GABA, serotonin, dopamine, octopamine, and neuropeptides including FMRFamide and locust adipokinetic hormone. Columnar neurons interconnect columns of the right and left CC hemispheres in a highly ordered fashion, suggesting a role of the CC in the coordination of neural activity between the two hemispheres. Systems of columnar neurons exhibit immunoreactivity with antisera against substance P, serotonin, and crustaceen cardioactive peptide.

In electrophysiological experiments, CC neurons responded to visual stimulation but more often to wind stimuli eliciting flight. Simultaneous recordings from flight muscles suggest that at least some CC neurons monitor flight motor activity. These data suggest that the CC plays a role in bilateral visual integration, receives information about ongoing motor activity and may be even involved in the control of the neuroendocrine system. Supported by DFG grant Ho 950/4-1.

492.9

LOCUSTS IN A FLIGHT SIMULATOR: THE ROLE OF HEAD MOVEMENTS DURING STABLE FLIGHT AND AVOIDANCE MANOEUVRES. D. Robert, Institute of Zoology, Basel University, Rheinsprung 9. CH-4051 Basel, Switzerland.

Flying insects strongly rely on vision to detect deviation from course and to maintain flight stability. Visually perceived deviations can have diverse causes: 1) aerodynamical disturbances and motor errors, and 2) active steering manoeuvres. The former (unintended) deviations activate the optomotor (compensatory) steering system while the latter (self-induced) do not. How can a locust initiate intentional turns without being restabilized to the original course by the optomotor system? Tethered locusts were flown in a flight simulator that electronically converts the steering force of the animal (measured by a torque meter) into the motion of a surrounding visual pattern driven by a servomotor. The locusts were in control of their visual environment (reafference control), and actively stabilized the panorama over long periods of time by means of compensatory steering manoeuvres. Under closed-loop conditions, preventing compensatory head (thus eye) movements impaired both the precision and the temporal coordination of stabilization manoeuvres.

This closed-loop paradigm allows one to test the robustness of the optomotor system to external or self-induced visual instabilities. Flying locusts exposed to pulsed ultrasound (resembling bat echolocation cries) actively steer away from the ultrasound source, thus provoking shifts of the panorama that were not compensated by the optomotor response. Compensatory and avoidance steering involved the same steering effectors, e.g. abdominal and hindleg ruddering and wingbeat modulation. In both cases, ruddering was directed to the side of the turn. During compensatory steering, the head moved to the side of the turn, while it moved to the side *away* from the turn during avoidance steering. For both reactions, head movements occurred 30-40 ms after torque production began and thus cannot be considered as initiating steering. Head turns during avoidance steering were shown to occur in the dark and to be independant of the sign of the visual reafference. This indicates a momentary cessation of visual control during avoidance steering. Head movements are proposed to proprioceptively assist opto- and audiomotor steering.

492.10

STEERING TORQUES GENERATED BY COLLISION AVOIDANCE MANOEUVRES DURING FLIGHT IN THE LOCUST. A. G. Johnson, R.M. Robertson and D.W. Lywood*. Departments of Biology and Biomedical Engineering, Queen's University, Kingston, Ontario, K7L 3N6, Canada.

When presented with objects in their immediate flight path, tethered flying locusts make asymmetrical movements of the wings on either side of the body. The side of the asymmetry correlates with the line of approach of the object. For objects approaching on the right of the midline, the right forewing is more elevated and separate from the right hindwing while the left forewing is more depressed and comes down in conjunction with, or in advance of, the left hindwing. These wing movements have been interpreted as steering movements. However it is unknown whether the described wing asymmetries generate the steering torques that would direct the animal around the closest edge of the object. We tested this by measuring the forces generated by flying locusts in response to approaching objects.

Locusts were attached to a tether which was mounted on a force transducer modified after an original design by Blondeau (J. Exp. Biol. **92**:155 1981). This device measured thrust, lift, side-slip, yaw, pitch, and roll forces. The locusts were suspended in the centre of a wind tunnel (20 cm square section, air speed 3 m/s). Objects (10 cm squares of black or patterned cardboard) were transported towards the head of the locust from 1.5 m away and 5 cm on either side of the sagittal midline at 1, 2, 3, 4 or 5 m/s. Wing movements were recorded using a video camera (60 frames/s) and could be correlated with the forces generated during the presentation of objects in the flight path. Preliminary results confirm that the wing asymmetries result in steering torques which would direct the animal around the closest edge of the approaching object.

492.11

VENTRAL GIANT INTERNEURON WIND FIELDS IN THE COCKROACH MODELED WITH CONSTRAINED BACK-PROPAGATION. R.D. Beer, G.J. Kacmarcik*, S. Chai*, R.E. Ritzmann and H.J. Chiel. Dept. of Comp. Eng. and Sci. and Dept. of Biology, Case Western Reserve University, Cleveland, OH 44106.

We are attempting to construct a physiologically accurate model of the escape system of the cockroach. At last year's meeting, we presented the basic components of a model constructed with the back-propagation training technique. Our model of the ventral giant interneurons (vGIs) was trained on the normal wind field responses and constrained by published patterns of connectivity from cercal afferents (Daley and Camhi, J. Neurophysiol. **60**:1350).

We have now refined this model by incorporating additional training data and constraints. The model is now trained on wind field data from both normal animals and animals with cercal ablations (Westin et al., J. Comp. Physiol. **121**:307; Volman and Camhi, J. Comp. Physiol. **162**:781). In addition, the sigmoidal responses of the model vGI neurons were fit to firing characteristics of actual vGI neurons. A series of intracellular experiments characterized current - frequency relationships of individual vGIs. Mean number of action potentials for varying levels of injected current were plotted.

A particularly interesting aspect of this system is the adaptation that occurs within vGI response patterns as a result of unilateral cercal ablation. With our more physiologically accurate model, we can now simulate this adaptation by retraining a lesioned model to the recovered wind fields. By comparing connection strengths before and after simulated adaptation, we hope to gain insight into possible loci of plasticity.

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492.12

INFLUENCE OF NON-GIANT SENSORY INPUTS ON THE ESCAPE RESPONSE OF THE COCKROACH R.E. Ritzmann, A.J. Pollack* and Sue E. Hudson*. Dept. of Biology, Case Western Reserve University, Cleveland, OH 44106.

The wind mediated escape system of the cockroach has become an important neuroethological model for orientation movements. Although it is clear that activity in the ventral giant interneurons (vGIs) is a primary determinant of the direction of the escape turn, there are several lines of evidence to suggest that other information is also factored into the decision.

We are investigating the influence of non-GI sensory modalities on electrophysiological parameters of the thoracic interneurons that receive vGI inputs (TI_As) and on escape movements. In the absence of vGI inputs, we have recorded vigorous responses in TI_As to wind directed at the head of the animal. We have also recorded responses to auditory stimulation that persists after the thoracic ganglia are isolated from both abdominal and anterior connectives. Responses to light have been recorded in at least one TI_A. Although the light response was labile, lasting for only 7 trials, it returned after 1 hour in darkness. A previously published account (Murrain and Ritzmann, J. Neuorbiol. **19:552**) demonstrated that proprioceptors also excite TI_As. Behaviorally, we have now shown that in response to ablation of the mesothoracic legs, a cockroach will alter its leg movements. As a result, after 2 days the animal can direct its turn in response to wind as well as a normal animal can.

Our data indicate that, although wind direction encoded in vGIs is critical to the escape movements, this information is interpreted in the TI_As in the context of a plethora of other sensory information. We are presently expanding on both the physiological and behavioral aspects of this study.

Supported by NIH grant NS 17411 to RER and ONR grant N00014-90-J-1545 to R.D. Beer.

492.13

EXPANDING THE BOUNDARIES OF THE COCKROACH "ESCAPE CIRCUIT": LESIONS AT THE CERVICAL LEVEL ALTER ESCAPE BEHAVIOR EVOKED BY TOUCH AND ALSO RESPONSES ELICITED BY WIND. A.P. Keegan, E. Mara, and C.M. Comer Dept. of Biological Sciences, University of Illinois at Chicago, Chicago, IL 60680.

The escape behavior of the cockroach (Periplaneta americana) has been known for many years to be triggered by wind stimulation of the caudal cerci, and to involve giant interneurons (GIs) ascending to the thoracic level of the CNS. We recently found that some predators appear not to be detected by wind cues, but rather by direct touch (Soc. Neurosci. Abst. 15:349) and that some of these tactile responses [e.g. those derived from the antennae] involve novel interneuronal pathways descending from the head ganglia to thoracic levels (Brain Res. 535:347).

We have now made lesions between the head ganglia and thoracic circuits and used high-speed videography to characterize both wind and tactually elicited behavior. We have found that such lesions alter antennal tactile escape responses, and also modulate cercal wind-evoked escape responses. When one neck connective was transected, animals responded less consistently to antennal tactile stimuli and the directionality of their turning was shifted: they often turned toward rather than away from stimuli on the side opposite the lesion. Animals with this type of lesion responded to cercal wind stimuli at normal levels, but the orientation of their turning was shifted in the same way as for touch.

This demonstrates lateralization of descending tactile pathways for escape, and also implies that pathways from head ganglia influence how thoracic circuits use ascending GI signals to initiate turning movements. Our data suggest that rostal modulation of GI-to-motor circuits is not likely to involve a phasic wind-derived signal, but rather a more tonic signal that can bias premotor and/or motor cells. Supported by NSF grant #BNS 89-09051 to C.M.C.

492.14

NEUROETHOLOGY OF SOUND PRODUCTION IN ARCTIID MOTHS: RHYTHMICITY AND CENTRAL CONTROL. J.H. Fullard. Insect Behaviour Group, Department of Zoology and Erindale College, University of Toronto, Mississauga, Ontario, CANADA L5L 1C6.

As part of their in-flight anti-predator defences, certain species of tiger moths (Arctiidae) emit trains of high frequency clicks when they hear the intense echolocation calls of an attacking bat. Sound production serves as a convenient and stereotyped response to acoustic stimuli that can be used to study the neural control of this adaptive behaviour. The moth produces its sounds by rhythmically buckling a tymbal on each side of its thorax once activated by auditory input. By monitoring the motor nerve that leads to the tymbal musculature a large single spike proceeds each tymbal sound emission by 3 - 10 ms. This motor neuron output continues in the absence of actual sound emission and persists when all nerves have been severed from their tymbal muscles. Since rhythmicity is preserved in deefferented preparations, I conclude that arctiid sound production is controlled by a central pattern generator (CPG), located in the moth's thoracic central nervous system and is independent of sensory influence. One-eared moths initiate sounds from the tymbal opposite to the stimulated ear and once the cycle is initiated it continues without interference from the other side. This indicates the presence of auditory invoked inhibitory effects on the contralateral CPG allowing the rhythmicity of the motor output to proceed without disruption. The period and phase characteristics of the deefferented preparation suggests a pair of asymmetrical CPGs, each of which contains an additional synapse on the side ipsilateral to the stimulated ear. A model for the tymbal CPG is provided.

492.15

FLIGHT MUSCLE ACTIVITY UNDERLYING PHEROMONE-MODULATED ZIGZAGGING FLIGHT IN MALE MOTHS, *Manduca sexta*. MA Willis & EA Arbas. ARLDN, Univ. of Arizona, Tucson AZ 85721.

Male *M. sexta* approach females for mating by flying a characteristic side-to-side zigzagging path. This upwind path results from self-steered maneuvers initiated and modulated by wind-borne pheromone released by the female.

To characterize motor patterns underlying flight maneuvers, we have recorded electromyograms (EMGs) from flight muscles of *M. sexta* males flying freely in female pheromone. EMG activity was synchronized with a video record of the zigzagging flight track in a laboratory wind tunnel (closed-loop conditions). We have also recorded the activity of these muscles, in the same individuals, during tethered flight activated by visual stimuli or pheromone (open-loop conditions). We recorded from three muscles: the left dorsal longitudinal muscle (DLM), a main wing-depressor; and the left and right third axillary muscles (AxM), which have been identified as "steering" muscles in earlier studies. Parameters measured from the EMGs were: burst period (ms), burst duration (ms), and spikes/burst for all three muscles; and the phase of AxM activity with respect to each DLM burst.

Characteristic asymmetries between bilateral AxMs were identified during zigzagging turns in free flight. The most typical is the appearance of an additional spike at a later phase of the wing beat cycle in the AxM on one side of the animal, but not the other. These may occur during only one, or a few wing beat cycles in a turn. We have observed similar, additional spikes during attempted turns in tethered males stimulated by air-borne pheromone alone. Phase shifts in AxM firing were observed during attempted turns activated with visual flow fields alone. These changes in AxM firing may contribute to the temporally regular turns that make up pheromone-modulated flight. [Supported by NIH grants DC-00348 and NS-07309.]

492.16

DIGGING BY THE SAND CRAB *BLEPHARIPODA OCCIDENTALIS*. Z. Faulkes*, D.H. Paul & S.M. Pellis[1]. Dept. Biol., U. Vic., Victoria, B.C. V8W 2Y2; [1]Dept. Psych., U. of Leth., Lethbridge, AB T1K 3M4.

The spiny sand crab, *B. occidentalis*, digs into the sand using its thoracic legs. We hypothesize this is homologous to walking in other crustaceans. We hope to understand how the crabs dig, how digging has evolved from walking, and how the nervous system generates the digging motor pattern. We are studying digging with Eshkol-Wachman movement notation (EW) and by EMG analysis. Leg movements of crabs held in water were videotaped from the side and below, and analyzed with EW.

Digging resembles walking in two respects. 1) Contralateral legs, including the chelae, move out of phase (but become synchronized when the crab is almost dug in). 2) Ipsilateral legs move in a metachronous front to back sequence of power strokes. Digging differs from walking in several ways. 1) Leg 4 plays a special role in digging and its control may be different from the others: a) the power stroke of Leg 4 is backward while those of Leg 2 and 3 are forward; b) the sequence of joint movements is proximal-distal in Leg 4, but distal-proximal in Legs 2 and 3. 2) Most joints make active movements; in walking, distal segments are largely carried along. 3) Each leg moves in different directions relative to the body; in forward walking legs move parallel to the midline. 4) The abdomen and the chelae move and contribute to locomotion. The abdomen cycles at about twice the rate of the thoracic legs; its small size and rapid movement suggest that it acts by "puddling," making the sand more fluid.

Research supported by NSERC.

493.1

CORRELATION OF APOMORPHINE- AND AMPHETAMINE-INDUCED TURNING BEHAVIOR WITH NIGROSTRIATAL DOPAMINE DEPLETION. J.L. Hudson*, C. G. vanHorne, I. Stromberg1, J. Clayton, S. Brock*, J. Masserano, B.J. Hoffer and G.A. Gerhardt. Depts. of Pharmacology and Psychiatry, Univ. Colorado Health Sci. Ctr., Denver, CO 80262 and Dept. of Histology1, Karolinska Institute, Stockholm, Sweden.

In the unilaterally 6-hydroxydopamine (6-OHDA)-lesioned rat model of Parkinson's Disease, some controversy exists concerning the use of apomorphine (APO)- vs. amphetamine (AMP)-induced rotations as indicators of nigrostriatal dopamine (DA) depletion. APO produces rotations contralateral to a lesion and is dependent upon the development of receptor supersensitivity, while AMP produces rotations ipsilateral to a lesion due to indirect DA agonist actions on the intact side. Our objective was to evaluate if either drug-induced behavior is more predictive of the extent of nigrostriatal DA depletion. Male and female Fischer 344 and Sprague-Dawley rats were unilaterally injected with 6-OHDA (9 micrograms/4 microliters/4min) into the medial forebrain bundle. The animals were tested twice each on APO (0.05 mg/kg) and AMP (5.0 mg/kg). Following the behavioral tests, right and left striatum (STR) and substantia nigra (SN) were analyzed for catecholamine content by HPLC-EC. The results showed that an animal that had greater than 99% (maximal) depletion of DA rotated well on either APO (500/hr) or AMP (300/hr). However, many sub-maximally lesioned rats were seen to rotate adequately on AMP but not on APO. We believe that APO-induced rotation behavior may be a better predictor of maximal DA lesions as compared to AMP. (Supported by USPHS grants NS09199 & AG06434)

493.2

DOPAMINE UPTAKE IN PREFRONTAL AND CINGULATE CORTEX AFTER CHRONIC AMPHETAMINE TREATMENT. M.A. Segall, G.A. Gerhardt, J.M. Masserano, Depts. of Pharmacology & Psychiatry, Univ. of Colorado Health Science Center, Denver, CO 80262.

Amphetamine (3mg/kg) given for 7 days to male rats induces a behavioral sensitivity to a single injection of amphetamine (1mg/kg) seven days after the last injection. In this study we found a 50 - 80% increase in the uptake of dopamine (DA) in prefrontal/cingulate cortex synaptosomes in rats chronically treated with amphetamine compared to saline controls. The increase in DA uptake is not seen 1 day after the last injection. There is also no increase in DA uptake into striatal or nucleus accumbens synaptosomes of chronically treated rats. Amphetamine blocks the uptake of DA in the control and chronically treated rats without any apparent change in sensitivity. With $1x10^{-7}$ M desipramine (DMI) blocking norepinephrine uptake sites, the increase in DA uptake in cortex was only about 30%. While with $1x10^{-7}$ M GBR 12909 blocking DA cells, there was a 70% increase in uptake of DA into norepinephrine sites. These data indicate that DA uptake into both DA and NE synaptosomes is increased in the medial prefrontal /cingulate cortex by chronic amphetamine treatment. (Supported by USPHS grants MH41551 and NS09199.)

493.3

FUNCTIONAL EFFECTS OF REPEATED APOMORPHINE ADMINISTRATION. L.J. Porrino[1] and F.E. Pontieri[2]. [1]Bowman Gray School of Medicine, Winston Salem, NC 27157 USA, [2]Department of Neurology, University of Rome, ITALY.

The repeated administration of apomorphine (APO) produces a progressive enhancement of its effects on motor behavior, in that stereotypic movements become more intense with repeated treatment. Using the 2-[^{14}C]deoxyglucose method, the distribution of alterations in functional activity associated with the repeated administration of APO was determined and compared to the distribution of changes associated with its acute administration. Male rats were treated with saline or APO (1 mg/kg, s.c.; x3). One week later APO or saline was administered and rates of glucose utilization measured in 3 groups: controls, pretreated and tested with saline; acute APO, pretreated with saline and tested with APO; and chronic APO, pretreated and tested with APO. The acute APO selectively increased rates of metabolism in portions of the nigrostriatal system, e.g. the globus pallidus, substantia nigra reticulata, and entopeduncular nucleus, and decreased metabolism in the lateral habenula, as reported previously (cf. McCulloch et al, 1982). In contrast, repeated APO treatment decreased metabolic rates in limbic areas, e.g. the nucleus accumbens, basolateral amygdala, hippocampus, and prefrontal cortex. Elevations in rates of metabolism, similar to those observed with acute APO treatment, were also seen in the nigrostriatal system, although these increases were somewhat attenuated when compared to the acute changes. The metabolic effects of repeated APO administration, then, are different from those of acute administration, and chronic APO treatment produces differential functional effects in the nigrostriatal and mesolimbic systems. Finally, the augmented behavioral response to repeated APO is not paralleled by corresponding increases in functional activity in any brain area examined.

493.4

ENVIRONMENTAL ENRICHMENT ALTERS AMPHETAMINE-STIMULATED DOPAMINE RELEASE AND SYNTHESIS IN THE NUCLEUS ACCUMBENS S.L. Bowling*, J.K. Rowlett & M.T. Bardo, Department of Psychology, University of Kentucky, Lexington, KY 40506.

In two experiments, rats were raised from postnatal day 21 to about day 60 in either an enriched (EC) or isolated (IC) condition. In the first study, amphetamine-stimulated release of dopamine (DA) was assessed in vitro from slices of the nucleus accumbens and striatum. DA and dihydroxyphenylacetic acid (DOPAC) levels were measured by high pressure liquid chromatography (HPLC). EC rats had less total DA and DOPAC than IC rats in the nucleus accumbens, but not the striatum. EC rats also released less DA than IC rats when stimulated by amphetamine (10^{-6}, 10^{-5} M). In the second study, an inhibitor of dihydroxyphenylalanine (DOPA) decarboxylase, NSD-1015 (100 mg/kg, i.p.) was administered after amphetamine (0, 0.5 or 2.0 mg/kg, s.c.). Tissue samples were assayed for DOPA using HPLC in order to estimate in vivo DA synthesis. In the nucleus accumbens, but not striatum, EC rats demonstrated less DOPA accumulation than IC rats. It was concluded that EC rats have a lower functional activity of the mesolimbic DA reward pathway relative to IC animals.
(Supported by USPHS grant DA05312.)

493.5

IN VIVO MECHANISM OF ACTION OF DEPRENYL: BIOCHEMICAL AND BEHAVIORAL STUDIES. C. Okuda, R. Kuczenski and D. S. Segal. Dept. of Psychiatry (0603), Univ. of California, San Diego, La Jolla, CA 92093.

In vivo microdialysis was used to concurrently measure the behavioral and striatal dopamine (DA) response following the administration of the irreversible type B selective monoamine oxidase (MAO) inhibitor, deprenyl (10 mg/kg), and its effects were contrasted to the action of an irreversible MAO-A inhibitor, clorgyline (4 mg/kg). Clorgyline administration demonstrated a response indicative of irreversible MAO inhibition, i.e., a gradual and enduring increase in extracellular DA and 3-methoxytyramine and a decrease in DOPAC and HVA which lasted for at least six hours. In contrast deprenyl administration produced rapid and transient changes in DA and its metabolites. This pattern of deprenyl effects closely resemble the neurochemical response following amphetamine administration (Kuczenski and Segal, J. Neurosci., 9:2051, 1989). Furthermore, our behavioral data demonstrated a stimulant-like increase in locomotor activity following deprenyl which was not observed following clorgyline. Taken together, these data suggest that systemic administration of deprenyl does not augment striatal extracellular DA by irreversible inhibition of MAO-B, but rather produces its effects on behavior and DA dynamics as a consequence of its conversion to amphetamine. (Supported by DA04157 and DA01568.)

493.6

THE ROLE OF DOPAMINE AND CALCIUM CHANNELS IN AMPHETAMINE-CONDITIONED LOCOMOTION. S.L. DiLullo*, K.F. McKenna, and M.T. Martin-Iverson. Neurochemical Research Unit, Dept. of Psychiatry, University of Alberta, Edmonton, Alberta, Canada T6G 2B7

Previous research has shown that dopamine (DA) D1 and/or D2 antagonists cannot block the establishment of amphetamine (AMP) -induced conditioned locomotion (Martin-Iverson and McManus, Br. Res. 521: 175, 1990). However, one DA receptor antagonist, pimozide, which is equipotent at blocking calcium (Ca) channels, has been shown to block stimulant-conditioned locomotion (Beninger and Hahn, Sci. 220: 1304, 1983). We directly tested the possibility that presynaptic catecholamine mechanisms are conditioned in rats by blocking the AMP-induced presynaptic release of DA and norepinephrine (NE) by pretreatment with alpha-methyl-para-tyrosine methyl ester (MPT, 50mg/kg,SC), or chemical lesioning of NE terminals with N-(2-chloroethyl) -N-ethyl-2-bromobenzylamine (DSP4, 50 mg/kg,IP). Secondly, we blocked L-type calcium channels with nimodipine (NIM, 10 mg/kg,SC) together or separately with DA D2 receptor blockade with haloperidol (HAL, .05-.2mg/kg,IP). All drug treatments were given during conditioning with AMP (1.5mg/kg, SC) but not on the test day. Both MPT and HAL blocked unconditioned AMP-induced locomotion and defecation, but did not block AMP-conditioned responses. DSP4 had no effect on either AMP-unconditioned or conditioned locomotion. NIM attenuated AMP-unconditioned but not conditioned locomotion. NIM and HAL treatments combined blocked AMP-unconditioned locomotion and attenuated AMP-conditioned locomotion. These results indicate that DA but not NE mediates the unconditioned effects of AMP. Actions on DA receptors alone, calcium-independent AMP-induced DA release, and NE do not mediate the establishment of AMP-conditioned behaviors. However a combination of DA D2 receptor blockade and blockade of L-type Ca channels attenuates the AMP-conditioned responses. Funding by AMHRF.

493.7

BEHAVIORAL CLAMPING ANALYSIS OF AMPHETAMINE-INDUCED EFFECTS ON STRIATAL SINGLE-UNIT ACTIVITY IN FREELY MOVING RATS: ROLE OF CORTICAL AFFERENTS. J.L. Haracz, J.T. Tschanz*, Z.R. Wang*, K.E. Griffith*, and G.V. Rebec. Prog. Neural Science, Dept. Psychology, Indiana University, Bloomington, IN 47405.

Our recordings of striatal single-unit activity in freely moving rats showed that amphetamine (AMP) typically excites motor-related and inhibits nonmotor-related neurons (Haracz et al., Brain Res., 489:365, 1989). Because motor-related cells regularly increase activity during movement, AMP-induced excitations may reflect a behavioral feedback response rather than a primary drug effect. This possibility was assessed with a new behavioral clamping method in which rated videotapes were used to match pre-AMP exploratory behaviors with similar post-AMP behaviors. Firing rates were compared within each match. AMP (1.0 mg/kg, sc) significantly enhanced movement-related activity of motor-related cells, suggesting a direct drug effect in exciting striatal neurons independent of behavioral feedback. This effect is abolished in rats with bilateral ablations of frontal and sensorimotor cortex, but not in sham-lesioned animals. Thus, AMP-induced excitations of striatal neurons require intact corticostriatal projections. Preliminary data from striatal nonmotor-related cells, however, indicate that such projections are not required for AMP-induced striatal inhibitions. These results suggest that AMP, possibly by enhancing dopamine neuromodulation, facilitates or inhibits the activity of striatal neurons that respectively receive substantial or little cortical input. [Supported by USPHS grant DA 02451.]

493.8

INCREASES IN EXTRACELLULAR DOPAMINE AND LOCOMOTION AFTER DIRECT INFUSION OF PHENCYCLIDINE INTO THE NUCLEUS ACCUMBENS OF THE RAT: A DIALYSIS AND BEHAVIORAL STUDY. L. D. McCullough and J. D. Salamone. Dept. of Psychology, Univ. of Connecticut, Storrs, CT 06269-1020.

Two studies were conducted to provide a further characterization of the neurochemical and behavioral effects of phencyclidine (PCP). The first experiment utilized in vivo microdialysis to measure extracellular dopamine (DA) concentrations after PCP administration into the nucleus accumbens (ACC). Rats were implanted with dialysis probes into the ACC and were tested for two days. Rats received either PCP (0.4×10^{-4} M dissolved directly into the perfusing medium) for two 45 min sessions, or continued perfusion with the normal medium, in a random order. PCP significantly increased extracellular DA levels. In a second experiment, chronic indwelling cannulae were implanted bilaterally into the ACC. Rats were injected with 1.0 µl of either saline or PCP (30.0 µg or 60.0 µg) in random order on 3 separate days, and placed in a motor activity chamber. PCP caused a dose-related increase in locomotion, with 60 µg causing the largest increases in motor activity. Little or no headweaving or ataxia was observed after injection of PCP. These results suggest that PCP can elevate extracellular DA concentrations by actions on the terminal regions of the DA neurons, and that these effects are related to some of the stimulant properties of PCP.

493.9

THE EFFECT OF INTRA-ACCUMBENS AMPHETAMINE ON LATENT INHIBITION USING AN ON-BASELINE, WITHIN-SUBJECT DESIGN. A.S.Killcross* and T.W.Robbins,(SPON: Brain Research Association). Dept. of Exp. Psychology, University of Cambridge, Cambridge, CB2 3EB, UK.

Latent inhibition (LI) is a phenomenon in which repeated, non-reinforced exposure to a stimulus retards subsequent conditioning to that stimulus. This is generally considered to be the result of learning to ignore or not to attend to the preexposed stimulus, and as such LI is commonly used to assess the influence of drugs and lesions on attentional processes. Several recent experiments have demonstrated that low doses of amphetamine, given acutely during preexposure and conditioning, abolish LI in a subsequent test session. These effects have been attributed to amphetamine-induced changes in mesolimbic dopamine (DA) function. The aim of this experiment was to test this hypothesis directly by examining the effects of intra-accumbens amphetamine on LI. In a within-subjects design with rats, LI was mapped across four conditioning trials using a conditioned suppression procedure. Animals that received bilateral infusions of d-amphetamine (1µl, 10µg/µl) immediately prior to preexposure and conditioning sessions showed a single trial retardation in conditioning, but subsequently developed a LI effect comparable to that of animals receiving control infusions of vehicle. These results provide no support for the hypothesis that the mesolimbic DA system plays a role in the attentional processes producing LI. It is likely that the decrements in LI found following acute, peripheral injections of amphetamine are due either to non-specific behavioural effects of the drug, or to effects of the drug on non-mesolimbic systems.

493.10

EXTRACELLULAR MONOAMINE LEVELS ARE DISSOCIATED FROM CHANGES IN LOCOMOTOR ACTIVITY FOLLOWING MEDIAL PREFRONTAL CORTEX ABLATION. E. Castañeda, D. Fiorino*, G. Mittleman and I.Q. Whishaw. Dep'ts. of Psychology, Arizona State University, Memphis State University & University of Lethbridge, Lethbridge, Alberta T1K 3M4.

Evidence suggests that some of the behavioral changes that follow cortical injury are produced by compensatory changes in the striatum. We examined the motor activity of rats that had received bilateral medial frontal cortex lesions and also examined extracellular monoamine levels in caudate nucleus and nucleus accumbens using *in vivo* microdialysis. Medial prefrontal cortex damaged rats displayed an attenuation in locomotor behavior during baseline measures and after an amphetamine injection (1.5 mg/kg, s.c.) one day after injury. In contrast, after two weeks recovery animals displayed heightened locomotion in relation to controls in response to amphetamine. There were no changes in extracellular levels of mesotelencephalic catecholamines (dopamine, DOPAC & HVA) at any time; and the serotonin metabolite, 5-HIAA, displayed a significant enhancement in nucleus accumbens extracellular levels, but only after an amphetamine challenge given on the day following medial prefrontal cortex lesions. The implications for non-dopaminergic mechanisms that might be responsible for recovery of behaviors traditionally thought to be mediated by mesotelencephalic dopamine systems will be discussed.

493.11

IBOTENIC ACID LESIONS OF THE MEDIAL PREFRONTAL CORTEX, DORSAL OR VENTRAL HIPPOCAMPUS DIFFERENTIALLY AFFECT RESPONSE TO FG-7142 IN THE RAT. Barbara K. Lipska, George E. Jaskiw, Daniel R. Weinberger. Clinical Brain Disorders Branch, IRP, NIMH, Neuroscience Center at St. Elizabeths, Washington, DC 20032.

The mechanism by which the anxiogenic ß-carboline FG-7142 reduces exploratory locomotor activity in rats is not known. FG-7142 has been shown to selectively enhance DA release within the medial prefrontal cortex (MPFC) and to reduce 5HT transmission in the hippocampus. Both these regions have been implicated in the control of exploratory activity. To assess the anatomical specificity of FG-7142-induced reduction of locomotion, we evaluated its effects in rats with ibotenic acid (5-6 µg/0.5 µl) lesions of MPFC, dorsal (DH) or ventral hippocampus (VH) as compared to the corresponding sham-operated animals (infused with buffered saline). We found that FG-7142 suppressed the locomotor behavior in control and VH lesioned rats. Its effect on locomotor reduction was potentiated in MPFC lesioned rats, but abolished in DH rats. These results suggest a specific role of the DH in mediating FG-7142-induced attenuation of exploration, possibly related to specific serotonergic projections to this region.

493.12

DISCRIMINATORY ACTION OF GABA AND VIGABATRIN ON ISOLATION-INDUCED OFFENSIVE BEHAVIOR IN MICE. M.Y.Hasan,R.D.E.Sewell and P.J.Nicholls. SPON: Brain Research Association, Division of Pharmacology, Welsh School of Pharmacy, UWCC, PO Box 13, Cardiff CF1 3XF,UK

GABA is involved in modulating mood and behavior and inhibition of GABA-transaminase reduces offensive behavioral expression in mice. Vigabatrin is an irreversible inhibitor of GABA-transaminase and increases GABA levels in the CNS. This study compares the effects of Vigabatrin with GABA on several behaviors including locomotion, non-social, social and in particular offensive profiles. These behaviors were assessed in male ICI-GB1 mice starting weight (20-25g,n=10) using a modified isolation procedure previously described by this laboratory. The behavioral interaction was observed between 3 week-isolated and group-housed individuals over a test period of 10 min. Pretreatment with GABA (<400mg/kg ip) or Vigabatrin (<600mg/kg ip) gradedly reduced the incidence of offensive encounters/animal, % animals displaying offense and delayed the onset of offense without modifying social, non-social and locomotor behaviors. Thus GABA and Vigabatrin anti-offensive activity is comparable and occurs at behaviorally selective doses.

493.13

PREVENTION BY MORPHINE OF PENILE ERECTION AND YAWNING INDUCED BY APOMORPHINE AND OXYTOCIN. M.R. Melis, R. Stancampiano* and A. Argiolas. B.B. Brodie Dept. of Neurosciences, Univ. of Cagliari, Via Porcell 4, 09124 Cagliari (Italy).

The effect of morphine administered systemically or into the paraventricular nucleus of the hypothalamus (PVN) on penile erection and yawning induced either by oxytocin or by the dopaminergic agonist apomorphine was studied in male rats. Systemic morphine (0.5-5 mg/kg i.p.) prevented in a dose-dependent manner penile erection and yawning induced by oxytocin (30 ng i.c.v.) or by apomorphine (80 μg/kg s.c.). Morphine (0.1-5 μg), but not U-69,593 (5 μg), injected into the PVN 10 min before oxytocin or apomorphine, prevented penile erection and yawning induced by the unilateral PVN microinjection of oxytocin (10 ng) or apomorphine (50 ng). The morphine effect was abolished by naloxone (1 and 3 mg/kg i.p.) 15 min before morphine. The results suggest that morphine prevents apomorphine- and oxytocin-induced responses by inhibiting the activity of oxytocinergic neurons through μ-type receptors in this hypothalamic nucleus. The finding raise the intriguing possibility that opiates inhibit sexual behavior by inhibiting central oxytocinergic transmission.

DRUGS OF ABUSE—AMPHETAMINE AND NICOTINE

494.1

ROTATIONAL BEHAVIOR AND CONCURRENT STRIATAL DOPAMINE OVERFLOW FOLLOWING AMPHETAMINE, PHENCYCLIDINE AND MK-801. A. Mele, D.J. Fontana, and A. Pert. BPB / NIMH, Bethesda, MD.

It has been suggested that some of the behavioral and pharmacological effects of PCP, like amphetamine, are determined through increases in dopaminergic (DA) function. The purpose of this study was to compare the effects of PCP, amphetamine, and the NMDA antagonist, MK-801, on rotational behavior and on concurrent striatal DA overflow in rats with unilateral nigrostriatal lesions. Rats were lesioned in the left MFB with 6-OHDA and implanted with a guide cannula in the striatum contralateral to the lesion. One week following surgery, a microdialysis probe was inserted into the striatum through the implanted guide. The animals were placed in a rotometer which had been modified to allow the collection of dialysate. Levels of DA and its metabolites were measured in the dialysate with HPLC-EC procedures at 20 min intervals. Following stabilization of DA levels, the animals were injected i.p. with either PCP (10 mg/kg), amphetamine (1 mg/kg), or MK-801 (0.25 mg/kg). All three drugs produced significant increases in rotational behavior ipsilateral to the lesion. Only amphetamine and PCP, however, elicited increases in striatal DA overflow. The effects of PCP on striatal DA were rather modest (approximately 50% increase over baseline at peak effect), whereas its effect on rotational output was quite dramatic (approximately 60 rotations/20 min at peak effect). Amphetamine, on the other hand, produced a more robust effect on DA (approximately 150% increase at peak effect) at the same time elevating rotational output to only 40 rotations at peak. MK-801 had behavioral effects similar to amphetamine, but did not produce any alterations in striatal DA. Thus, there appears to be a significant dissociation between the effects of these drugs on rotational behavior and their ability to alter striatal DA function. It is suggested that the modest DA actions of PCP contribute little to determining its effects on nigrostriatal functions.

494.2

METHAMPHETAMINE-INDUCED STIMULUS PROCESSING DEFICITS. B.G. Cooper*, A.E. Butt, D.J. Hardy*, & G.K. Hodge. Psychology Dept., University of New Mexico, Albuquerque, NM 87131-1161.

Neurotoxic doses of methamphetamine (MA) resulted in cognitive deficits in rats tested in an operant discrimination task. Animals were shaped to bar-press and then received four s.c. injections (2 hours between injections) of either saline or MA (10 mg/kg or 12 mg/kg). One week post-injection, animals were tested in an operant discrimination task. The first phase of the task required animals to press a bar for a food reward during the presentation of a 10-sec target light. A 10-sec period of darkness followed the target light; bar-presses during this period were recorded as errors. During the second phase of testing, a 10-sec distractor light followed the target light instead of darkness. Errors were penalized in this phase by delaying the onset of the target light by an additional 10 sec. During the third phase of testing, the target window was reduced to 3 sec, with distractor light and penalty conditions remaining constant. Results from the second phase of testing showed an initial increase in errors in the 10 mg/kg group, followed by rapid recovery; the 12 mg/kg group showed a more prolonged increase in errors with subsequent recovery. The third phase of testing was characterized by a pronounced increase in errors in both MA groups. Results suggest the inability of MA-treated rats to alter response patterns as task parameters change.

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494.3

MDMA'S EFFECTS ESTABLISH A CONDITIONED PLACE PREFERENCE AND ELICIT SIGNS OF SEXUAL AROUSAL AMONG RATS. E.J. Bilsky* and L.D. Reid. Rensselaer Polytechnic Institute, Troy, NY 12180.

Studies of methylenedioxymethamphetamine (MDMA) are of interest because of its potential addiction liability and its neurotoxicity. Previously, we have demonstrated that MDMA establishes a positive conditioned place preference (CPP) among rats. Doses of 30 microgram/kg MDL 72222, a selective serotonin 5-HT3 antagonist, and 30 mg/kg CGS10746B, two compounds that attenuate the release of dopamine, blocked the establishment of a MDMA CPP. The data lead to the suggestion that MDMA's ability to elicit positive affect (and, thereby, positive reinforcement) is modulated by dopaminergic pathways. In observing rats being conditioned with a 6.3 mg/kg dose of MDMA, the ejaculation of seminal plugs was noted. With the first injections, in one experiment: (a) rats receiving saline or naltrexone (56 mg/kg, SC, 4 hr before conditioning) almost never left plugs in the place of conditioning, while (b) 7 of 12 rats receiving only MDMA, and (c) 12 of 12 rats receiving MDMA and naltrexone left plugs.

494.4

***IN VITRO* METABOLISM OF 6-HYDROXY-3,4-(METHYLENEDIOXY)METHAMPHETAMINE TO THE NEUROTOXIN, 2,4,5-TRIHYDROXYMETHAMPHETAMINE.** H.K. Lim*, W. Stevens and R.L. Foltz*, *Center for Human Toxicology, Dept. Pharmacology and Toxicology and Dept. Anatomy, University of Utah, Salt Lake City, UT 84112.

Since O-dealkylation has been reported as a primary *in vivo* metabolic pathway for 3,4-(methylenedioxy)methamphetamine (MDMA) in the rat, it is likely that the previously identified aromatic hydroxylated metabolites of MDMA (Lim and Foltz 1990) are further metabolized by O-dealkylation to compounds similar in chemical structure to the potent neurotoxin, 6-hydroxydopamine. Thus, this study examines the *in vitro* rat liver metabolism of 6-hydroxy-3,4-(methylenedioxy)methamphetamine (6-OH-MDMA) to 2,4,5-trihydroxymethamphetamine (THM). THM was found to cyclize to 5,6-dihydroxy-1,2-dimethylindole (DHDMI) when incubated in fortified rat liver S-9 fraction (Lim and Foltz, 1990). Selected reaction monitoring of the daughter ion at m/z 218 from DHDMI provided a highly sensitive method for the detection of THM. Comparison of the daughter ion profiles corresponding to the *in vitro* incubate containing 6-OH-MDMA and control indicated the presence of a peak not found in the control. Conclusive identification of this peak as DHDMI was from co-injection with synthetic DHDMI. Addition of SKF-525A, 1-aminobenzotriazole and quinidine resulted in 100, 76 and 100 % inhibition of the*in vitro* metabolism of 6-OH-MDMA. This study conclusively identified THM as an *in vitro* metabolite of 6-OH-MDMA. Furthermore, the metabolism of 6-OH-MDMA to THM is cytochrome P-450 dependent; the specific cytochrome P-450 isozyme P450IID6 appears to be involved. (Supported by NIDA grant 1RO1 DA 05860-01).

494.5

EFFECT OF 2,4,5-TRIHYDROXYAMPHETAMINE ON MONOAMINERGIC SYSTEMS IN THE RAT BRAIN. I.M. Elayan*, M. Johnson, G.R. Hanson, K.H. Lim*,R.L. Foltz* and J.W. Gibb. Department of Pharmacology and Toxicology. University of Utah, Salt Lake City, Ut 84112.

This study was designed to test the effect of a new amphetamine derivative, 2,4,5-trihydroxyamphetamine (THA), on the monoaminergic systems in the rat brain after intraventricular injection. In the long-term study, male Sprague-Dawley rats (200-280 g) were sacrificed one week after the injection of three different doses of THA (0.25, 0.5, or 1.0 µmole in 20 µl of 0.1% ascorbate in saline). A control group was injected with the same volume of the vehicle. Hippocampal tryptophan hydroxylase (TPH) activity was markedly reduced to 5%, 1%, and 7% of control by 0.25, 0.5, and 1.0 µmole THA, respectively. TPH activity in the striatum was decreased to 74%, 81%, and 56% of control and sriatal tyrosine hydroxylase (TH) activity was reduced to 67%, 10%, and 19% of control, with the same doses, respectively. Hippocampal norepinephrine levels were reduced to 10%, 18%, and 16% of control. In the short-term experiment, the rats were sacrificed 3 hours after the injection of THA (1.0 µmole) or saline. Hippocampal and striatal TPH activity were decreased to 8% and 79% of control, respectively. Striatal TH activity was reduced to 75% of control. Hippocampal norepinepherine was reduced to 16% of control. This study demonstrates that THA is a potent compound that decreases hippocampal and striatal TPH and TH activity when administered intraventricularly. (Supported by USPHS grants DA 00869, DA 04222 and DA 05860).

494.6

FUNCTIONAL INTERACTIONS OF LIMBIC AFFERENTS TO THE VENTRAL STRIATUM WITH MESOLIMBIC DOPAMINE: STUDIES OF CONDITIONED REINFORCEMENT AND LOCOMOTOR ACTIVITY IN THE RAT. L.H. Burns*, B.J. Everitt, and T.W. Robbins. Depts. of Experimental Psychology and Anatomy, Univ. of Cambridge, Cambridge, U.K. (SPON: European Brain and Behaviour Society).

Intra-accumbens d-amphetamine enhances the behavioral control of reward-related stimuli (or conditioned reinforcers) via dopaminergic mechanisms. This study examines the contributions of different limbic afferents to the ventral striatum in the amphetamine-induced potentiation of conditioned reinforcement. Rats were trained to associate a light+noise stimulus (the CR) with reward and then tested in extinction for the acquisition of a new response, maintained by this conditioned reinforcer. Excitotoxic lesions of either the basolateral amygdala or the ventral subiculum suppressed responding maintained by the conditioned reinforcer, with the greatest effect at the higher doses of intra-accumbens d-amphetamine. Similar results were found following excitotoxic lesions of the ventral striatum itself or following intra-accumbens infusions of low doses of NMDA receptor or quisqualate/AMPA receptor agonists and antagonists. Control experiments assessed the effects of these treatments on sucrose consumption and locomotor activity. The results suggest that the behavioral control of reward-related stimuli is mediated by limbic afferents and their interaction with dopaminergic mechanisms in the ventral striatum.

494.7

INFLUENCE OF CORTICOSTERONE ON AMPHETAMINE SELF-ADMINISTRATION. P.V. Piazza, V. Deroche*, S. Maccari*, J.M. Deminière*, M. Le Moal and H. Simon. Lab. des Comportements Adaptatifs INSERM U259 - Univ. Bordeaux II. Domaine de Carreire 33077 Bordeaux Cedex-France.

Individual vulnerability to the reinforcing properties of drugs of abuse appears to be an essential characteristic predisposing humans to addiction. In rat we have previously shown that individuals with a higher locomotor reactivity and a longer corticosterone release in response to novelty (High Responders, HR) have a higher predisposition to acquire amphetamine self-administration (SA)(Science 1989 245:1551-1513). Animals with weak behavioral and endocrinological responses to novelty (Low Responders, LR) do not develop SA. In the present study we have investigated whether corticosterone levels have a direct influences on amphetamine SA. When animals naturally resistant to develop amphetamine self-administration where injected with corticosterone they developed this behavior. Such an effect was present both when corticosterone (0.36mg/Kg i.v.) was administered after 8 days of testing for SA ($P<0.01$), during which resistant animals do not develop SA, and when the hormone (50lg/20µl) was present in the SA solution in addition to amphetamine (10lg/20µl) ($P<0.05$). Corticosterone also modified SA of individuals that spontaneously acquired this behavior. In this case, and with both the protocols described above, the hormone decreased the intake of the drug of HR animals. This apparent opposite effect of corticosterone in the two groups of individuals is similar to the phenomenon observed when the amount of drug delivered for each injection is increased: there is a faster acquisition of this behavior and a decrease of the number of drug injections during SA. Thus, it is suggested that corticosterone acts on SA by increasing the reinforcing properties of amphetamine. The possible direct reinforcing effect of corticosterone is under investigation and will be discussed.

494.8

CENTRAL CORTICOSTEROID RECEPTORS AFFINITY IS REDUCED IN RATS PREDISPOSED TO DEVELOP AMPHETAMINE SELF-ADMINISTRATION. S. Maccari*, P.V. Piazza, J.M. Deminière*, L. Angelucci, H. Simon and M. Le Moal. INSERM U.259, Rue Camille St Saëns, 33700 Bordeaux, France, and Farmacologia II, Univ. Roma, P.le A. Moro,2 00185 Rome, Italy.

Recently, we suggested that reduced corticosterone feedback in the rat may be involved in the predisposition shown by some individual animals to develop amphetamine self-administration (SA) of amphetamine. This hypothesis was based on the following observations: i) animals which are predisposed to acquire SA have a longer secretion of corticosterone after stress; ii) life events, which induce a longer secretion of corticosterone after stress, also increase likelihood to acquire SA; iii) an experimental increase of corticosterone levels also increase the propensity to amphetamine SA. Since corticosteroid receptors are involved in corticosterone feedback sensitivity, we examined the relation between individual differences in amphetamine SA and characteristics of corticosteroid receptors in hippocampus, amygdala and hypothalamus. Rats were selected on the basis of reactivity to novelty and designed as: 1) High Responder (HR) rats, with high locomotor reactivity, prolonged corticosterone secretion in response to novelty, and fast acquisition of amphetamine SA; 2) Low Responder (LR) rats, with low locomotor response and short corticosterone secretion in response to novelty, which fail to acquire amphetamine SA. The HR animals showed a lower affinity of type I receptors in the hippocampus ($P<0.01$) and amygdala ($P<0.01$), and a reduced affinity of type II receptors only in the hippocampus ($P<0.05$). The receptors in hypothalamus were not different in the two groups. These data suggest that a reduced affinity of corticosteroid receptors in hippocampus and amygdala may be responsible for the longer secretion of corticosterone after stress observed in the HR animals. Thus, it is suggested that pharmacological manipulations of corticosteroid receptors may open new therapeutic strategies for compulsive drug use.

494.9

INFLUENCE OF CORTICOSTERONE ON INDIVIDUAL BEHAVIORAL FEATURES PREDICTING THE PROBABILITY TO ACQUIRE AMPHETAMINE SELF-ADMINISTRATION V. Deroche*, P.V. Piazza, S. Maccari*, J.M. Deminière*, M. Le Moal and H. Simon. Lab. des Comportements Adaptatifs INSERM U259 - Univ. Bordeaux II. Domaine de Carreire 33077 Bordeaux Cedex-France.

In previous work (Science, 1989,245:1551-1513), we have shown that rats with a higher predisposition (High Responders, HR) to acquire amphetamine self-administration (SA) show higher locomotor activity in response to novelty and to amphetamine administration and also during the nocturnal period. Indeed animals with a weak behavioral response to these environmental and pharmacological challenges (Low Responders, LR) did not develop SA. HR animals also have a longer corticosterone secretion in response to novelty than LR groups. In the present study we have investigated whether corticosterone levels may have a direct influence on locomotor activity and on locomotor responses to amphetamine. The higher locomotor activity of HR animals seems to be partially dependent on plasma corticosterone levels. Thus, when HR animals are adrenalectomized and implated with corticosterone pellets, which maintain corticosterone levels in a physiological but fixed range, the locomotor activity of HR animals drop to the level of the LR's ($P<0.05$). Moreover, the addition of corticosterone (50mg/ml) to the drinking water of LR's increased their nocturnal locomotor activity to the level of HR animals ($P<0.01$). Corticosterone levels also may influence the locomotor response to amphetamine. Thus, when HR and LR animals do not differ in corticosterone levels amphetamine administration induces in the two groups the same increase in locomotor activity. Conversely when HR animals show higher corticosterone levels (i.e. after 120 min of exposure to novelty) amphetamine induces in this group of rats a greater increase of locomotor response ($P<0.01$). Furthermore, the addition of corticosterone in drinking water for 15 days (50mg/ml)induced an increase of locomotor response to amphetamine ($P<0.01$).These results suggest that corticosterone levels is an important biological feature determining individual differences in behaviors related to the predisposition to psychostimulant SA.

494.10

THE BEHAVIORAL EFFECTS OF ACUTE AND CHRONIC ADMINISTRATION OF 4-METHYLAMINOREX (4-MAX) ON ACTIVITY IN THE RAT. Batsche, K., Ashby, C. R. Jr, Pan, H. S., Kimura, C., Schwartz, J., and Wang, R. Y. Department of Psychiatry, SUNY Stony Brook, Putnam Hall, South Campus, Stony Brook, N.Y. 11794-8790.

The compound 4-methylaminorex is a phenylisopropylamine derivative with 4 isomers: trans 4R, 5R; trans 4S, 5S; cis 4S, 5R; and cis 4R, 5S. This compound produces sympathomimetic, stimulant, and anorectic effects similar to amphetamine and psychological effects similar to amphetamine and cocaine. We show here that acute subcutaneously administration of the cis 4R, 5S isomer of 4-MAX (.3, 1, and 3 mg/kg) in rats caused dose-dependant increases in horizontal activity. The drug effect for 3 mg/kg lasted for more than 3 hrs with the peak of activity around 1.5 hrs following injection of the drug. The locomotor activity for the dose 10 mg/kg was reduced relative to that of 3 mg/kg but was still larger than any other dosage assessed. At 10 mg/kg stereotyped sniffing was markedly increased. Our preliminary results show that the acute effects of the 4R, 5S isomer at 3 mg/kg were significantly attenuated by the dopamine D2 receptor antagonist eticlopride at .5 mg/kg IP. Chronic treatment with 4R, 5S isomer (3 mg/kg) for 7 days and rechallenged following 1 day drug withdrawal caused a marked desensitization in the horizontal activity. By contrast, in the same treatment paradigm followed with 10 days withdrawal and rechallenged with 4-MAX (4R, 5S), no desensitization was observed. In fact, the horizontal activity peaked earlier in this group than the control indicative of a sensitizing response. In conclusion, similar to amphetamine, 4-MAX dose-dependently increases locomotor activity. Unlike amphetamine, however, there was a sign of desensitization following chronic treatment.
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494.11

EFFECT OF 4-METHYLAMINOREX ON MIDBRAIN DOPAMINE CELLS. C. Vogel, C.R. Ashby, Jr., H. Pan, L. Fishkin, Y. Minabe and R.Y. Wang. Dept. of Psychiatry and Behav. Sci. SUNY at Stony Brook, Putnam Hall-South Campus, Stony Brook, NY 11794-8790

4-methylaminorex (4-MAX, U4Euh), a phenylisopropylamine derivative, is a potent psychostimulant and anorectic agent, similar to amphetamine. It consists of four isomers: trans 4R, 5R, trans 4S, 5S, cis 4R, 5S and cis 4S, 5R. The aim of the present study was to compare the potency and efficacy of isomers of 4-MAX to inhibit the firing of midbrain dopamine (DA) cells. Single cell recording techniques were used to record DA cells in both the substantia nigra pars compacta (A9) and the ventral tegmental area (A10) in chloral hydrate anesthetized Sprague Dawley rats. All 4-MAX isomers dose-dependently suppress the firing of both A9 and A10 DA cells. In A10 DA cells the most potent isomer was trans 4S, 5S (IC50 = 0.56 mg/kg, i.v.) followed by the cis isomers (IC50s for cis 4S, 5R and 4R, 5S were 6.5 and 6.1 mg/kg, respectively) and the trans 4R, 5R isomer (12.8 mg/kg produced 43% inhibition). The same rank order of potency holds for the A9 DA cells. However, the trans 4S, 5S appears to be 6.6 fold less potent in inhibiting A9 compared to A10 DA cells. This result is surprising because the S(+)amphetamine stimulus generalized to all 4-MAX isomers and S(+)amphetamine appears to suppress equi-potently both A9 and A10 DA cells. Although systemically administered S(+)amphetamine has been shown to stimulate DA release more effectively in the nucleus accumbens as compared to the caudate, whether the latter finding can be extended to 4-MAX isomers remains to be demonstrated. In conclusion, our results show that similar to the action of S(+)amphetamine, 4-MAX isomers dose-dependently suppress the firing of both A9 and A10 DA cells. Unlike S(+)amphetamine, 4S, 5S isomer at low doses appears to exert its action preferentially upon A10 DA cells. (Supported by USPHS grants MH-41440 and MH-00378.)

494.12

REPEATED ADMINISTRATION OF AMPHETAMINE, AT LOW DOSES, CAUSES SUBSENSITIVITY OF A10 SOMATODENDRITIC DOPAMINE AUTORECEPTORS. R.S.J. Nassar and F.J. White. Wayne State Univ. Sch. Med., Dept. Psychiatry, Cellular and Clinical Neurobiology Program, Neuropsychopharmacology Laboratory, Lafayette Clinic, Detroit, MI 48207.

Repeated administration of amphetamine (AMPH), at low doses, produces an enhancement of its locomotor stimulating effects. This sensitization involves enhanced transmission within the mesoaccumbens dopamine (DA) system, including increased DA releasibility. Recent evidence indicates that an additional action of repeated AMPH within the ventral tegmental area (VTA) is necessary for the initiation of sensitization. We have suggested that subsensitivity of impulse-regulating somatodendritic DA autoreceptors, and the resulting increased activity of A10 DA neurons within the VTA, may be important triggering events leading to other, more persistent, neuronal alterations responsible for maintaining sensitization. Previous studies of DA autoreceptor subsensitivity have used AMPH doses which are higher than those necessary for the induction of sensitized locomotion. Therefore, the present study determined the ability of a low d-AMPH dosing regimen (1.0 mg/kg/day, i.p., 5 days on/2 days off/5 days on), known to produce locomotor sensitization, to cause subsensitivity of A10 DA autoreceptors. Anesthetized rats that had received either repeated AMPH or saline were prepared for single cell recording 3-4 days after the last injection. The inhibitory effects produced by i.v. challenge with either the mixed D1/D2 receptor agonist apomorphine or the D2/D3 receptor agonist quinpirole on A10 DA cell firing were significantly reduced in the AMPH-treated rats. Thus, somatodendritic A10 DA autoreceptor subsensitivity occurs following repeated administration of AMPH at doses which produce sensitization to its locomotor stimulant effects. (Supported by MH-40832 and DA-04093).

494.13

DIFFERENTIAL BRAIN REGIONAL DISTRIBUTION OF [^{3}H]D-AMPHETAMINE AFTER PERIPHERAL ADMINISTRATION R. Zaczek, C. Dersch and N.M. Appel. NIDA Addition Res Ctr, Balto., MD 21224

While much work has been done on the pharmacokinetic and distribution profiles of d-amphetamine (d-AMPH) subsequent to peripheral administration, controversy remains as to whether d-AMPH accumulates uniformly or unevenly across brain regions. We examined the in vivo accumulation of [^{3}H]d-AMPH after peripheral administration and found that both dose and time can be confounding factors when addressing the question of its brain distribution. At times up to 12 min post subcutaneous injection of 5mg/kg [^{3}H]d-AMPH , the regional distribution of d-AMPH appeared uniform. However, after 30 minutes a distinct differential distribution d-AMPH in brain became apparent. At 45 minutes, the areas with highest levels of d-AMPH were the neocortex and hippocampus (38 ± 7 and 38± 4 pmoles/mg wet wt, respectively). Areas with the lowest levels of d-AMPH were the pons-medulla and the cerebellum (26 ± 3 pmoles/mg wet wt). At lower doses (0.5mg/kg) of [^{3}H]d-AMPH, the distribution of the drug in brain appeared uniform. We found two reasons for the discrepancy between the distribution of tritium after injections of 0.5 and 5 mg/kg [^{3}H]d-AMPH. First, unmetabolized [^{3}H]d-AMPH made up a much smaller percentage of the total tritiated compounds found after 0.3 mg/kg (30 ± 18%) than after 3 mg/kg [^{3}H]d-AMPH (80 ± 15%). Second, rats injected with 0.5 mg/kg [^{3}H]d-Amph and subsequently subjected to perfusion fixation possessed identical brain regional distributions of [^{3}H]d-AMPH as rats injected with 5 mg/kg [^{3}H]d-Amph. These data suggest that the apparent uniform regional distribution after the low dose was due to serum contaminating brain. We conclude that d-AMPH accumulates differentially into various brain areas after peripheral administration. The rostro-caudal gradient of d-AMPH accumulation is reminiscent of the phenomenon of AMPH sequestration we have previously described.

494.14

ALTERATIONS IN MESOLIMBIC AND NIGROSTRIATAL DOPAMINE OVERFLOW FOLLOWING CHRONIC AMPHETAMINE. K.M. Wozniak, A. Mele, M. Linnoila and A. Pert.LCS/NIAAA and BPB/NIMH, Bethesda, MD 20892.

Certain behavioral effects of amphetamine increase in intensity and duration with repeated administration. The precise mechanism underlying this phenomenon of "behavioral sensitization" is unknown, although there is evidence for enhanceed synaptic accumulation of dopamine (DA) in chronically treated rats following an acute challenge. Using in vivo microdialysis, we have examined and compared the effect of various doses of amphetamine, applied both to the terminal regions and systemically, on DA overflow in rats chronically treated with amphetamine as compared to saline. Focal application of amphetamine into the striatum resulted in similar increases in DA in both treatment groups when administered 24 hr. after cessation of a 7-day (3 mg/kg, i.p., daily) treatment regimen. Similarly, there was no difference in either the n. accumbens or striatum between the two treatment groups following a systemic challenge of amphetamine (1 mg/kg, s.c.), either 24 hr. or 72 hr. after the chronic regimen. In contrast, a lower challenge dose of amphetamine (0.25 mg/kg, s.c.) elicited a larger increase in extracellular DA in the amphetamine-pretreated rats than in the control group, when administered 3 days after the chronic treatment regimen. Hence our preliminary data suggest that a pharmacological sensitization of dopamine systems after chronic amphetamine, which is apparently absent following certain acute challenges, can be unmasked following a systemic dose that produces relatively modest changes in dopamine. We are further investigating the exact nature of this process.

494.15

CORTICOSTERONE ACCELERATES THE DEVELOPMENT OF TOLERANCE TO NICOTINE-INDUCED ANALGESIA IN RATS A.R. Caggiula, L.H. Epstein*, S.M. Antelman*, S. Knopf* S. Saylor* and K.A. Perkins*. *Depts. of Psychology and Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA 15260*

We have previously shown that conditioned tolerance develops to some of the behavioral and endocrine effects of nicotine and Collins et al (1990) have recently suggested that a conditioned release of corticosterone (CORT) may contribute to this form of tolerance since chronic CORT reduces nicotine responsiveness in mice. Two studies tested the feasibility of this idea in male rats. In one, a single sc injection of 0.75 mg/kg of CORT prevented the analgesic response to sc 0.66 mg/kg of nicotine bitartrate (free base) given 5 min later, when compared to untreated controls. In a second study, males were given CORT (1 mg/kg), vehicle (ETOH + distilled water) or no injection 10 min before nicotine (.75 mg/kg) and tested for nicotine-analgesia every other day for 10 days. Significant tolerance developed more rapidly (1st vs 3rd injections) in the CORT pretreated rats compared to animals given only nicotine. A similar acceleration was produced by the vehicle pretreatment, which induced a significant release of endogenous CORT. These data are consistent with the hypothesis that a conditioned CORT response could contribute to the development of tolerance to some of nicotine's effects.

494.16

EXPOSING RATS TO A SINGLE BRIEF STRESSOR TWO WEEKS EARLIER MODIFIES THE RESPONSE OF PLASMA ACTH AND GLUCOSE TO ETHANOL (ETOH). S.M. Antelman*, A.R. Caggiula, S. Knopf*, D.J. Edwards and H. Barry III *Depts of Psychiatry, Psychology and Pharmacology/Physiology, Univ. of Pittsburgh, Pittsburgh, Pa. 15213*

We have shown that one exposure to a stressor can induce an extremely long-lasting alteration in the subsequent responsiveness of the organism to pharmacological or nonpharmacological stressors. This effect progresses with time since the first stressor and has been demonstrated using CNS, endocrine and immune endpoints, among others. The present study inquired whether one exposure to a stressor [an injection of isotonic saline or 200 or 750 mg/kg, 2-deoxy-d-glucose (2DG)] 2 weeks earlier would alter the effect of ETOH (1g/kg) on the stress-responsive hormones, ACTH, corticosterone (CORT) and glucose. These were measured at 5, 15 & 30 min. following ETOH, as were adrenal weights. At 5 min., ETOH itself significantly elevated both ACTH and glucose and this elevation was prevented in animals injected with saline 2 weeks earlier. At 15 min., significant elevation of ACTH and glucose occurred only in rats preexposed to 1 or both of our stressors 2 weeks earlier. Earlier stress failed to influence either the CORT response to ETOH or adrenal weights. Supported by MH24114 from NIMH & P50AA08746 from NIAAA.

494.17

RE-EXAMINATION OF PENTYLENETETRAZOL (PTZ)-LIKE EFFECT OF NICOTINE (NIC) AND ITS WITHDRAWAL. C.M. Harris, Dept. Pharmacol., New York Col. Osteo. Med., Old Westbury, NY. 11568

Two groups of rats were trained in a 2-lever choice task with food reward to discriminate the anxiogenic drug PTZ (12 mg/kg or 20 mg/kg) from saline. NIC substituted fully for PTZ and this effect depended upon both dose of NIC and training dose of PTZ. Tolerance to this subjective effect of NIC was evident during chronic infusion of NIC via osmotic pump for 7 days at 0, 10, 20 and 30 mg/kg/d. Chronic NIC dose-dependently reduced lever-pressing, food consumption, weight, and defecation. The magnitude of the weak PTZ-like stimulus previously found during withdrawal (Harris et al., Psychopharmacol. 90:85, 1986) depended neither on dose of chronic NIC nor on training dose of PTZ. These results indicate that NIC-withdrawal is not detected in a systematic manner by the PTZ discrimination. In contrast, the direct PTZ-like effect of NIC is both robust and systematic, suggesting that the acute anxiogenic effect of NIC may be mediated by a mechanism common to PTZ, while the withdrawal symptom "anxiety" is not.

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494.18

PEMPIDINE-INDUCED ANTAGONISM OF PHYSIOLOGICAL AND BEHAVIORAL RESPONSES TO NICOTINE. W. Cao, S. Borch*, M.J. Marks, A.C. Collins. Institute for Behavioral Genetics, Univ.of Colorado, Boulder, CO 80309

Previous studies have demonstrated that pempidine both competitively and noncompetitively antagonizes central actions of nicotine. The purpose of the present study was to determine if genetic factors influence the sensitivities of mouse strains to pempidine blockade of the effects of nicotine in vivo. Both time course and dose response relationships for pempidine antagonism of effects of nicotine were determined. C57BL and DBA mice were pretreated with saline or pempidine (0.5-6.0 mg/kg, ip) at various time points prior to nicotine administration (1.5 mg/kg, ip). The responses to nicotine were measured using a battery of physiological and behavioral tests that includes alterations in respiratory rate, acoustic startle response, Y-maze activity, heart rate and body temperature. Dose response experiments (15 min. preinjection) indicated maximal inhibition at a pempidine dose of 1.0 mg/kg. Possible toxicological actions were seen at higher doses. The 1.0 mg/kg dose of pempidine was used to determine a time course for nicotine antagonism; maximal antagonism was seen 20 min following pempidine injection. Pempidine antagonism of nicotine's effects persisted for at least 90 min following acute treatment. Strain differences in sensitivity to pempidine-induced antagonism of nicotine's actions were defined. The results indicate that pempidine is very similar in potency to the classical nicotine antagonist, mecamylamine, and that is a useful agent for determining the function of brain nicotine receptors. Supported by DA-00116 and DA-03194.

494.19

ANIMAL MODEL OF NICOTINE DEPENDENCE AND ABSTINENCE SYNDROME P.N. Maultsby*, J.R. Lake, C.W. Cortes*, J.G. Lanier*, L.K. Roberts*, O.B. Wilson and D.H. Malin. Univ. of Houston-Clear Lake, Houston, TX 77058 and Baylor College of Medicine, Houston, TX 77030.

Very few animal models are currently in use for the recognized clinical problem of nicotine dependence and abstinence. This study introduces a rapid and convenient model using the rat. Male rats (250g) were continuously infused s.c. for 7 days via Alzet osmotic minipump with 0, 3 or 9 mg/kg/day nicotine tartrate in saline. Rats were observed before, during and after drug infusion on a modified checklist of opiate abstinence signs based upon a pilot experiment with nicotine-abstinent rats. All 3 groups showed few signs both before infusion and at the end of infusion, but there were large, significant and dose-related differences 16 hours after pump removal. ANOVA showed significant drug, time and interaction effects.

OVERALL ABSTINENCE SIGNS (M ± SEM)			
Nicotine Tartrate (mg/kg/day):	0	3	9
Last Infusion Day:	1.7±0.3	2.2±2.0	1.7±1.0
16 Hours Post-Withdrawal:	10.7±2.9	23.4±5.2	43.6±8.3

Most frequently observed signs during withdrawal included: teeth chatter, writhes/gasps, ptosis, tremors, shakes, and yawning. Sharp post-nicotine drop in locomotor activity and increases in food consumption and weight gain provided additional evidence of an abstinence syndrome. (Supported by Neuromedical Technology, Inc.)

DEVELOPMENTAL DISORDERS OF THE NERVOUS SYSTEM II

495.1

ABNORMAL GANGLIOSIDE GD3 SYNTHESIS IN MUTANT MOUSE EMBRYOS. Seyfried, T.N. and Novikov, A.*, Boston College, Biology Dept., Chestnut Hill, MA 02167.

Gangliosides are a family of sialic acid-containing glycolipids that are proposed to function in cellular differentiation and adhesion. Although present in most cells and tissues, ganglioside concentration is greatest in neurons of the CNS. It is generally agreed, that neuronal ganglioside composition is regulated through two main metabolic pathways. The "a" pathway (comprising gangliosides GM3, GM2, GM1, and GD1a) and the "b" pathway (comprising GD3, GD2, GD1b, GT1b, and GQ1b). We previously found significant ganglioside abnormalities in E-11 and E-12 t^{w1}/t^{w1} mutant mouse embryos. The t^{w1} mutation is part of the T-locus complex on chromosome 17. These mutants express failed neural differentiation in the developing neural tube. The mutant ganglioside abnormalities involve reductions in "b" pathway gangliosides (especially GQ1b) and elevations in "a" pathway gangliosides. We proposed that these abnormalities could arise from a biosynthetic defect in the "b" pathway. To test this hypothesis we examined the in vitro activity of sialyltransferase 2 (SAT-2: EC2.4.99.8) in the normal (+/+ and $+/t^{w1}$) and t^{w1}/t^{w1} mutant embryos at E-12. This enzyme catalyzes the initial step for the synthesis of "b" pathway gangliosides. The enzyme assay was conducted for 4 hours at 37°C and contained (in 50 µl), substrate (GM3, 5 nmol), detergent (CF-54, 50 µg), donor (CMP-[^{14}C]-NeuAc, 25 nmol) cacodylate-HCl buffer (pH 6.43, 10 µmol containing 1 µmol MnCl2) and P2 embryo protein (50-100 µg). The labeled ganglioside products were separated using HPTLC and quantitated using liquid scintillation. The relative specific activity of SAT-2 (expressed as pmol ^{14}C-NeuAc incorporated into GD3/mg protein/hr) was significantly lower in the t^{w1}/t^{w1} embryos (227 ± 7, n=3) than in the +/+ (536 ± 24, n=6) or $+/t^{w1}$ (618 ± 33, n=6) embryos. The absence of an intermediate activity in the $+/t^{w1}$ embryos suggests that the reduced activity in the t^{w1}/t^{w1} mutants may be a pleiotropic effect of the mutation. (Supported by NIH grant 24826)

495.2

VITAMIN A INDUCED ALTERATIONS IN SECONDARY NEURULATION. T. Inagaki*, W. Goossens*, R.G. Higbee, D.G. McLone, and P.A. Knepper. Div Neurosurgery, Children's Memorial Med Center, Northwestern University, Chicago, IL 60614.

Vitamin A, in the proper amount and correct time, is crucial for embryonic development but if administered in excess, is a potential teratogen. To test the effects of vitamin A on secondary neurulation and canalization process, pregnant C-57 BL/6J mice were given IP injections of 5000 IU (Aquasol A) on gestation day 8.5 and 9.0 (during the time of closure of the posterior neuropore). On successive days, embryos were examined for neurulation defects and were processed for fluorescence microscopy using a battery of FITC-lectins and low light video microscopy. The majority of vitamin A embryos were abnormal, with absent tails (18%), kinked/short tails (64%) or caudal neural tube defects (2%). Light microscopy of tail-less embryos revealed alterations in the deposition of the basal lamina, delays in expression of sialic acid-containing glycoconjugates on the apical neuroepithelium and, in most embryos, the presence of multiple neuroepithelial rosettes with morphological features of neural tubes. These results suggest that excess vitamin A (or its active metabolites) altered the pre-set developmental timetable responsible for secondary neurulation.

(Supported in part by Kiwanis.)

495.3

DIFFERENTIATION PROCESS OF IV VENTRICLE OUTLETS IN MICE. K. Wang*,W. Goossens*, R.G. Higbee, D.G. McLone, and P.A. Knepper. Div Neurosurgery, Children's Memorial Med Center, Northwestern University, Chicago, IL 60614.

The differentiation process of the outflow mechanisms of cerebrospinal fluid (CSF) depends on remodeling of the primitive ventricular system. To examine the time at which the outlets of the IV ventricle become functional, embryonic C-57 BL/6J mice (gestation days 13, 14, 15, and 16) were investigated by: 1) injection of a CSF marker (0.5% potassium ferrocyanide-0.5% ammonium citrate [1:1] into the lateral or mesencephalic ventricles, followed by fixation and treatment with 2% hydrochloric acid (Prussian Blue reaction) and vibratome sections; or 2) injection of methyl methacrylate:monomer:catalyst: promoter (90:10:10:1, respectively) into the central canal, followed by fixation and scanning electron microscopy. The results of the Prussian Blue reaction product indicate that the outlets of the IV ventricle are functional by gestation day 15, and SEM revealed that a ciliated slit-like opening appeared in the lateral recesses of the IV ventricle. Studies are in progress to apply these approaches to determine whether the IV ventricle outlets and arachnoid development occur normally in genetic models of hydrocephalus (hy 3/hy 3) and the Chiari II malformation (dSp/dSp) in mice. (Supported in part by Kiwanis.)

495.4

LACTOSAMINE-CONTAINING GLYCOPROTEINS IN EMBRYONIC NORMAL AND ABNORMAL (NTD) SPINAL CORD. C.S.K. Mayanil*, W. Niforatos*, R. Higbee, D.G. McLone, and P.A. Knepper. Div Neurosurg, Northwestern Univ, Chicago, IL 60614.

Carbohydrates play roles in many cellular functions involved in neurulation, including cell recognition, adhesion, and differentiation. The tomato lectin, which recognizes lactosamine-containing glycoconjugates, was used to isolate glycoproteins from embryonic normal and abnormal (neural tube defect [NTD]) spinal cord. Neuroepithelial proteins were extracted in phosphate-buffered saline-2 mM EDTA-4 mM 2-mercaptoethanol-0.3 M lactose, followed by centrifugation at 100,000 x g for 1 hr. Supernatants were concentrated in centricon-10 to remove the lactose; 2 mg of protein was applied onto a pre-equilibrated tomato-lectin Sepharose 4B column. The bound proteins were eluted with chitotriose and chitotetraose in a ratio of 5:1. To date, we have analyzed bound fractions from rostral and caudal spinal cord proteins of normal and abnormal gestation day 14 delayed splotch embryos with NTD. As ascertained by SDS-PAGE, a set of lactosamine-containing proteins is characteristic of normal versus abnormal spinal cord; notably, a 15 kD and a 60 kD protein were not observed in the caudal NTD spinal cord. Studies are in progress to further characterize these glycoproteins and determine whether they function as carbohydrate-binding proteins. (Supported in part by Kiwanis.)

495.5

EFFECTS OF ACUTELY ADMINISTERED NALTREXONE ON SOCIAL BEHAVIOR OF AUTISTIC CHILDREN. I.F Borghese*, B.H. Herman, G.S. Asleson*, I. Chatoor*, M.B. Benoit*, P. Papero* & G. McNulty*. Brain Res Cen, CHNMC & Dept Psychiat, GWUSM, Washington, DC 20010.

Our group has hypothesized that heightened brain opioid activity and a dysregulation in POMC may underlie autism (Chamberlain & Herman, Biol. Psychiat 28:773 (1990)). Accordingly, we investigated the acute effects of placebo(P) and the opiate antagonist, naltrexone(N) (0.5, 1.0, 1.5, 2.0 mg/kg) in 13 autistic children (2F, 11M, 3-12y.o.).

The Childhood Autism Rating Scale(CARS) was used as an autism measure. Social behavior was assessed using the BRC Social Proximity Test where each subject was tested for two 10min sessions ("No Toys", "Toys") in a playroom with a volunteer(V) and a third 10min with mother(M). The room was divided into four quadrants(Q) (V in Q2).

CARS scores (n=5) were significantly lower in response to N (1.0, 1.5, and 2.0 mg/kg) than P ($p<.001$). ANOVA failed to indicate any significant effect of N on eye contact in either "No Toys" or "Toys" (n=13). We analyzed the effects of P vs N (1.0) on the pattern of Qs crossed (n=7). In the "No Toys" and "Toys" session, no significant effect of N (1.0) was obtained on the % time spent in Q2 with V. These data suggest that significant effects of N in autistic children can be obtained on a well established rating scale for autism. However, both eye contact and social proximity data failed to confirm the hypothesis of opioid involvement in the social dysfunction characteristic of autistics. Supported by grants from FDA, NICHD, and DuPont (to BHH)

495.6

PROLONGED ADMINISTRATION OF PIRACETAM TO DEVELOPING MICRENCEPHALIC RATS HAS LITTLE SALUTARY EFFECT ON MAZE LEARNING. A. Rabe, D. Dobrogowska*, A. Heaney*, and M.H. Lee. NY State Institute for Basic Research in Developmental Disabilities, Staten Island, NY 10314.

Rats with severe micrencephaly (induced by a single in utero exposure to 30 mg/kg of methylazoxymethanol acetate on gestation day 15) have a life-long impairment in maze learning. In search of early interventions that could ameliorate the defect, we tried the cognition-enhancing drug piracetam which improves learning in adult rats with MAM-induced micrencephaly. We injected 18 micrencephalic and 17 normal Long-Evans rats with piracetam from day 2 to 2 mo of age. Two doses of the drug (50 or 100 mg/kg) or vehicle were injected s.c. five days a week. Body weight, developmental landmarks, and reflexes were monitored. Animals were trained to find a hidden platform in a water tank between the ages of 50 to 60 days. Piracetam had no effect on development in either group of rats. The drug had no effect on learning in the controls. Its effects on the micrencephalic animals (who were impaired on this task) were not clear-cut even though the high dose appeared to speed acquisition and lower the asymptote.

495.7

PATTERNS OF IMPAIRMENT IN DEVELOPMENTAL DYSCALCULIA. S.M. Sokol, P. Macaruso* and T.H. Gollan*. IHP Neurolinguistics Laboratory, Massachusetts General Hospital, Boston, MA 02108.

Utilizing a cognitive model developed in studies of acquired dyscalculia, we analyzed the number processing and calculation performance of several developmentally impaired children. Associations and dissociations of function in these children's data support the general architecture of the model as well as provide insight into the computational structure of individual sub-components.

Specifically, the ability to process numbers dissociated from the ability to calculate. Within the number processing system, Arabic and verbal subsystems proved functionally distinct, and within these subsytems, syntactic and lexical components were isolated which possess different representational properties. Finally, within the calculation system, mechanisms responsible for fact retrieval were found to be autonomous of those responsible for procedural planning and execution.

In addition to providing new data on the incidence and nature of developmental dyscalculia, our studies highlight the utility of a cognitive neuropsychological approach to the study of developmental impairments.

495.8

SUDDEN INFANT DEATH SYNDROME: POSTNATAL CHANGES IN THE NUMERICAL DENSITY AND TOTAL NUMBER OF NEURONS IN THE HYPOGLOSSAL NUCLEUS. J.R. O'Kusky and M.G. Norman*. Dept. of Pathology, Univ. of British Columbia, Vancouver, B.C., Canada V5Z 1M9.

It has been suggested that sudden infant death syndrome (SIDS) results from an abnormal development of neurons in brainstem nuclei involved in respiratory control. Tissue samples of brainstem were taken at autopsy from 27 SIDS victims and from 14 control infants without neurological disease (36-94 postconceptional weeks). Morphometric analyses were performed on serial Nissl sections through the hypoglossal nucleus. The volume of the nucleus, the numerical density (N_V, cells per mm^3) and total number of neurons, and the average neuronal profile area were measured. Normal development was characterized by increases in volume and neuronal profile area. The N_V of neurons decreased, while the total number of neurons remained relatively constant. In SIDS cases the rate of increase in volume was greater than in controls (36%). The rate of increase in neuronal profile area was greater for motoneurons (42%), but not for interneurons. The N_V of neurons decreased more rapidly than in controls, but the total number of neurons did not differ. These abnormalities in growth indicate a greater volume of neuropil in the hypoglossal nucleus of SIDS cases. Synapse counts by electron microscopy are in progress. (Supported by the MRC of Canada)

495.9

A DOUBLE BLIND STUDY OF FOLINIC ACID (LEUCOVORIN) FOR THE TREATMENT OF FRAGILE X SYNDROME. W. J. Pizzi, C.M. Strom* and R. Brusca*. Northeastern Illinois University & Illinois Masonic Hospital, Chicago, IL 60625.

Fragile X Syndrome is the most common inherited disease that causes mental retardation. Symptoms routinely include mild to severe retardation and hyperactivity with attention deficits, and reports of autistic-like behavior. Several uncontrolled case studies have reported dramatic remediation of symptoms following folic acid therapy; however, double blind, controlled studies have failed to report positive effects. We conducted a study of 19 Fragile X males whose diagnosis had been confirmed by karyotype analysis. Subjects were administered dl-Leucovorin, 15 mg/day by mouth or a placebo for 3 months, followed by a crossover phase. There were no significant differences between placebo and Leucovorin in the behavior of subjects evaluated on the Vineland Adaptive Behavior Scales; Peabody Picture Vocabulary Test; ACTERS, and; parent questionnaires. There were no significant differences in blood parameters or side effects for the duration of the study. While formal measures were negative, 50% of the parents reported improvement in their sons.

495.10

AGE-RELATED RESPONSES TO OXYGEN DEFICIENCY IN PRETERM AND FULLTERM NEONATAL RATS. M. Dyer and C. R. Almli. Develop. Neuropsychobiol. Lab., Wash. Univ. Sch. Med., St. Louis, MO, 93110

Perinatal oxygen deficiency is associated with neural and neurobehavioral pathology. Behavioral responses of pre- and fullterm neonatal rats to acute oxygen deficiency were investigated.

Oxygen deficiency was produced by placing fullterm (P0; vaginal delivery) and preterm (E20, E21; Cesarean section delivery) neonates in chambers until they reached a criterion of 3 successive gasps separated by at least 15 sec each. Control rats were placed in unsealed chambers. Pups were resuscitated by brushing. The procedure was repeated for E21 and P0 rats. Time to reach gasping criterion and survival through 24 hrs postnatal were studied.

Results showed age-related differences in response to oxygen deficiency. Younger rats took significantly more time to reach the criterion. Pups born at less than 4 grams body weight showed lower survival rates. Neuropathology and transmitter changes due to oxygen deficiency are under study. (Conducted under NIH Guide for Care and Use of Laboratory Animals).

EPILEPSY: HUMAN STUDIES AND ANIMAL MODELS II

496.1

AMYGDALAR SCLEROSIS: A NOVEL PATTERN IN TEMPORAL LOBE EPILEPSY. L.P. Hudson, D.G. Munoz, J.P. Girvin and L. Miller. Departments of Pathology and Clinical Neuroscience, University of Western Ontario, London, Canada.

Mesial temporal sclerosis (MTS), a stereotyped pattern of hippocampal cell loss and gliosis, is the sole pathological abnormality in approximately 65% of temporal lobectomies for intractable temporal lobe seizures. Although a small portion of the remaining cases can be related to vascular, malformative, or neoplastic lesions, up to 22% of these resections demonstrate no recognizable pathology. We report a novel pattern of temporal lobe pathology associated with the development of temporal lobe epilepsy. Four en bloc temporal lobe resections without any evidence of hippocampal damage demonstrated cell loss and gliosis in the amygdala. This group of patients exhibited significant preoperative clinical differences from those patients found to have MTS in terms of age at onset of seizures, and performance on standard neuropsychological tests. Although MTS is invariably accompanied by a severe degree of amygdalar pathology, there have been no previous reports of isolated amygdalar pathology in temporal lobe epilepsy.

496.2

ELECTROPHYSIOLOGICAL COMPARISON OF HUMAN AND RODENT DENTATE GRANULE CELLS MAINTAINED IN VITRO. A. Williamson, G.M. Shepherd and D.D. Spencer, Sections of Neurobiology and Neurosurgery, Yale University School of Medicine, New Haven, CT 06510.

We have studied the electrophysiological properties of dentate granule cells in both human and guinea pig hippocampi using intracellular recording techniques in slices maintained in vitro. The human tissue was removed for treatment of extrahippocampal lesions and is morphologically normal compared to autopsy controls. There were no differences in any of the membrane properties examined including the membrane potential, input resistance, slope conductance and membrane time constant.

However, we noted differences in the response to depolarizing current injections. The guinea pig granule cells showed robust spike frequency adaptation, and directly evoked spike trains were followed by long afterhyperpolarizations (AHPs) which had both fast and slow components. By contrast, the human cells showed very little spike frequency adaptation and had prominent fast AHPs but the slow AHPs were very small. When 100 msec long depolarizing current pulses which produced four action potentials were given from membrane potentials between -60 and -65 mV, the AHPs were between 5 and 10 mV in amplitude (mean 6.52 mV, n=27) and were 2.5 sec long in the guinea pig but only 2 to 3 mV in amplitude (mean 2.23 mV, n=15, from 8 patients) and 0.5 sec long in the human cells. The conductance underlying the slow AHP was present in the human cells as prolonged trains were followed by an AHP which could be reduced by NE, as has been shown for the slow AHP in rodent granule cells. This may represent an important species difference as the slow AHP is modulated by a number of neuroactive compounds. These data suggest that human granule cell activity may be modulated to a lower degree than the guinea pig cells by compounds such as ACh, NE, 5-HT and HA. Supported by NIH NSO6208 (AW and DDS) and NIH DC00086 and ONR N00014 (GMS)

496.3

PARVALBUMIN AND CALBINDIN IMMUNOREACTIVE NEURONS IN EPILEPTIC AND NORMAL HUMAN HIPPOCAMPAL FORMATION. M. Vonau* and I. Török, School of Anatomy, University of New South Wales, Kensington, NSW 2033, Sydney, Australia.

The parvalbumin and calbindin-D28K immunoreactive (P-IR and C-IR) cells and terminals were examined in hippocampal tissue removed from patients with temporal lobe epilepsy and postmortem controls. A differential distribution of both calcium binding proteins was found in the various cytoarchitectonic regions. The highest density of P-IR neurons was found in the CA1 region in small to large multipolar neurons. Many of the latter had extensive dendritic processes across the full depth of the CA1 cortex. While CA2 contained moderate numbers of P-IR cells there was a conspicuous paucity of such cells in CA3. In CA4 there were numerous large P-IR multipolar cells with dendrites radiating in all directions. The pyramidal layer in CA1-3 contained a dense plexus of P-IR terminals with specially high density in the superficial sublayer in CA1. In the dentate gyrus, P-IR neurons were present in the molecular and polymorphic layers while there was a distinct and dense terminal labelling in the granule cell layer. C-IR was present in the majority of granule cells, clearly delineating their dendrites in the molecular layer. In addition, medium to large multipolar cells were labelled throughout the dentate gyrus. In CA1 of the hippocampal cortex, large numbers of pyramidal and non-pyramidal neurons were weakly C-IR. Terminal labelling was mostly discernible in the stratum radiatum in the CA3 area. Comparison of the sclerotic and non-sclerotic regions with each other and with normal controls clearly demonstrated the loss of P-IR and C-IR neurons together with the pyramidal and granule cells. Supported by the Australian Brain Foundation.

496.4

TYPICAL ABSCENCE LIKE ATTACKS INDUCED BY SIMULTANEOUS BILATERAL STIMULATION OF THE CENTROMEDIAN (CM) THALAMIC NUCLEI IN MAN. F. Velasco, M. Velasco*, L. Coronel*, A.L. Velasco*. Functional Neurosurgery Service. Hospital General, S.S. and Division of Neurophysiology, Nat. Med. Cent IMSS. México, D.F. P.O. Box 73-032.

This work was performed on epileptic patients with intractable generalized seizures and with recording/stimulating electrodes implanted in both CM nuclei as part of a neuroaugmentive procedure for seizure control. Prior to the onset of chronic electrical stimulation the correct placement of the electrodes was corroborated by electrophysiological studies. Simultaneous bilateral electrical stimulation of CM (3/s, 1.0 ms., 1.2-1.5m Amp and trains of 20s or more) induced a lapse of blank store, arrest of spontaneous ongoing activily, initial increase in reaction time, followed by blocking of single responses and "Number Cancellation" test. Concomitant 3/s spike and wave complexes appeared in the EEG at the frontal and central regions time locked to the individual stimulus presentation spike (P15 N20) and wave (N60) components had an amplitude relation of 1:5-10 uV. Unilateral stimulus induced ipsilateral spike and wave scalp discharges without clinical correlations.

Subcortical (centroencephalic) vs. cortical theories have been proposed to explain the physiopathology of generalized non convulsive seizures. Present results favors the centroencephalic theory.

496.5

ENHANCED PAIRED-PULSE INHIBITION IN HUMAN EPILEPTOGENIC AMYGDALA AND HIPPOCAMPAL PATHWAYS. S.U. Khan, C.L. Wilson, G.J. Kricorian* and M.F. Levesque. Department of Anatomy and Cell Biology, Department of Neurology, Division of Neurological Surgery and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

Both the animal kindling model of epilepsy and depth recordings of seizure onsets in human temporal lobe epilepsy implicate the amygdala nucleus in epileptogenesis. Wilson et al. (Neurosci. Abst. 1989, 15:236) demonstrated greater paired pulse suppression of stimulation evoked population EPSPs in the epileptogenic hippocampal formation (HF) than in the nonepileptogenic HF. They attributed this suppression to enhanced GABAergic inhibition produced by principal neuron loss in the hippocampus. Since amygdala and HF have multiple connections, we decided to examine the excitability of these pathways.

For this purpose, we employed paired stimuli in a study of 13 medically intractable complex-partial epilepsy patients with depth electrodes implanted in the amygdala and the HF for diagnostic monitoring prior to surgery. Conditioning and test stimuli were delivered to five specific HF sites while recording evoked field potentials from microelectrodes in the amygdala. Stimulation was also delivered to the amygdala during recording from microelectrodes in the HF sites. Paired-pulses were delivered at inter-stimulus intervals of 20 to 1600 msec, and response depression or facilitation was measured by dividing the test by conditioning evoked response slope. The epileptogenic sites were defined as those on the side of all recorded seizure onsets and the nonepileptogenic sites as those on the side of no seizure onsets.

The slope of evoked field potentials was significantly suppressed on the epileptogenic side in comparison to the slope on the nonepileptogenic side ($F = 9.07$, $df=1$, $p < .005$), at interstimulus intervals of 20 to 200 msec. These results indicate interictal inhibition is enhanced in the epileptogenic amygdala, a functional difference consistent with that observed in the nonepileptogenic HF.

Supported by NIH grant NS02808.

496.6

INTERACTION OF ORTHODROMIC AND ANTIDROMIC STIMULI IN THE DENTATE GYRUS OF EPILEPTIC PATIENTS IN VITRO. K. Uryno, M.J. O'Conner* and L.M. Masukawa. Departments of Neurology, Research and Surgery, Graduate Hospital and Departments of Neurology and Neurosurgery, University of Pennsylvania Medical School, Philadelphia, PA. 19146.

Neuronal loss and mossy fiber reorganization occur during the course of epilepsy. Alterations in circuitry are thought to be associated with functional changes. In order to elucidate changes in function and circuitry, field responses of granule cells to paired pulse stimuli were examined in the dentate gyrus of hippocampal slices from temporal lobe epilepsy (TLE) patients and normal rats. Paired pulse stimuli (orthodromic pair, antidromic pair and combinations of orthodromic and antidromic pulses) were applied at various interstimulus intervals (ISI) via electrodes placed in the molecular layer and in the hilus.

In slices of TLE patients, the first stimulus (either orthodromic or antidromic) inhibited the second antidromically evoked population spike at short ISIs. This inhibition was stronger in the slices which showed hyperexcitability of granule cells. The inhibition was enhanced by bicuculline, and blocked by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX). 2-amino-5-phosphonovalerate (APV) partially reduced the inhibition. Neither the inhibition nor its enhancement by bicuculline was significant in rat slices. Orthodromic paired pulse inhibition was well preserved in some slices that showed antidromic paired pulse inhibition in TLE patients. These results suggest that mossy fibers make functional synapses with inhibitory interneurons (possibly non-GABAergic), in addition to excitatory synapses with granule cells. Presence of the new inhibitory synapses may precede the progressive decrease or loss in orthodromic paired pulse inhibition observed in TLE patients.

Supported by NIH grant NS23077 to LMM.

496.7

RELATIONSHIPS BETWEEN EVOKED FIELD RESPONSES AND TIMM-SULFIDE STAINING IN THE DENTATE GYRUS OF TEMPORAL LOBE EPILEPTIC PATIENTS. L.M. Masukawa, K. Uruno, L.J. Burdette and M.J. O'Connor*. Depts. of Neurology and Surgery, Graduate Hospital, Philadelphia, PA 19146.

To address the question of the functional significance of changes in mossy fiber termination, we examined the relationships between Timm-sulfide staining and evoked field responses to orthodromic and antidromic stimuli in *in vitro* brain slices from the dentate gyrus of epileptic patients who underwent temporal lobe resective surgery. We used the same population of patients for these measures to account for variation in their clinical histories. The following physiological parameters were significantly correlated with the intensity of Timm-silver staining in the molecular layer or hilus: 1) early and late inhibition as measured by paired pulse stimulation; 2) change in the orthodromic response during 1 Hz stimulation; and 3) abnormality of the antidromic waveform. These results are consistent with the possibility that reorganization of mossy fiber terminals from the hilus into the molecular layer may underlie the recorded physiological parameters. Supported by NIH Grant NS-23077 to LMM.

496.8

DYNAMIC ANALYSIS OF CARDIAC RATE DURING COMPLEX PARTIAL SEIZURES. R.C. Frysinger, S. Garg*, M. Levesque and R.M. Harper. Dept. of Anatomy and Cell Biology, Div. of Neurosurgery and the Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

Heart rate has been shown to change dramatically and systematically during epileptic seizures. Such changes may involve mesial temporal structures such as hippocampus and amygdala, which have been shown to play a role in autonomic control as well as epileptogenesis. Heart rate patterns can thus serve as identifying features of seizure discharge. We examined peri-ictal beat-by-beat cardiac interval records of patients undergoing continuous seizure monitoring. Poincaré plots (RR_{n+1} vs RR_n) of intervals were calculated, and these plots indicated marked dynamic changes in interval to interval variation during a seizure. To quantify variability at low (0-.03 Hz), mid (.03-.15 Hz) and high (> .15 Hz) frequencies, cardiac interval records were interpolated to provide a linear time series, and FFTs were calculated on successive 16.38 second periods around each seizure. We derived two measures from each period: broad-band power (a measure of total variability), and the ratio of low to high frequency variation (an inferential measure of the relative contributions of sympathetic and parasympathetic sources). Results indicate that preictal cardiac variability is state-dependent, moderate and variable in frequency dominance. At seizure onset a large increase in broad-band power and in the low/high ratio occurs, suggesting a dramatic increase in sympathetic input. Low frequency power decreases over the course of the seizure, while higher frequencies increase, probably reflecting significant co-activation of sympathetic and parasympathetic sources. These techniques provide quantitation of degree and type of autonomic activation associated with seizures, an important factor in seizure characterization and cardiovascular risk assessment. The computationally simple Poincaré plots provide a qualitative display of the dynamic characteristics of the variability which could be provided in real time.

Supported by NS 02808

496.9

ULTRASTRUCTURAL ORGANIZATION OF SOMATOSTATIN INTERNEURONS IN THE FASCIA DENTATA OF HIPPOCAMPI FROM PATIENTS WITH EPILEPSY. M. F. Philips*, C. Pappas*, D. D. Spencer and N. C. de Lanerolle. Section of Neurosurgery, Yale Univ. Sch. of Med., New Haven, CT. 06510

Hippocampi surgically removed from patients with medically intractable Temporal Lobe Epilepsy (TLE) can be classified into two groups. Those from subjects with an extra-hippocampal temporal lobe lesion (TTLE) have a relatively normal hippocampus in contrast to those who have no identifiable lesion (CTLE) [de Lanerolle et al., Brain Res., 495: 387-395, 1989]. Somatostatin-like immunoreactivity (SLI) in the fascia dentata (FD) of TTLE was localized in interneuron-like cell bodies in the hilus. Dendrites and axonal processes of these somata ramify in the hilus and some extend through the granule cell layer into the molecular layer (ML). The density of fibers in the inner ML is low compared to the outer ML. In CTLE, SLI somata in the hilus are reduced, but there is an increase in immunoreactive fibers throughout the width of the ML. The participation of SLI elements in the synaptic organization of the FD was studied by an electron microscopic analysis of SLI elements. In TTLE immunostained dendrites were located in the hilus and inner molecular layer. SLI dendrites were often the center of simple to complex glomeruli with several unlabeled, mostly round vesicle containing terminals. SLI myelinated axons and terminals were commonly seen only in the outer molecular layer and the hilus. The labeled terminals usually contained round vesicles. One main difference in CTLE was the presence of labeled terminals in all regions of the ML. (Supported by NS 27081)

496.10

CARBACHOL STIMULATED PHOSPHOINOSITIDE TURNOVER IN HUMAN BRAIN TISSUE FROM PATIENTS WITH EPILEPSY. E. W. Johnson, D. D. Spencer, and N. C. de Lanerolle. Section of Neurosurgery, Yale Univ. Sch. of Med., New Haven, CT 06510.

Using [^{3}H]cytidine as a precursor, it has been demonstrated that PI turnover in tissue slices can be stimulated by a variety of compounds resulting in the accumulation of membrane bound [^{3}H]cytidine diphosphate diacylglycerol ([^{3}H]CDP-DAG)(Science 249: 802 (1990)). The non-specific levels of non-membrane bound precursor and radiolabeled nucleic acids are reduced by washing. The autoradiographic images generated from these labeled sections reflects the degree of PI turnover in fairly discreet neuroanatomical regions.

Living human brain tissue was obtained from patients undergoing surgery for medically intractable temporal lobe epilepsy. Slices were prepared from either hippocampus or neocortex and preincubated with [^{3}H]cytidine. The slice was then incubated with LiCl +/- carbachol. The slice was cut into 20um sections before being washed and apposed to film.

The addition of carbachol resulted in an overall increase in [^{3}H]CDP-DAG accumulation in the hippocampus compared to LiCl stimulation alone. The increase was greatest in the dentate gyrus and in some parts of the CA fields. Carbachol stimulation of the neocortical tissue also resulted in increased labeling particularly in the outer layers of the cortex. (Supported by NS27081)

496.11

STIMULATION EVOKED POPULATION SPIKES IN HUMAN EPILEPTOGENIC HIPPOCAMPAL FORMATION SHOW ENHANCED INHIBITION. C.L. Wilson, S.U. Khan, H.C. Hu*, and M.F. Levesque. Department of Anatomy and Cell Biology, Dept. of Neurology, Div. of Neurological Surgery and Brain Research Inst., UCLA School of Medicine, Los Angeles, CA 90024.

Previous studies have shown that more pronounced paired pulse suppression of stimulation evoked population (pop) EPSPs occurs in the human epileptogenic hippocampal formation (HF) than in the nonepileptogenic HF (Wilson et al. 1989, Neurosci. Abst. 15:236). Similar results were obtained during perforant path stimulation in the kindled rat (e.g.Tuff et al. Brain Res. 1983, 277:79-90). However, the animal studies were based on measures of increased inhibition of the perforant path pop *spike*, not on pop *EPSPs*, (the latter show less modulation in seizure models; Sloviter 1991 Hippocamp,1:31-40).

In the present study, 6 HF sites were implanted with depth electrodes for surgical diagnostic recording in 23 complex partial seizure patients. Paired stimuli were delivered to one or more of these sites, in turn, while adjacent sites were monitored for pop spikes. Pop spike amplitude was the average of spike positivity and negativity. Paired pulse interval was varied from 20 to 800 msec and response suppression or facilitation compared between the epileptogenic vs nonepileptogenic HF as defined by depth electrode seizures.

Population spike amplitude in the epileptogenic HF was found to be significantly more suppressed than in the nonepileptogenic HF ($F=14.867$ $df=1$ $p<.0002$), further supporting the finding of enhanced inhibition in the epileptogenic hippocampus observed in pop EPSP studies. Supported by NIH grant NS02808.

496.12

Electrocorticography and simultaneous recording of regional cerebral blood flow with laser doppler technique from the human epileptic focus.

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In comparison with normal brain tissue epileptic zones are characterized by altered metabolism and blood flow as visualized with PET and SPECT technique, respectively. Intracerebral microdialysis has been used in epilepsy patients to correlate levels of aminoacids and energy related metabolites to *i.a* seizure activity (Ronne-Engström et al 1991, J Cereb Blood Flow Metab, in press).The relationships between seizure activity, blood flow and biochemical events are unclear. In order to investigate the relation between the corticographic(ECoG) pattern and the regional cerebral blood flow with a high spatial and temporal resolution a combined subdural strip electrode and single fiber laser doppler probe was designed. The "lasertrode" was utilized in patients with focal epilepsy subjected to preoperative ECoG and videomonitoring of seizures. Relative flowmetry (Pf3, Perimed, Sthlm,Sweden) was performed intermittently in periods of eight hours/day. Six partial seizures have been recorded with ECoG and flowmetry. All started in the medial subtemporal region and had a duration of 1-3 min. Significant changes of the regional blood flow occurred in strict correlation to the ECoG seizure pattern.The flow returned to pre seizure levels within 1 min after termination of the seizures.Thus,as investigated by laser doppler flowmetry, there is no evidence for prolonged changes of the regional cerebral blood flow in the epileptic focus as defined by ECoG.The results indicate that this new method can be employed for investigations of the dynamics in regional cerebral blood flow in relation to the neuronal activity.

496.13

THE FREEZE LESION EPILEPSY MODEL ADAPTED TO RATS. W.D. Knowles and W.R. Barrett. Epilepsy Basic Research Program, Cleveland Clinic Foundation Research Institute, Cleveland, OH 44195.

The cortical freeze lesion epilepsy model has been used to study epileptogenesis primarily in cats. We report the successful adaptation of this model to rats, which have practical advantages.

Three Sprague-Dawley rats received neocortical freeze lesions and two rats received sham treatments. The lesions were located approximately in the motor cortex (1.5 mm posterior to bregma and 3.4 mm lateral.) The lesions were made by applying a 1 mm stainless steel probe that was in contact with a dry ice/95% ethanol slurry, to the dura for 30 seconds, then flushing with room temperature saline to remove the probe. Chronic EEGs were recorded from epidural skull screws embedded in an acrylic headpiece/connector.

No abnormal EEG or seizures were observed in the sham treated rats. EEG spikes and polyspikes could be recorded in freeze lesioned rats as early as one day post lesion. Behavioral seizures were observed starting 5-7 days post lesion, and continued as long as the animals were observed (1-3.5 months.) The seizures began with motionless staring, followed by clonus of the vibrissae, mouth, head, and eyes (Racine stages 1 or 2.) The seizures lasted from 5 seconds to 3.5 minutes, and were followed by restlessness, grooming and chewing. The EEG showed widespread low amplitude fast signals, followed by wide spread rhythmic high amplitude spikes and oscillations. Seizure frequency varied from one every 5 to 10 minutes to one every 15-20 minutes.

496.14

A SYSTEM FOR PRODUCING BENZODIAZEPINE TOLERANCE IN MICE USING OSMOTIC PUMPS. **C.D. TORCHIN*, I.M. KAPETANOVIC, AND H.J. KUPFERBERG*. Epilepsy Branch, NINDS, NIH, Bethesda, MD 20892.**

Tolerance to the anticonvulsant effects of benzodiazepines limits their use in epilepsy treatment. Animal models demonstrating tolerance have been developed, but they require repetitive injections over several days or use silastic capsules which must be made for each drug and do not provide a constant infusion rate. Alzet® 2001 osmotic mini-pumps deliver at a constant rate (1 μl/hr) and dosage can be easily adjusted. Various solvents; PEG400, propylene glycol, 2% Tween, 50% DMSO, saline, Molecusol®, and 0.5% methyl cellulose, were tried and were found unsuitable because diazepam was not maintained in solution or proconvulsant activity was seen. Tetraglycol was chosen as it did not demonstrate these shortcomings. Anticonvulsant activity was evaluated by PTZ iv tail infusion using forelimb clonic seizures as the endpoint. For naive NIH:NGP(S) mice, an infusion of 15 mg/ml at 0.2 ml/min produces forelimb clonus in about 30 seconds. All mice challenged with PTZ received an ip bolus of diazepam, 7.5 mg/kg, 30 minutes pre PTZ. Pumps containing vehicle, 15 μg/μl, 30 μg/μl, or 60 μg/μl diazepam were implanted sc in groups of six mice. Along with 6 naive mice, each group was challenged with PTZ 24 and 72 hours post implantation. There was no difference in the amount of PTZ required for clonus in naive or vehicle mice at 24 or 72 hours. Comparing 72 hours to 24 hours, protection was reduced by 15% in 15 μg/μl mice, 40% in 30 μg/μl mice, and 50% in 60 μg/μl mice. These decreases in protection show that tolerance to the anticonvulsant effects of diazepam developed while using osmotic mini-pumps. This model could be useful to screen putative anticonvulsant drugs for development of tolerance.

EPILEPSY: ANTICONVULSANT DRUGS

497.1

THE GABA UPTAKE INHIBITOR TIAGABINE INCREASES EXTRACELLULAR BRAIN LEVELS OF GABA IN AWAKE RATS. A.Fink-Jensen, M.D.B.Swedberg, M.E.Judge, P.Suzdak, L.Hansen* and P.G.Nielsen*. Novo Nordisk A/S, CNS Division, Denmark.

Tiagabine (NO-328) is a selective GABA uptake inhibitor in-vitro (IC50=67nM) and a potent anticonvulsant in rodents. Tiagabine is currently in Phase II clinical trials as an antiepileptic. The present study examined the in-vivo mechanism of action of tiagabine using microdialysis to measure changes in the extracellular brain levels of GABA in awake Sprague Dawley rats. Tiagabine was administered at 11.5 or 21.0 mg/kg i.p. (these doses of tiagabine correspond to the ED50 and ED85 doses, respectively, for inhibiting pentylenetetrazole-induced seizures). A microdialysis probe was placed in either the globus pallidus or the substantia nigra (two areas previously shown to receive a strong GABAergic innervation). Tiagabine produced a significant increase in extracellular GABA levels in globus pallidus with peak values of 310% of basal level (after 21.0 mg/kg) and 240% of basal level (after 11.5 mg/kg). A significant increase in extracellular GABA levels was also found in the substantia nigra - one of the major output channels of the basal ganglia - where the ED85 dose of tiagabine (21.0 mg/kg) produced a peak value of 200% compaired with basal level. The present microdialysis study shows that tiagabine increases extracellular brain levels of GABA demonstrating that it also in-vivo acts as a GABA uptake inhibitor.

497.2

ANTICONVULSIVE ACTIVITY OF ANTAGONISTS AT THE GLYCINE MODULATORY SITE ON THE NMDA RECEPTOR COMPLEX. **Alfred C. Nichols and K. Lemone Yielding*. Dept. of Pharmacology, Univ. of Texas Medical Br., Galveston TX, 77550.**

Halogenated derivatives of indole-2-carboxylic acid (Salituro et al., 1990) and kynurenic acid (kyn) (Kemp et al., 1988) can act as antagonists at the glycine modulatory site on the NMDA receptor complex. Twenty-two kynurenic acid derivatives were synthesized, and, along with nine indole derivatives and two pyridine dicarboxylic acids, were tested for anticonvulsive activity in the Antiepileptic Drug Development Program of NINCDS. In addition, each compound was tested for glycine receptor binding at a concentration of 10uM using the method of Snell et al. (1988). Although twelve of the kynurenate derivatives showed glycine receptor binding under the conditions of this assay, only four demonstrated any anticonvulsant activity (at 300 mg/kg). These were 7-Cl-8-methyl-kynurenate, 5,7-dichlorokynurenate, the ethyl ester of 7-Cl-kyn, and the ethyl ester of 5,7-difluoro-kyn. Three indole derivatives demonstrated anticonvulsant activity at the 100 mg/kg dose level: the ethyl ester of 5-methoxy-indole-2-carboxylic acid, 5-fluoro-indole-2-carboxylic acid and its ethyl ester. The two 5-fluoro derivatives showed competition binding in the glycine assay. However, the 5-methoxy-indole compound was inactive in this assay suggesting that its anticonvulsive activity is not due to interactions with the glycine site of the NMDA receptor complex.

497.3

VALPROIC ACID ENHANCES THE IPSP IN THE RAT AMYGDALA. L.-M. Tian and K. A. Alkadhi. Department of Pharmacology, University of Houston, Houston, TX 77204-5515.

Valproic acid (VPA) has been widely used clinically as an antiepileptic drug, but the mechanism of its action has not been established. In this study, we have examined the action of VPA on rat amygdala neurons *in vitro* using intracellular recording techniques. Amygdala slices (500 μm) were prepared from male Sprague-Dawley rats and bathed in artificial cerebrospinal fluid (ACSF).

The resting membrane potential and input resistance of the amygdala basolateral nucleus neurons were -65 to -70 mV and 40 to 80 mΩ respectively. Addition of VPA (30-100 μM) to the superfusate caused hyperpolarization (3-5 mV) and an increase in the membrane input resistance of amygdala neurons. Stimulation of the amygdalo-fugal pathway evoked inhibitory postsynaptic potentials (IPSP). This IPSP could be eliminated by hyperpolarizing the membrane to -75 mV or by addition of the $GABA_A$ antagonist bicuculline (10 μM) in the superfusate. The IPSP amplitude increased 1-3 fold after 10 minutes of superfusion with VPA (100 μM) when the membrane potential was artificially held at the resting level.

When slices were bathed in bicuculline (10 μM) for 5 min, an epileptiform burst of action potentials was observed upon subthreshold stimulation of the amygdalo-fugal pathway. The bicuculline-induced epileptiform response was completely abolished 15 minutes after addition of VPA (100 μM). These preliminary results suggest that VPA changes the membrane properties of the amygdala basolateral nucleus neurons probably by modifying the $GABA_A$ receptor function.

497.4

PD117302, AN OPIOID KAPPA AGONIST, AND MK801 PARTIALLY BLOCK MAXIMAL ELECTROSHOCK CONVULSION-INDUCED INCREASES IN *c-fos* mRNA IN RAT BRAIN. J.R. Dave*, L. Robles, K. Miller*, E.W. Bernton* and F.C. Tortella. Neuropharmacology Branch, Walter Reed Army Institute of Research, Washington, DC 20307 and Medicis Pharmaceutical Corp. New York, NY 10017.

It has been known that seizure activity is associated with increases in the proto-oncogene *c-fos* mRNA and *c-fos* protein in brain. The present study was undertaken to determine if the novel anticonvulsant kappa opioid drug, PD117302 (Tortella et al, Life Sci., 46, PL 1, 1990), and the NMDA antagonist MK801, would block seizure-induced *c-fos* mRNA in rat brain. *c-fos* mRNA levels, measured by Northern blot analysis, were greatly increased in a time-dependent manner following maximal electroshock (MES). Increased levels were measured within 15 min after MES, reaching a maximum in 30 min and declining to control levels within 2 hr post-convulsion. A maximal stimulation in *c-fos* mRNA of 10-15 fold was observed in the cerebellum, followed by hippocampus (5-7 fold) and cerebral cortex (4-6 fold). Administration of anticonvulsant doses of PD117302 (16 and 32 mg/kg, s.c.) and MK801 (0.2, 1.0 and 2.0 mg/kg, s.c.) alone produced no significant changes in *c-fos* mRNA levels in these three brain areas. However, both drugs (PD117302, 32 mg/kg; MK801, 0.2 mg/kg) administered 30 min prior to MES significantly blocked *c-fos* mRNA induction with the greatest effect observed in the cerebellum and a minimum effect in the cerebral cortex. Seizure-induced increases in *c-fos* mRNA levels and its partial blockade by anticonvulsant drugs suggest a possible role of *c-fos* in the mechanism of action of these drugs.

497.5

CARAMIPHEN ANALOGS: RELATIONSHIP OF σ BINDING AFFINITIES WITH ANTICONVULSANT ACTIVITY. J.T. Allen[1], D.L. DeHaven-Hudkins[1], J.F. Stubbins[2,*], R.L. Hudkins[2] and F.C. Tortella[3]. [1]Dept. Enzymology & Receptor Biochemistry, Sterling Research Group, Malvern, PA 19355, [2]Dept. Medicinal Chemistry, Medical College of Virginia, Richmond, VA 23298 and [3]Walter Reed Army Institute of Research, Washington, DC 20307.

Caramiphen is an antimuscarinic agent which is a more effective antidote to organophosphate poisoning than atropine, and also potently blocks MES-induced seizures in mice and rats. The anticonvulsant mechanism of caramiphen has been hypothesized to be due to high-affinity binding to dextromethorphan (DM)-labelled σ recognition sites in brain. We initially examined four properly chosen para-substituted caramiphen analogs to determine the influence of aromatic substituent parameters (i.e. σ, π) on σ receptor binding and also to determine if there was a correlation of σ binding affinity with anticonvulsant activity. Some of the analogs potently inhibited σ binding but were devoid of anticonvulsant activity. Of the compounds evaluated in MES only the 4-amino derivative showed anticonvulsant activity (ED_{50} = 3 mg/kg), although in σ binding assays its affinity was less than that of caramiphen. Comparable structure-activity data has previously been reported for 3-substituted DM analogs. In both series, while no apparent correlation exists between σ affinity with anticonvulsant activity, this information may reveal the common modes of binding of caramiphen and DM analogs to the σ recognition site.

497.6

CROSS TOLERANCE BETWEEN CARBAMAZEPINE AND VALPROATE IN AN AMYGDALA KINDLED SEIZURE PARADIGM. S.R.B. Weiss, R. Lewis*, E.Sohn*, A. Berger*, R.M. Post. Biological Psychiatry Branch, NIMH, Bethesda, Md. 20892

We have previously demonstrated the importance of the peripheral-type benzodiazepine receptor (P-BzR) in carbamazepine's (CBZ) anticonvulsant effects on amygdala kindled seizures. In order to evaluate potential common mechanisms of action, we evaluated cross-tolerance between CBZ and other anticonvulsant drugs. When tolerance to CBZ was induced, animals showed a lack of anticonvulsant response to PK-11195 (an antagonist at the P-BzR) and sodium valproate (VPA), but not to diazepam (DZP). Since the P-BzR is found in greatest concentrations in the olfactory bulb in rats, and VPA has recently been shown to be distributed almost exclusively in this area following *in vivo* administration (Hoeppner, 1989), we examined what effect lesions of the olfactory bulb would have on anticonvulsant responsivity. Rats with lesions of the olfactory bulb developed amygdala kindled seizures at rates equivalent to rats with sham lesions, and they showed equal anticonvulsant responses to CBZ, VPA, and DZP.

Our results suggest that while the P-BzR is important for the anticonvulsant efficacy of carbamazepine, receptors in the olfactory bulb are not critical for this effect. Similarly, although radiolabeled VPA is highly localized to the olfactory bulb, this structure is not necessary for the anticonvulsant effects of VPA. Potential common mechanisms of anticonvulsant action of CBZ and VPA are suggested by our findings of cross tolerance and require further investigation.

497.7

LAMOTRIGINE AND PHENYTOIN INTERACTIONS ON IONIC CURRENTS PRESENT IN N4TG1 AND GH3 CLONAL CELLS. D.G. Lang* and C.M. Wang. Division of Pharmacology, Burroughs Wellcome Co., Research Triangle Park, NC 27709.

Lamotrigine (LTG) is a novel anticonvulsant with a unique chemical structure; it has a pharmacological profile similar to phenytoin (PHT; Miller, et al., *Epilepsia* 27:483-489, 1986). LTG, like PHT, inhibits veratrine-induced glutamate release from rat brain cortical slices, presumably by inhibiting Na^+ channels (Leach, et al., *Epilepsia* 27:490-497, 1986).

We have examined the tonic effects of LTG and PHT on voltage-dependent Na^+ currents in N4TG1 mouse neuroblastoma cells and voltage-dependent Ca^{2+} and K^+ currents in GH3 rat pituitary cells using the whole cell mode of the patch clamp technique. Both LTG and PHT produced an inhibition of the Na^+ current with IC_{50} values of 90 μM and 58 μM, respectively. Low threshold, transient Ca^{2+} currents were inhibited by PHT (IC_{50} = 50 μM), but 100 μM LTG caused only a 15 ± 7.0% inhibition. High threshold, sustained Ca^{2+} currents were not significantly affected by either 100 μM LTG (+9.5 ± 4.9%) or 100 μM PHT (-8.0 ± 8.7%). Ca^{2+}-activated K^+ currents were not significantly affected by either 100 μM LTG (-3.0 ± 4.2%) or 100 μM PHT (-7.0 ± 7.0%). Transient, rapidly inactivating voltage-dependent K^+ currents were significantly inhibited by 100 μM LTG (-19 ± 3.8%) and by 100 μM PHT (-25 ± 3.6%). These data support the hypothesis that the primary site of action of the inhibition of glutamate release by LTG is Na^+ channels and not Ca^{2+} or K^+ channels.

497.8

MEMANTINE AND ATROPINE PROTECT AGAINST SOMAN-INDUCED SEIZURES. W.-D. Dettbarn, M.J. McLean, R.C. Gupta* and A.W. Wamil. Neurology Dept., Vanderbilt Univ. Med. Ctr., Nashville, TN 37212

A single sublethal dose of the nerve agent, SOMAN (100 μg/kg, sc), produced limbic seizures in rats. Pretreatment with memantine (MEM, 18 mg/kg, sc) ± atropine sulfate (ATS, 16 mg/kg,sc), but not ATS, prevented seizures. MEM, but not ATS attenuated seizure activity in progress. MEM±ATS or ATS had no effect on acetylcholinesterase (AChE) activity in crude brain homogenates. Pretreatment with MEM reduced inhibition of AChE activity by SOMAN, but not edrophonium (cationic site) or decamethonium (peripheral site inhibitor), suggesting action at a different modulatory site. MEM limited action potential firing frequency, and both MEM and ATS blocked ACh responses recorded intracellularly from cultured neurons. Thus, protection against SOMAN seizures by MEM ± ATS resulted from a combination of biochemical and pharmacological effects.

497.9

VOLTAGE-SENSITIVE CALCIUM CHANNELS (VSCC's) OF MOUSE CORTICAL ASTROCYTES ARE ATTENUATED BY PROTOTYPE ANTICONVULSANTS. J.A. Edwards[1], D.M. Woodbury[2] and H.S.White[1]. [1]Dept. of Pharmacology & [2]Dept. of Physiology, University of Utah, Salt Lake City, Utah 84108

Previous investigations in this laboratory have demonstrated that primary cultures of mouse cortical astrocytes express VSCC's which are effectively blocked by the L-type channel blocker nimodipine and the N-type channel blocker Ω-conotoxin. Since a number of anticonvulsants have been shown to block VSCC's of neurons, the present study was initiated in order to assess the effect of these drugs on VSCC's of cultured astrocytes using the fluorescent probe indo-1. Astrocytes grown on 25 mm cover slips and maintained in tissue culture for 20-31 days were "loaded" with indo-1 (5 μM) for 20 min. and fluorescence measured according to the method of Peeters et al. Depolarization of differentiated astrocytes with elevated KCl (55 mM) resulted in a significant increase in the free $[Ca^{2+}]_i$ concentration as estimated by an increase in the 410/480nm fluorescence intensity ratio (Grynkiewicz et al.,J.Biol.Chem.260:3440-3450, 1985). Pretreatment for 5 min. with therapeutic concentrations of phenobarbital (10 μM); valproate (1 μM); diazepam (1 μM); ethosuximide (100 nM); phenytoin (3 μM) and flunarizine (10μM) attenuated the peak KCl-induced Ca^{2+} transients by 84, 82, 81, 78, 71, and 60%, respectively. Whether these drugs are blocking Ca^{2+} influx through a direct interaction with the channel (L or N) or through an effect on second messenger systems is currently under investigation. In summary, the present results suggest that the VSCC's of astrocytes are amenable to pharmacological manipulation by anticonvulsant compounds. Supported by a grant from the NINDS of the NIH (2R01-NS-22200).

497.10

ANTICONVULSANT EFFICACY OF CALCIUM ANTAGONIST AGAINST CHEMICALLLY-INDUCED SEIZURES IN DEVELOPING RAT. S.K. Sobrian, N.L. Robinson* and L.E. Burton*. Dept. Pharmacology, Howard Univ. Col. Med., Washington, DC 20059.

Calcium channel antagonists (CAs) are effective anticonvulsants in several reflex and generalized models of epilepsy. The present study determined if nifedipine (NIF) and verapamil (VER) would protect Sprague-Dawley rats from convulsions induced by pentylenetetrazol (PTZ), picrotoxin (PIC) or bicuculline (BIC). At 15 or 30 days of age, pups were pretreated i.p., with either phenobarbital (15 mg/kg, 45 min), NIF (20 mg/kg, 30 min) or VER (20 mg/kg, 10 min) prior to s.c. injection of PTZ (50 mg/kg), PIC (3.15 mg/kg) or BIC (2.7 mg/kg). Controls received 0.1 ml/10G body weight DMSO. Three indices of convulsive behavior were measured for 30 minutes. At PND 15, NIF reduced head/limb movements (HLM) and body rotation (BR) induced by PTZ, BIC and PIC; in contrast, NIF pretreatment enhanced seizure-like activity (SLA) induced by PIC and BIC but not PTZ. VER reduced HLM movements induced by all 3 chemical convulsants and BR following BIC and PIC; PIC induced SLA was enhanced by VER pretreatment. At PND 30, NIF pretreatment enhanced SLA of PTZ and PIC; it was ineffective in reducing other indices. VER had no effect on PTZ, BIC or PIC convulsions. The results suggest that in developing rats CAs exhibited limited anticonvulsant efficacy and may exascebrate some chemically induced seizures. (Supported by PHS Grant #5RO1NS20725).

497.11

A ROLE FOR SEROTONIN IN THE ANTICONVULSANT EFFECT OF ANTIEPILEPSIRINE IN GENETICALLY EPILEPSY-PRONE RATS. Q-S. Yan*, P.C. Jobe and J.W. Dailey. Department of Basic Sciences, University of Illinois College of Medicine at Peoria, Peoria, Illinois 61656.

Previous reports from our laboratories demonstrate that antiepilepsirine (3,4,-methylenedioxycinnamyl piperine, AE), which is a clinically used antiepileptic agent in China, is an effective anticonvulsant in genetically epilepsy-prone rats (GEPRs) and that the anticonvulsant effect of AE in moderate seizure GEPRs (GEPR-3s) might be mediated, at lesst in part, by activation of CNS serotonergic neurons (FASEB. J. Abs. 3: A292, 1989; Neurosci. Abs. 15: 24.8, 1989). In order to further test this hypothesis linking the anticonvulsant action of AE in GEPRs to enhanced serotonin release, we evaluated the effects on dialyzable serotonin of i.p. AE in severe seizure GEPRs (GEPR-9s) and of i.v. AE in GEPR-3s. Under anesthesia, guide cannulae were stereotaxically placed over hippocampi of both GEPR-3s and GEPR-9s. To inject i.v. AE without stress, the GEPR-3s were also implanted with a jugular catheter. After recovery from surgery, dialysis probes were inserted into the guides of the conscious and unrestrained animals and the hippocampus was dialyzed with artificial CSF. Either AE (120 mg/kg i.p. in GEPR-9s; 40 mg/kg i.v. in GEPR-3s) or vehicle was administered after establishing basal release and dialysis was continued for 5 hours. Significant increases in dialyzable serotonin (approximately 200-250% over the basal values) were seen after the injection of AE in both GEPR-3s and GEPR-9s. The peak increases in serotonin produced by i.p. AE corresponded temporally with the time of peak anticonvulsant effect for this drug. These results confirm our earlier observation and are consistent with a role for serotonin in the anticonvulsant effect of AE in GEPRs. (Supported in part by a grant from Tsumura Juntendo Fdn.)

497.12

CESSATION OF BICUCULLINE-INDUCED FOCAL SEIZURES VIA LIDOCAINE: BEHAVIORAL AND ELECTROPHYSIOLOGICAL QUANTIFICATION. S.E. Krahl, E.J. Barea, and D.C. Smith. Department of Psychology, Southern Illinois University, Carbondale, IL 62901.

Earlier investigations in our laboratory have shown lidocaine to be an effective agent in the cessation of ongoing seizures when microinfused into an experimentally-produced focus. While the effectiveness of this technique has been verified behaviorally (1), quantification of the ECoG has not been performed to date. Although behavioral measures are useful in assessing abnormal discharges which have spread to the motor cortex, ECoG measures provide information about seizure activity occurring in other cortical and subcortical areas as well as about ictal events which may not be expressed as motor seizures. The present study used both behavioral and electrophysiological measures to quantitatively evaluate the effectiveness of the lidocaine technique.

Ten Long-Evans rats (250-274 g) were implanted with bilateral cannulae in the area tempestas (AT), and with cortical electrodes overlying the frontal and parietal cortices. Seizures were induced via a unilateral microinfusion of 0.5 μl of bicuculline (200 ng/μl) into the AT. Fifteen minutes later, 1 μl of either lidocaine or saline was microinfused into the same area. The results showed that lidocaine significantly reduced both the behavioral seizure, as measured on the Racine motor rating scale (2), and the integrated ECoG amplitudes to baseline levels. Saline did not cause a deviation in either measure from seizure levels. These results indicate that lidocaine may prove to be a useful means by which to arrest ongoing focal seizures.

(1) Smith, D.C., & Browning, R.A. (1987). *Soc. for Neurosci. Abstr.* 13:365.
(2) Racine, R.J. (1972). *EEG and Clin. Neurophys.* 32:281-294.

497.13

CLASSIFICATION OF SUBSTANTIA NIGRA PARS RETICULATA (SNpr) NEURONS BASED ON RESPONSES TO IONTOPHORETIC GABA AND FLURAZEPAM. H.C. Rosenberg, H. Zhang and X. Weng*. Dept. of Pharmacology, Med. Coll. of Ohio, Toledo OH 43699.

Previous work showed that intranigral GABA agonists and benzodiazepines (BZs) had different effects on some experimental seizures (Zhang et al., 1989,1991). It was suggested that SNpr neurons might be differentiated based on their responses to GABA and BZs. Male, Sprague-Dawley rats were anesthetized, and anesthesia maintained by a constant i.v. infusion of chloral hydrate. Multi-barreled glass electrode assemblies were used to record spontaneous activity of single SNpr neurons. GABA and flurazepam (FZP), a benzodiazepine, were applied iontophoretically, using several currents for each agent. Recording sites were marked for histological confirmation. As expected, both FZP and GABA, as a function of increasing current, decreased the rate of neuronal discharge. Based on the maximum effect obtained with increasing currents of each agent, the neurons could be divided into categories. Most SNpr neurons could be completely silenced by GABA, though their sensitivities varied widely. About half the SNpr neurons were inhibited >90% by GABA, but could be inhibited only about 50% by FZP. Most of the other neurons were inhibited >90% by both FZP and GABA. A few neurons were relatively insensitive to FZP and GABA, and a few others could be inhibited more by FZP than by GABA. The data suggest that SNpr neurons can be differentiated by their relative sensitivities to FZP and GABA. Histological examination suggested some anatomical segregation according to BZ sensitivity. Supported by DA02194.

497.14

BIDIRECTIONAL CONTINGENT CROSS TOLERANCE BETWEEN THE ANTICONVULSANT EFFECTS OF PENTOBARBITAL AND ETHANOL IN AMYGDALA-KINDLED RATS. C.K. Kim, J.P.J. Pinel and N.R. Roese. Department of Psychology. University of British Columbia, Vancouver, B.C. V6T 1Y7

The amygdala-kindling model of epilepsy was used to examine contingent tolerance and cross tolerance to the anticonvulsant effects of pentobarbital (15 mg/kg, IP) and ethanol (1.5g/kg, IP) in male Long-Evans rats. In Experiment 1, rats received a pentobarbital injection and a convulsive stimulation once every 48 hr on each of 10 trials. Pentobarbital was injected either 1 hr before or 1 hr after each stimulation. Only rats receiving pentobarbital before each stimulation became tolerant to pentobarbital's anticonvulsant effect. Cross tolerance to the anticonvulsant effect of ethanol was also found to be greater in the pentobarbital-before-stimulation rats. Experiment 2 assessed the transfer of tolerance from ethanol to pentobarbital. Results mirrored those of Experiment 1: Convulsive stimulation during the periods of ethanol exposure facilitated the development of tolerance to the anticonvulsant effect of ethanol and its transfer to pentobarbital. The development of tolerance and cross tolerance to anticonvulsant drug effects is facilitated by the administration of convulsive stimulation during periods of drug exposure. This supports the drug-effect theory of tolerance. (MRC and NSERC grants to JPJP)

498.1

REDUCED BLOOD FLOW IN THE TEMPORAL LOBES IN ALZHEIMER'S DISEASE. J.L. Eberling, W.J. Jagust, B.R. Reed*, M.G. Baker, Donner Laboratory, Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720.

Alzheimer's disease (AD) is characterized by reduced cerebral blood flow and metabolism, especially in parietal and temporal cortex. This has been reported with both single photon emission computed tomography (SPECT) and positron emission tomography (PET). While some studies report more severe involvement of the parietal cortex, others report that the temporal cortex is most severely affected. We used SPECT to study regional cerebral blood flow (rCBF) in 50 mildly and moderately demented AD patients and nondemented control subjects in order to evaluate rCBF as a function of disease severity. Subjects were scanned at two tomographic levels using the blood flow tracer [123I] -N-isoproyl-p-iodoamphetamine (IMP). Regions of interest (ROIs) were quantitatively analyzed by constructing ratios of the regional radioactivity count density for each ROI to the average radioacitivity count density in occipital cortex. Data were analyzed with multivariate analyses of variance (MANOVA) and post-hoc tests to evaluate group differences for each ratio. Pearson product moment correlations were used to evaluate the relationship between disease severity, as measured by the MMSE, and rCBF for the AD patients. Results of the MANOVA showed that rCBF in temporal cortex was significantly ($P<0.0001$) lower than controls for both the mild and moderate AD patients, while rCBF in parietal cortex was significantly ($p<0.0001$) lower than controls for the moderate AD patients only. Disease severity was associated with rCBF in dorsolateral frontal and parietal cortex ($r = .51$ and $r = .48$, respectively), but not with rCBF in temporal cortex. These results suggest that temporal cortex is affected earliest and most severely in AD, and that other cortical areas become involved as the disease progresses.

498.2

PROLONGED MR T2 TIMES IN THE HIPPOCAMPUS AND AMYGDALA MARK THE PRESENCE AND SEVERITY OF ALZHEIMER'S DISEASE. S.J. Kirsch, R.W. Jacobs, L.L. Butcher, and J. Beatty. Behavioral Neuro-science Program, Department of Psychology, UCLA, Los Angeles, CA 90024.

Magnetic resonance (MR) imaging is a well-accepted visualization tool that produces high quality macroscopic images of body tissues based on the excitation of hydrogen nuclei. Three fundamental aspects of the precessing hydrogen nuclei contribute to an MR image: proton density, T1 relaxation, and T2 relaxation. Thus, the data from which those macroscopic images are constructed arise from molecular properties of the tissue. By quantitatively estimating such properties, information concerning molecular characteristics of tissues can be deduced.

Taking this approach, we measured T2 relaxation times in the left and right hippocampus (HC) of 13 patients with Alzheimer's disease (AD), 9 subjects with multi-infarct dementia (MID), 11 elder normals (EN), and 23 young normals (YN). Using an Insturmentarium Magnaview MRI operating at 0.04T (SE: 1000/130 and 1000/200), T2 values for all AD patients exceeded that of any nondemented individual, regardless of age. Further, HC T2 values were normal for the 9 subjects with MID. Finally, the degree of T2 prolongation was highly correlated with the severity of functional and cognitive impairment of the AD patients. A second study is now underway in which preliminary observations show that T2 times are also prolonged in the amygdala of AD patients, but not YN,EN, or MID subjects. These results suggest that HC, and possibly amygdala, T2 prolongation may provide a specific anatomical marker by which AD pathology may be characterized and followed *in vivo*.

498.3

COMPARISON OF NEURODEGENERATION IN ALZHEIMER'S DISEASE AND COGNITIVELY IMPAIRED STATES USING MRI. J.P. Kesslak, S.F. Nagata*, C.W. Cotman, and O. Nalcioglu*. Departments of Neurology, Psychobiology & Radiological Sciences, Univ. California, Irvine, CA 92717

Magnetic resonance imaging (MRI) is a non-invasive method that can be used to monitor and quantify neuroanatomical changes. Neural degeneration in Alzheimer's disease (AD) and cognitively impaired (CI) patients affects a variety of structures, including the hippocampus and parahippocampal gyrus. Structural changes may be associated with behavioral deficits. MR images of AD (n=27), CI (n=19) and age-matched controls (n=12) were used to quantify volumes of discrete brain areas and correlated with a measure of general cognitive function, the Mini Mental State Exam (MMSE). The CI group included vascular dementia, Wernicke-Korsakoff, multi-infarct dementia, and age-related memory impairment. Scanning was done on a 1.5 Tesla GE Signa Unit using a head coil. Three series of scans were taken: Spin Echo-1) sagittal, T1 weighted; 2) transverse T2 weighted; and 3) coronal T1 weighted Inversion Recovery (IR). The coronal IR images provided high resolution and contrast to best visualize structures. Peliminary analysis, using ANOVA, indicated AD and CI subjects had significantly smaller hippocampus and parahippocampus, and larger ventricles than controls ($p > 0.05$), but AD and CI did not differer from each other. AD subjects also had significantly smaller parietal lobes than controls and smaller temporal lobes (excluding hippocampus and parahippocampus) than CI and controls ($p > 0.05$). Hippocampal and temporal lobe volumes had a high correlation with MMSE ($r = .51$ and .50, respectively). Ventricle volumes had an inverse correlation (-.58) with MMSE scores. The results support previous observations indicating the integrity of the hippocampus and parahippocampus is important for cognitive functioning. MRI is useful in determining the neuroanatomical changes associated with dementia, but limited for differentiating between AD and CI which requires a battery of neurological and neuropsychological tests. MRI provides an excellent means to examine brain structure-function relationships and assessing the potential cognitive capacity with respect to the degenerative state.

498.4

Education provides a cognitive reserve against the clinical manifestations of Alzheimer's Disease: Evidence from regional cerebral blood flow. Y. Stern, G. Alexander*, I. Prohovnik* and R. Mayeux. Depts. of Neurology and Psychiatry, College of Physicians and Surgeons of Columbia Univ., New York, NY 10032.

The finding that dementia is most prevalent in individuals with fewer years of education has suggested the specific hypothesis that education protects against the consequences of Alzheimer's disease (AD). We tested this hypothesis by asking: do individuals with more years of education have a more advanced state of the underlying dementing process before it is clinically evident? In AD, there is a specific pattern of cerebral blood flow reduction in the temporoparietal area. We measured regional cerebral blood flow, using the ^{133}Xenon inhalation technique, in 3 groups of patients with probable AD matched for severity of dementia, but with varying levels of education (N=58). Although global flow was comparable across the three groups, the temporoparietal perfusion deficit was significantly greater in the group with the highest level of education ($p<.01$). This suggests that AD was more advanced in the higher education group although measures of the clinical severity of the dementia were comparable across groups. We conclude that increased education is associated with a reserve which compensates for the neuropathological changes of AD and delays the onset of its clinical manifestations.

498.5

ATP UTILIZATION IN DORSAL PREFRONTAL CORTEX OF ALZHEIMER'S DISEASE BRAIN IS CORRELATED WITH SEVERITY OF DEMENTIA. K. Panchalingam, J. W. Pettegrew, G. Branthoover*, M. Tretta*, Laboratory of Neurophysics, Dept. of Psychiatry, U. of Pittsburgh, Pittsburgh, Pa 15261.

Both *in vitro* and *in vivo* ^{31}P NMR studies have shown increased brain levels of phosphomonoesters (PME) in early stages of Alzheimer's disease. Phosphodiesters (PDE) are elevated in late stages of the disease. In this study, we report results of *in vivo* studies that demonstrate possible correlations between the degree of dementia and energy metabolism in the dorsal prefrontal cortex of mildly to moderately demented (Mattis 92-125, Blessed 1.5-13, MMS 16-24) male and female patients (N=14,ages over 65 years) with probable AD (NINCDS-ADRDA criteria). The results shows significant increase in ATP with degree of dementia (Blessed $p=0.03, r=0.4$, Mattis $p=0.02$, $r=0.4$). Female patients 63 years and older show similar correlations (Mattis $p=0.01, r=0.7$, Blessed $p=0.05$, $r=0.5$). In addition, the results indicate that the PME levels first increase and then decrease as the disease progressed. Normal controls show no change in membrane phospholipid metabolism or energy metabolism. These results support an important role for altered energy metabolism in the clinical symptoms of AD. ^{31}P NMR is non-invasive and therefore ideally suited to follow the natural course of the disease and response to therapies *in vivo*.

498.6

PATTERNS OF INTERCORRELATIONS OF REGIONAL CEREBRAL METABOLIC RATES OF GLUCOSE (rCMRglc) IN PATIENTS WITH DEMENTIA OF THE ALZHEIMER TYPE (DAT). N.P. Azari, S.I. Rapoport, C.L. Grady, M.B. Schapiro* and B. Horwitz. Lab. of Neurosciences, NIA, NIH, Bethesda, MD, 20892.

A correlational analysis of resting rCMRglc data obtained using positron emission tomography (PET) with [18F]flurodeoxyglucose, examined differences between DAT and control (CTRL) subjects. 22 age- and sex-matched CTRLs and 19 mild/moderate DATs were scanned with a Scanditronix PC1024-7B tomograph. The brain was divided into 65 regions of interest (ROIs). DATs had lower metabolic rates than CTRLs, and a greater variability of metabolic asymmetry. Correlations were performed on normalized (to whole-brain) values. DATs had fewer total correlations than CTRLs and showed decreased fronto-parietal, frontal-sensorimotor, frontal-limbic, and sensorimotor-parietal correlations. ROIs most involved in the decreased DAT correlations were dorsal left premotor, ventral left sensorimotor, right/left inferior parietal, right/left insula, and anterior cingulate. The results, confirming and extending a prior correlation analysis using a lower resolution PET scanner and different DAT and CTRL subjects (Horwitz et al., 1987), demonstrate decreased corticocortical interactions in DAT.

498.7

DETECTION AND MEASUREMENT OF NEURAL THREAD PROTEIN (NTP) IN CEREBROSPINAL FLUID (CSF): A POTENTIAL ANTEMORTEM MARKER FOR ALZHEIMER'S DISEASE. J. Chong, L. Cantrell*, B. Miller, M. Husain*, S. Riesing*, H. Ghanbari. Neuropsychiatric Markers R&D, Abbott Laboratories, Abbott Park, IL 60064.

Elevated levels of pancreatic thread protein (PTP)-immunoreactivity have been reported in brains of patients with Alzheimer's Disease (AD). Antibodies to PTP showed cross-reactive epitopes with a protein in AD brains. Further analysis of this neural thread protein-like immunoreactivity (NTP) revealed a larger protein species (20 KD) both in the brain and cerebrospinal fluid (CSF) as compared to the pancreatic form of the protein (14kD). We have developed an automated microparticle enzyme immunoassay (MEIA) for NTP. The reagents for this assay have been optimized and stabilized. Up to 22 samples can be analyzed within 45 minutes with a sensitivity of 60 picogram/ml. Titration curves of both normal and AD CSF demonstrated a linear relationship as did normal and AD brain extracts. Preliminary results indicate that clinically diagnosed probable AD CSF specimens have much higher NTP levels than those of non-AD CSF.

498.8

DETECTION AND MEASUREMENT OF ALZHEIMER'S DISEASE ASSOCIATED PROTEIN (ADAP) IN CEREBROSPINAL FLUID (CSF): AN ANTEMORTEM MARKER FOR ALZHEIMER'S DISEASE. H. Ghanbari, B. Miller, G. Robertson*, J. Chong, S. Riesing*, M. Hardey*. Neuropsychiatric Markers R&D, Abbott Laboratories, Abbott Park, IL 60064.

Alzheimer's disease (AD) is a neurodegenerative disease characterized by a chronically deteriorating course of impaired intellectual function and memory loss. The definitive diagnosis of AD is made by pathological examination of postmortem brain tissue in conjunction with a clinical history of dementia. The antemortem diagnosis of AD, however, is by exclusion. The misdiagnosis rate is about 30%. We have demonstrated in a large population of patients (n > 400) that ADAP is present in AD brain tissues and not in non-AD. Studies involving the distribution of ADAP in the brain have revealed that ADAP is elevated in the brain regions associated with memory processing/storage. Hence, we expect ADAP in CSF to be a good antemortem marker for AD. The ADAP concentration in CSF is in the femtomole/-ml range. Using ADAP extracted from brain tissue as a model, we have developed a chemiluminescent immunoassay (CLIA) suitable for measuring ADAP in CSF. Preliminary results indicate that ADAP level is significantly higher in AD CSF specimens as compared to non-AD CSF specimens.

498.9

LABELLING OF ACTIVATED MICROGLIA AND NEURAL MACROPHAGES BY ANTIBODY PRESENT IN THE CSF OF ALZHEIMER'S DISEASE PATIENTS. A. Dahlström[1], E.A. Ling*[2], R. Polinsky*[3] and A.McRae[1] Dept. of Histology[1], Univ. of Göteborg, Göteborg Sweden, Dept. of Anatomy[2], Nat. Univ. of Singapore, Singapore 0511, NINDS NIH[3], Bethesda MD USA.

Cerebrospinal fluid from Alzheimer's disease patients (AD-CSF) contains an antibody that recognizes amoeboid microglial cells, an activated form of microglia and macrophages in the developing rat brain. This study examined if AD-CSF would mark (A) the activated microglia, and (B) neural macrophages induced experimentally in adult rats. In (A), minute amounts of (2µl) of the toxic lectin Ricinus communin agglutinin (RCA-60, 0.05%) was injected into the sciatic nerve in anesthetized adult rats. This caused a selective destruction of the ventral horn neurons in the lower lumbar cord. The rapid neuronal cell death elicited a drastic response of the indigenous microglia. In (B), trace amounts of Kainic acid (KA) was applied extradurally over the sensorimotor cortex. Underlying cortical neurons were destroyed, which attracted a massive influx of neural macrophages. Rats were overdosed with barbital and perfusion fixed with 4% paraformaldehyde, 1-3 weeks after the operations. Tissue samples were incubated with non-diluted AD-CSFs and processed for immunocytochemical visualization with the avidin-biotin method. In RCA-treated samples, the activated microglia associated with the ventral horn neurons undergoing lysis were intensively stained. Many neuronal cell 'ghosts' were surrounded by AD-CSF positive cells. Contralateral microglial cells were not stained. In KA-treated samples, numerous active neural macrophages most probably derived from monocytes were also strongly stained. Electron microscopic studies showed that the immunoreactivity was detected at their plasma membrane. Some of the immunoreactive cells showed features indistinguishable from microglia. AD-CSF contains an antibody that specifically marks membrane antigens in the activated microglia and neural macrophages induced by experimental procedures, which strongly suggest the immunological involvement of monocytic cell lineage in AD.

PARKINSON'S DISEASE: HUMAN STUDIES

499.1

THE EFFECT OF ENZYME INHIBITORS ON THE APPARENT AUTOXIDATION RATE OF DOPAMINE AND RELATED CATECHOLS OF THE BRAIN.

B. Fornstedt and A. Carlsson*, Dept. of Pharmacology, University of Göteborg, Göteborg, Sweden.

The occurrence of 5-S-cysteinyl-dopamine (5-S-cysteinyl-DA), 5-S-cysteinyl-3,4-dihydroxyphenylalanine (5-S-cysteinyl-DOPA) and 5-S-cysteinyl-3,4-dihydroxyphenylacetic acid (5-S-cysteinyl-DOPAC) in dopaminergic brain areas of several mammalian species has been reported by this laboratory. The data indicates that DA and related catechols undergo *in vivo* autoxidation to reactive quinone forms, which then interact with thiol groups as that in cystein.

A substantial increase in 5-S-cysteinyl-DA has been found in the striatum following different time periods of reserpine treatment (5 mg/kg, i.p.). It was suggested that reserpine's inhibition of vesicle storage caused an increase in autoxidation of the unsheltered cytosolic pool of DA. Co-administration of pargyline (75 mg/kg, i.p., 18 h) did not change the elevation in 5-S-cysteinyl-DA levels, although DA available for autoxidation must have been increased. However, following pargyline treatment alone 5-S-cysteinyl-DA levels rised significantly.

The turnover rate appears to be lower for the 5-S-cysteinyl adducts than for their parent catechols as indicated in studies on enzyme inhibition as well as in other studies. Following 1 h of monoamine oxidase or tyrosine hydroxylase inhibition the levels of DOPAC and DOPA, respectively, decreased significantly whereas no effect was oberved for the correlated adduct's levels. Following 18 h of pargyline treatment the 5-S-cysteinyl-DOPAC concentration had decreased drastically, although not as much as the concentration of DOPAC. Alpha-Methyltyrosine treatment for 8 h (250 mg/kg, i.p.) of reserpine pre-treated animals resulted in significantly decreased levels of all three cysteinyl adducts.

499.2

LOCALIZATION OF SUPEROXIDE DISMUTASE AND GLUTATHIONE PEROXIDASE IN ANATOMICAL AREAS ASSOCIATED WITH NEURODEGENERATIVE DISORDERS. F.J. Denaro and J.S. Schneider. Dept. of Neurology, Texas Tech University Health Sciences Center, Lubbock, TX 79430. Dept. of Neurology, Hahnemann University, Philadelphia, PA 19102.

Superoxide dismutase and glutathione peroxidase are two key enzymes which protect cells from destructive oxygen radicals. There is a growing body of evidence which implicates potentially damaging oxygen free radicals in disease processes. Examples of such diseases could be the neurodegenerative disorders of the brain, such as Parkinson's Disease and Alzheimer's Disease. Previously we developed antibodies to these enzymes (*J. Cell Biochem.* 12A, pp 44, 1988) and by means of immunocytochemistry identified superoxide dismutase and glutathione peroxidase containing cells in the human and rodent brain (Soc. for Neuroscience Abs., 1989). In the present study, we focus on areas strongly correlated with two important neurodegenerative disorders, Alzheimer's Disease (hippocampus, nucleus basalis) and Parkinson's Disease (substantia nigra and ventral tegmental area). It has been possible to identify the protective enzymes of interest in melanin-containing cells of the substantia nigra in primates and in cells of the cortex and hippocampus (granule cell layer). The sub-regional localization of these enzymes in these anatomical regions may have important implications for the pathophysiology of these neurodegenerative diseases.

499.3

ALLELIC VARIATIONS IN MAO GENES MARK ACTIVITY STATES AND MAY PREDICT VULNERABILITY TO PARKINSON DISEASE. C. Konradi (1), G.S. Hotamisligil (1,2),* A.S. Girmen (1),* S. Fink (1), J. Baenziger (3),* J. Sullivan (3),* J. Growdon (1), & X.O. Breakefield (1,2). (1) Neuroscience Ctr. & Neurology Dept., Mass. General Hosp., Boston, MA; (2) Dept. Genetics & Neuroscience Prog., Harvard Med. Sch., Boston, MA; (3) Depts. Psychiatry & Pathology, Alcohol Research Ctr., Indiana Univ. Med. Ctr., Indianapolis, IN.

Monoamine oxidases A and B are involved in degradative deamination of biogenic amines, including catecholamine neurotransmitters. These isozymes are encoded in homologous genes near each other on chromosome Xp11.3. Alleles for the MAOA gene are marked by several RFLP's, as well as a (GT)n repeat (Black et al, NAR, 1991); those for the MAOB gene, by a (GT)n repeat. A strong association has been found in control males between levels of MAO-A activity, measured in human skin fibroblasts, and specific alleles for the MAOA gene. Similar comparisons are underway for MAO-B activity, measured in platelets, and MAOB alleles. Individuals inherit widely varying levels of MAO-A and MAO-B activities. These different activity states may predispose individuals to certain diseases, like Parkinson disease, where drug models have implicated MAO in the pathogenic process. We have found a statistically significant difference in the frequency of alleles for MAOA and MAOB in over 60 Parkinson patients and 60 controls. This finding implicates endogenous levels of MAO-A and MAO-B activity in the disease process, and suggests that allelic status for these genes may be an index of susceptibility.

499.4

CALBINDIN-D_{28K} IN THE HUMAN HYPOTHALAMUS: RELATIONSHIP TO TYROSINE HYDROXYLASE (TH) IMMUNOREACTIVE NEURONS. M.K. Sanghera and D.C. German. Dept. of Psychiat., UT Southwestern Med. Cntr., Dallas, TX.

Calbindin-D_{28K} (CaBP), a calcium-binding protein, is localized within the midbrain dopaminergic (DA) neurons in the same regions that are spared in Parkinson's disease (PD) (German et al., Neurosci. Abstr. 16:696, 1990). Hypothalamic DA neurons do not appear to be degenerated in PD (Matzuk & Saper, Ann. Neurol. 18:552, 1985). The present experiment sought to determine if CaBP is localized in the same hypothalamic nuclei as the DA neurons. Immunocytochemical staining, with antibodies against TH (1:1000) and CaBP (1:1000), and computer imaging techniques were used to map the distribution of CaBP- and TH-containing cells. CaBP cells were located throughout the hypothalamus, however, often not confined within specific hypothalamic nuclei. The TH-containing cells were found within such nuclei as the arcuate, peri- and paraventricular nuclei. There was no substantial overlap in the distributions of CaBP- and TH-containing cells. These data suggest that CaBP does not protect the hypothalamic DA neurons from degeneration in PD. Supported by American Parkinson Disease Association, and AG-08013.

499.5

DOPAMINE DEPENDENT DECREASE IN ENKEPHALIN AND SUBSTANCE P LEVELS IN BASAL GANGLIA REGIONS OF POSTMORTEM PARKINSONIAN BRAINS. S. P. Sivam. Dept. Pharmacol./Toxicol., NW Ctr. for Med. Ed., Indiana Univ. Sch. Med., 3400 Broadway, Gary Indiana 46408-1197.

This study examined whether a relationship exists between the degree of dopamine (DA) loss and the changes in opioid (Met-enkephalin, ME; dynorphin A (1-8), DYN) or tachykinin (substance P, SP) peptidergic systems in basal ganglia (caudate and putamen) and limbic (frontal cortex) regions of postmortem tissue samples derived from patients who died of Parkinson's disease (PD). The levels of peptides were determined by radioimmunoassays. The levels of DA, 5-hydroxytryptamine (5-HT) and their metabolites were determined by HPLC. The degree of loss of DA in PD tissues was classified into two major categories, those with less than 80% and those with more than 80% DA loss as compared to control. It was found that the samples with more than 80% of DA loss exhibited lower levels of ME in caudate and SP in putamen; no changes were observed in DYN levels. A decrease in 5-HT level was also observed in caudate and putamen of samples that showed more than 80% DA loss. The frontal cortical region exhibited no changes in the levels of peptides. It appears that there is a DA-dependent secondary loss of enkephalin and tachykinin peptides in PD. In view of the involvement of these peptidergic systems in the regulation of behavior, movement and memory, derangements in these peptidergic systems should be considered as additional factors in the progression of symptoms of PD. Supported by USPHS grant NS 26063

499.6

DISTRIBUTION OF BINDING SITES FOR [^{3}H]CP-96,345 , A NONPEPTIDE NK_1 RECEPTOR ANATAGONIST IN HUMAN BRAIN: ALTERATIONS IN PARKINSON'S AND ALZHEIMER'S DISEASE. L. Rioux, B.S. Neal and J.N. Joyce. Departments of Psychiatry and Pharmacology, Univ. of Penn. Sch. Med., Philadelphia , PA

CP-96,345, a nonpeptide substance P antagonist, has been tritiated and determined to have high affinity (1.9 nM) for NK_1 receptors in guinea pig (McLean et al, Science 251:437, 1991). We have examined the distribution of this compound in postmortem human brain and examined alterations in Parkinson's (PD, n = 3) and Alzheimer's (AD, n=4) disease. Tissue sections were pre-incubated for 15 min at 22°C in 50 mM tris-HCl, containing 5mM $MnCl_2$ and 0.02% BSA (pH 7.3). Tissue section wre incubated in the same buffer with 5 nM [^{3}H]CP-96,345 at 4°C for 30 min with adjacent sections incu-bated in the presence of 5 µM CP-93,009-1. Following rinses and drying, the slides were apposed to ^{3}H-sensitive film for 60 days. Computer aided analysis indicated that nonspecific binding was 50% of the total binding at this concentration and certain regions of the human brain contained relatively high numbers of sites. The regions with the highest densities included the caudate (CN), putamen (PUT) and nucleus accumbens (NAS) (200-225 fmol/mg protein). Lower binding was observed in globus pallidus (40 fmol/mg P) and frontal cortex (100 fmols/mg P). The nucleus basalis (50 fmols/mg P) and regions of the medial temporal lobe, including the amygdala (50 fmol/mg P); entorhinal cortex (150 fmol/mg P), subiculum (175 fmol/mg P), CA1-CA3 (175 fmol/mg P), and dentate gyrus (200 fmol/mg P), showed high levels of binding. Binding was decreased in all regions of the striatal complex in PD and AD. In AD cases the binding was decreased by 17% in CN , 52% in PUT and by 32% in NAS and globus pallidus. In the PD cases the loss 68% in CN, 46% in PUT and 72% in NAS. Analysis of the medial temporal lobe is pending. **Funded by a grant from Pfizer Central Research.**

499.7

DISTURBANCES IN PRE- AND POSTSYNAPTIC DOPAMINE SYSTEMS IN PARKINSON'S AND ALZHEIMER'S DISEASE. A.M. Murray, F. Weihmueller*, J. F. Marshall*, and J.N. Joyce. Departments of Psychiatry and Pharmacology, Univ. of Penn. Sch. Med., Philadelphia , PA ; *Dept. Psychobiology, Univ. California, Irvine CA.

Parkinsons disease (PD) is most clearly associated with disturbances of motor function such as bradykinesia, tremor and rigidity. Increasing attention has been paid to the contribution of post-synaptic dopamine (DA) systems within the striatum to the development of the motor symptoms and the loss of responsiveness to treatment regimens. Many patients also develop severe dementia, which may also be related to disturbances in DA function. Patients developing Alzheimers disease (AD) also may develop PD symptoms, but only some of them show Lewy bodies or significant losses of pigmented neurones in the substantia nigra. In order to explore the role of pre and post-synaptic elements of the DA system to the clinical picture associated with AD, we utilized quantitative autoradiography to identify alter-ations in the density of high-affinity uptake sites for DA ([^{3}H]mazindol), and two DA receptor sub-types, D1 ([^{3}H]SCH23390) and D2 ([^{125}I]epidepride), in post-mortem tissue from patients with PD, AD, PD with AD and age-matched controls. Loss of pigmented neurons in PD was associated with a 90% loss of DA uptake sites in the motor region of the striatum (putamen) with less significant reductions seen in the caudate (40% rostral and 60% caudal). In contrast only a 20% loss of sites was seen in the putamen of the AD cases with a greater loss in the caudate (30-35%). In PD cases, an increase (46-51%) in D2 receptor density was seen in the region of almost complete loss of DA uptake sites (putamen) with a 10% reduction in the caudate nucleus. AD cases showed a 12-20% decrease of D2 receptors in the putamen. Preliminary analysis of D1 receptors showed slight decreases in AD striatum with no significant differences in PD brains. **Funded by MH43852, MH 43880, AG 09215.**

499.8

MITOCHONDRIAL FUNCTION IN PLATELETS AND MUSCLE IN PARKINSON'S DISEASE. D. Bravi*, J.J. Anderson, F. Dagani*, T.L. Davis*, R. Ferrari*, M. Gillespie*, and T.N. Chase, Experimental Therapeutics Branch, NINDS, NIH, Bethesda, MD 20892 and Institute of Pharmacology, University of Pavia, Pavia, Italy.

Recent evidence suggests that reductions in mitochondrial metabolism exist in substantia nigra, platelets, and skeletal muscle in Parkinson's disease (PD). However, it is unclear if these alterations occur in all cells of the body or specifically in dopamine cells of the substantia nigra. In this investigation, mitochondrial respiration and electron transport chain enzyme activity was studied in both platelets and muscle isolated from seven subjects suffering from PD and matched normal controls. Oxygen consumption rate in the presence of ADP induced by NADH-generating substrates (Complex I activity), succinate + rotenone (Complex II-III activity), and TMPD + ascorbate (Complex IV activity) in digitonin permeabilized platelets and isolated muscle mitochondria were not significantly different between control and PD subjects. Likewise, activities of the enzymes succinate cytochrome c reductase (Complex II-III activity) and cytochrome oxidase (Complex IV activity) in platelet and muscle mitochondria were not significantly different between control and PD subjects. These results suggest that there are no differences in mitochondrial function in peripheral tissues in Parkinson's disease and any mitochondrial defect may be localized to the substantia nigra.

499.9

ANTI-NEURONAL ANTIBODIES AND OTHER MARKERS OF IMMUNE SYSTEM ACTIVATION IN PARKINSON'S DISEASE. D.A. Loeffler[1]*, C.M. Brickman*[1], G. Kapatos[3], J.B. Peter*[2], P.A. Lewitt*[1] Dept. of Psychiatry, Wayne State University[3], Detroit, MI 48201 and The Clinical Neuroscience Program[1], Sinai Hospital, Detroit, MI 48235 and Specialty Labs, Inc.[2], Santa Monica, CA 90404-3900.

Anti-neuronal antibodies have been reported in serum and cerebrospinal fluid (CSF) from patients with Parkinson's Disease (PD), though further clues for immune mechanisms in this disorder have not been explored. With CSF from unmedicated PD subjects (n=17), normal controls (n=13), and patients with multiple sclerosis (n=10) and other medical disorders (n=18), we measured several indicators of immune system activation, including interleukin-1-beta (IL-1b), soluble interleukin-2-receptor (sIL-2R), tumor necrosis factor-alpha (TNF), and beta-2-microglobulin (B-2-M). Two methods were investigated for evaluating possible anti-neuronal immunoglobulins: an enzyme-linked immunosorbent assay (ELISA) detecting antibodies against plasma membrane antigens of a human neuroblastoma cell line (Sk-N-Mc), and an immunocytochemical technique for detecting antibody binding to neurons in sections of fixed rat midbrain. Three of the PD CSF samples produced detectable staining of rat midbrain cells; no antibodies against neuroblastoma antigens or cardiolipin were found in these samples. IL-1b, sIL-2R, and TNF were not detected in PD CSF samples; B-2-M was detected in all PD CSF samples but did not differ from B-2-M in normal CSF. Our data do not indicate that local immune system activation is present in PD.(Supported by the Student Fellowship Program of the Michigan Parkinson Foundation and NIH grants NS-27892, PAL, and NS-26081, GK).

499.10

FORCE CONTROL IN PARKINSONIAN HANDWRITING. H.L. - Teulings*, Nijmegen Institute for Cognitive Research, Nijmegen, The Netherlands, and G.E. Stelmach, Department of Exercise Science, Arizona State University, Tempe, AZ 85287-0404, USA.

The aim of this study was to quantify motor control deficits in Parkinsonian handwriting. Using a digitizing tablet, and performing signal-to-noise analyses and computer simulations, six idiopathic Parkinson's disease subjects and matched controls were examined as they executed a writing pattern. The analysis revealed that the movement deficits, often noted in Parkinsonians, were primarily due to an impaired force-amplitude component rather than to an impaired stroke-duration component. Computer simulations which altered the force-amplitude and force-duration components supported the signal-to-noise analysis. Applying the simulations as predictors, Parkinsonians were found to have few problems with sharp movement reversals during cursive script while exhibiting difficulty in maintaining a straight baseline over the writing pattern. The data are interpreted as demonstrating that Parkinson's disease alters the ability to regulate force amplitude.

499.11

SLEEP DEPRIVATION IMPROVES OCULAR AND MOTOR DEFECTS OF PARKINSONS DISEASE. E.M. DeMet, C. Reist, E. Coskinas*, A. Chhoeu*, C.-C. Chen*, K. Sokolski*. Depts. Psychiatry, Univ. California, Irvine, CA 92717 and Long Beach VA Hospital, Long Beach, CA 90822.

The retina and substantia nigra arise from a common embryological source. Degenerative changes in both tissues occur in Parkinsonism. Retinal function may be assessed through the use of electrooculograms obtained under conditions of light and dark adaptation. Some evidence suggests that sleep deprivation (SD) may improve the motor dysfunction of Parkinsonism. The present study sought to determine if similar changes also occur in retina. Motor function and ocular potentials were measured in patients with Parkinsons disease prior to and following SD. This treatment substantially improved motor function (>40%) and significantly increased ocular potentials. Improvements in these measures were highly correlated (r>0.9) and were approximately equally distributed over the range of the group response. The results confirm that SD therapy may provide temporary relief of motor disabilities. Parallel actions in retina and striatum, and the anatomical isolation of these tissues, further suggest that the SD effect may be expressed through induction of an endogenous humoral agent. Such an agent could provide a basis for the development of more effective treatments for Parkinsons disease.

499.12

DOPAMINE DEPENDENT SPATIAL WORKING MEMORY DEFICITS IN PARKINSON'S DISEASE.

A.M.Owen*, M. Beksinska*, M.James*, K.W.Lange*, B.A.Summers*, P.N.Leigh, N.P.Quinn, C.D.Marsden* & T.W.Robbins. Institutes of Psychiatry and Neurology, London, U.K. and Dept. of Expt. Psychol., Univ. of Cambridge, Cambridge, U.K.

Groups of patients with idiopathic Parkinson's disease (PD), either medicated or unmedicated, were compared with matched controls on a computerised battery of tests designed to assess different aspects of visuo-spatial memory and learning. In a test of spatial working memory known to be sensitive to frontal lobe damage, significant impairments were found only in the medicated PD patients and particularly in those patients with more severe clinical symptoms. These medicated patients 'later in the course' of the disease were also impaired in both a delayed matching to sample procedure and at learning the locations of a number of patterns on the screen. In contrast, spatial recognition was significantly impaired in medicated PD patients with both mild and severe clinical symptoms.

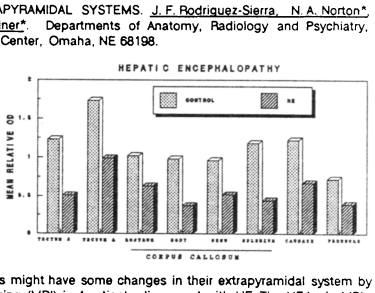
In a parallel experiment, this same battery of tests was given to 10 PD patients 'on' and 'off' L-Dopa therapy. Significant impairments following withdrawal of medication were only evident in the test of spatial working memory.

These results suggest that there are multiple memory impairments in PD which may differentially depend on the clinical severity of the disease. In addition, the selective impairment in spatial working memory following L-Dopa withdrawal suggests that those tests of memory function most sensitive to frontal lobe damage also depend most heavily on dopaminergic substrates.

499.13

PATIENTS WITH HEPATIC ENCEPHALOPATHY SHOW ALTERATIONS OF BRAIN ACTIVITY THAT APPEARS TO RELATE TO EXTRAPYRAMIDAL SYSTEMS. J. F. Rodriguez-Sierra, N. A. Norton*, J. R. McConell* and C. B. Greiner*. Departments of Anatomy, Radiology and Psychiatry, University of Nebraska Medical Center, Omaha, NE 68198.

Hepatic Encephalopathy (HE) is an alteration in mental state due to chronic liver disease. Although the etiology of HE is varied the expression of the disease is fairly typical: from subtle abnormalities of intellectual and motor function to coma and death. During the progression of the disease, we have noted that the patient displays signs of agitation with accompanying extrapyramidal signs of activity. We investigated whether HE patients might have some changes in their extrapyramidal system by using Magnetic Resonance Imaging (MRI) in 4 patients diagnosed with HE. The HE brain MRIs were compared to the MRIs of patients that did not have HE. All the images were scanned and the reflectances were analyzed by densitometry using the JAVA (Jandel Scientific) software system. Our results show that there were significant differences in areas of the extrapyramidal system, but that in addition some major fiber tracts were also affected: particularly the corpus callosum and the cerebral peduncles. The tectal area showed a significant effect using both axial (A) or sagittal (S) approaches. There were no significant differences in areas of the cerebral cortex, nor in the thalamus. There was some changes in the hippocampus and anterior portion of the internal capsule, but due to our small number of patients, the differences were not statistically significant. It appears that the HE patient is experiencing some alteration in the extrapyramidal pathways as well as in interhemispheric pathways. It remains to be determined whether the small effects in the hippocampus can be replicated as they would explain some of the behavioral deficits experienced by these individuals. (This work was partially supported by a grant from the NIH HD-13219 and funds from the University of Nebraska Medical Center Department of Radiology).



499.14

A SENSITIVE AND SPECIFIC RADIOIMMUNOASSAY (RIA) FOR THE QUANTIFICATION OF PERGOLIDE. R. R. Bowsher*, J. A. Compton*, J. M. Apathy*, R. L. Wolen* and D. P. Henry. Lilly Lab. for Clinical Research, Eli Lilly & Co., Indianapolis, IN 46202.

Pergolide mesylate, [(Methylthio)methyl]-6-propylergoline monomethanesulfonate (LY127809), is a synthetic ergot alkaloid with potent dopaminergic (D1 and D2) activity and is marketed under the trade name Permax ® for use in the treatment of Parkinson's Disease. The low level of pergolide found in plasma (≤1 ng/ml) after oral dosing has complicated the development of analytical methodology for pergolide. Accordingly, we developed and validated a monoclonal antibody-based competitive RIA specifically optimized for the measurement of pergolide in plasma (Table I). Briefly, samples (0.5 ml) are incubated 20 h at 10 C with 75 ng of tritiated pergolide (0.1 ml) and anti-pergolide MoAb diluted 1:50,000 (0.1 ml). Separation of bound and free labelled pergolide is achieved by adsorbing the free fraction with 1% dextran-coated 5% charcoal. Plasma levels are then estimated from a standard curve of reference pergolide ranging from 5 to 5,000 pg/ml.

Table I

Sensitivity	< 25 pg/ml
ED50	168 pg/ml
Inter-assay %CV	11.3-15.5%
Recovery	82-97%

Displacement experiments involving 4 pergolide metabolites found during animal studies and 5 synthetic analogs indicated the RIA is specific for pergolide and permitted defining the MoAb binding epitope. We conclude the RIA is a valid method for quantifying therapeutic concentrations of pergolide in plasma from man, rat and monkey.

500.1

THE USE OF MICROWAVE (MW) FIXATION FOR THE DETERMINATION OF MOUSE BRAIN METABOLITES DURING HIGH +Gz (HEAD-TO-FOOT) EXPOSURE IN A SMALL ANIMAL CENTRIFUGE (SAC). A. R. Shahed, B. B. Stavinoha*, J. A. Barber* and P. M. Werchan*. Up. Tech. Corp., UTHSC, San Antonio, Krug Life Sci. and Armstrong Laboratory, Brooks AFB. TX 78235

The mechanism or effects of acceleration induced loss of consciousness (G-LOC) observed in pilots of high performance aircraft are not fully understood. It has been proposed that cerebral blood flow may cease due to zero carotid artery pressure within 2s after >5 +Gz exposure. **HYPOTHESIS:** G-LOC may result from global ischemia and subsequent energy depletion. **METHODS:** A SAC equipped with a 2450 MHz, 10 KW, MW system with rotating waveguide was used for brain fixation. Mice were exposed to either varying +Gz (15 to 35) forces or duration (0-35s) and brains were fixed at specific time points during and 60s post run. Brain glycolytic and high energy phosphates were measured. **RESULTS:** The G-tolerance data show that the levels of glucose, C-P and ATP decreased and lactate accumulated as +Gz force increased. The time curve shows significant changes in glucose (-85%), C-P (-73%) and ATP (-22 %), lactate (+220%), AMP (+67%) and adenosine (+10 fold) 35s after the onset of 35 +Gz. The level of these metabolites were not restored 60s after the SAC run. **CONCLUSIONS:** (1) MW is an ideal tool for brain fixation during +Gz exposure. (2) G-LOC may occur to conserve energy resources during the cessation of cerebral blood flow in a high +Gz environment.

500.2

EFFECT OF LOW FREQUENCY PULSING ELECTROMAGNETIC FIELDS (PEMFs) ON FOCAL CEREBRAL ISCHEMIA. G.A. Grant, R. Cadossi*, and G.K. Steinberg. Dept. of Neurosurgery, Stanford Univ. Sch. of Med., Stanford, CA 94305

There is evidence that electromagnetic stimulation may accelerate the healing of tissue damage following ischemia. We studied the effect of exposure to PEMFs on cerebral injury in a rabbit model of transient focal ischemia (2 hr. occlusion of left ACA, ICA, and MCA, followed by 4 hrs. of reperfusion). PEMF exposure(240 V, 75 Hz) was initiated 10 min. after the onset of ischemia and continued throughout reperfusion(6 exposed, 6 controls). Magnetic resonance imaging(MRI) and somatosensory evoked potentials(SEPs) were used to measure the degree of ischemic injury. Exposure to the PEMF did not significantly reduce the area of MRI injury (exposed, 16.1 vs. control, 17.1). Although exposure showed a trend toward recovery of SEPs, this finding was not statistically significant (exposed, 54.9% vs. control, 27.7%). Preliminary data suggest that exposure to a PEMF of short duration has no beneficial role in the treatment of stroke, although more prolonged exposure might prove efficacious. (AHA Grant 881069 and IGEA).

500.3

PROTEIN OXIDATION AND INHIBITION OF GLUTAMINE SYNTHETASE IN THE CEREBRAL CORTEX FOLLOWING CARDIAC ARREST AND RESUSCITATION. G. Fiskum[1,2], R.E. Rosenthal[2*], and P.E. Starke-Reed[3*]. Dept. of Biochemistry and Molecular Biology[1], Emergency Medicine[2], and Medicine[3]. George Washington Univ. Medic. Center, Wash., D.C. 20037.

Proteins, e.g. glutamine synthetase, are an important site of attack by free radicals and metal-catalyzed oxidation systems. In this study, site-specific protein oxidation, as reflected by generation of carbonyl groups (CG), and glutamine synthetase activity (GS) were measured in protein extracts from the temporal cortex of chloralose-anesthetized, adult female beagles subjected to: A. sham-procedure (SP); B. 10 min of electrically-induced cardiac arrest (CA); C. 10 min CA followed by defibrillation and 2 hour of restoration of spontaneous circulation (CA/2 hr ROSC) and; D. 10 min CA followed by 24 hr ROSC (CA/24 hr ROSC). The following results were obtained: (values represent means ± S.E., n = 5).

	SP	CA	CA/2hr ROSC	CA/24hr ROSC
CG (nmoles mg^{-1} protein)	3.1±0.2	2.4±0.2	5.7±0.3	4.4±0.4
GS (units mg^{-1} protein)	87±4	84±8	59±8	75±3

These results suggest that free radical-mediated protein oxidation and associated inhibition of the activity of GS, an important enzyme in glutamate metabolism, may contribute to the molecular basis of brain damage in a clinically-relevant model of global cerebral ischemia and reperfusion. (Supported by a grant from Sigma Tau, S.p.A.).

500.4

LITHIUM EFFECTS ON DECAPITATION-INDUCED RELEASES OF INOSITOL PHOSPHATES, DIACYL-GLYCEROLS AND FREE FATTY ACIDS IN MOUSE CEREBRUM. T-A Lin*, J-P Zhang* and G.Y. Sun, Biochem. Dept. Univ Missouri, Columbia, MO 65212.

Decapitation-ischemia is known to cause a rapid hydrolysis of poly-phosphoinositides with concurrent increases in inositol phosphates, free fatty acids (FFA) and diacylglycerols (DAG) in brain. In this study, the effects of lithium administration on these events were examined. Adult C57Bl/6J mice were administered i.p. either lithium (8 meq/kg body wt) or saline 4 hr prior to decapitation and then subjected to decapitation ischemic treatment (5, 35, 65, 125, 305 sec). The cerebrum was taken for analysis of inositol phosphates by ion chrmatography, Ins(1,4,5)P_3 by radioreceptor binding assay and FFA and DAG by gas-liquid chromatography. In control mice, decapitation induced a sequential turnover of Ins(1,4,5)P_3 to Ins(1,4)P_2 and Ins(4)P with peak activities at 35, 65 and 125 sec, respectively. Lithium administration did not alter the turnover of Ins(1,4,5)P_3 but increased the levels of Ins(1,4)P_2, Ins(1)P and Ins(4)P. Decapitation resulted in an immediate increase in the levels of DG but the increase in FFA levels showed a lag time of 35 sec. Lithium treatment resulted in a decrease in the initial phase of FFA release which was reflected in an increase in the level of DAG. These results suggest that lithium may interfere with the release of FFA in brain through the DG-lipase pathway.

500.5

EFFECTS OF RAT FOCAL CEREBRAL ISCHEMIA ON LEVELS OF INS(1,4,5)P_3 G.Y. Sun, T-A Lin*, T-N Lin*, Y.-Y. He*, and C.Y. Hsu. Biochem Dept, Univ Missouri-School of Medicine, and Div. Restoratory Neurology, Baylor College of Medicine, Houston, TX.

Severe and mild focal cerebral ischemia in the right and left middle cerebral artery (MCA) could be induced by temporary occlusion of the right MCA and both common carotid arteries. Poly-phosphoinositide (PI) breakdown in the MCA cortex was dependent on the severity of the ischemic insult (Stroke 22: 495, 1991) and is marked by a transient increase in the level of Ins(1,4,5)P_3, followed by rapid conversion to Ins(1,4)P_2 and Ins(4)P. In this study, the levels of Ins(1,4,5)P_3 in the left and right MCA cortex were determined using the radioreceptor binding assay. Ischemia for 60 min resulted in marked decreases in levels of Ins(1,4,5)P_3 in the right (2.7 ± 1.9 nmol/g wt, n=7) and to a lesser extent, the left (7.1 ± 2.8, n=7) MCA cortex as compared to sham-operated controls (16-18 nmol/g wt). After 60 min ischemic treatment, reperfusion for 24 hr failed to restore the levels of Ins(1,4,5)P_3 in the right MCA cortex. On the other hand, ischemia for 30 min followed by reperfusion resulted in complete restoration of the levels of Ins(1,4,5)P_3 to control levels in both the right and left MCA cortex. The biochemical findings in this study are in agreement with earlier observation of irreversible neuronal injury to the right MCA cortex following severe ischemia for 60 min or longer (Am J. Physiol. 256:H589, 1989).

500.6

PERSISTENCE OF SEROTONIN AND CHOLECYSTOKININ IMMUNOREACTIVE NERVE FIBERS IN THE GERBIL HIPPOCAMPAL FORMATION FOLLOWING CEREBRAL ISCHEMIA. T. Kondoh[1], J. Howard[2], P.L. Faris[2], B.K. Hartman[2], Y.J. Li[1], S.M. Onifer[3], and W.C.Low[1], Depts. of [1]Neurosurgery and [2]Psychiatry, Univ. of Minnesota Medical School, Minneapolis, MN 55455 and [3]Dept. of Neurological Surgery, Univ. of Miami School of Medicine, Miami, FL 33101.

Serotonin (5HT) and cholecystokinin (CCK) -immunoreactive nerve fibers were studied in the hippocampal formation following the induction of transient forebrain ischemia in the mongolian gerbil. Transient cerebral ischemia was produced by occluding bilaterally the carotid arteries for a period of 10 min. Nerve fibers within the ischemic hippocampus were visualized using antibodies raised against 5HT and CCK. Pyramidal neurons in the dorsal CA1 region of the hippocampal formation had degenerated extensively 6-12 months after the ischemic episode. 5HT- and CCK-immunoreactive fibers were found to persist throughout the ischemic CA1 field in the absence of their post-synaptic targets. These observations suggest that 5HT and CCK fibers are resistant to ischemic injury. Moreover their persistence after ischemia suggests that they may be available to establish host-to-graft fiber connections with transplanted hippocampal pyramidal cells in attempts to reconstruct neural circuits that are damaged following ischemic injury.

500.7

CEREBROVASCULAR AND EVOKED POTENTIAL EFFECTS OF NEONATAL HYDROCEPHALUS. U.S. Vasthare*, J.P. McAllister, P.M. Hale* R.H. Rosenwasser* and J.S. Way. Depts. of Physiology, Anatomy and Neurosurgery, Temple University School of Medicine, Philadelphia, PA 19140.

This study was designed to assess the effects of infantile hydrocephalus and surgical decompression on cerebral blood flow (CBF) and somatosensory evoked response (SSER). Hydrocephalus was induced in 10 day old kittens by intracisternal injection of kaolin and verified with ultrasound; saline-injected or normal animals served as age-matched controls. Radiolabelled microspheres were used to measure CBF in severely hydrocephalic animals; standard SSER measurements were performed on control and hydrocephalic animals, as well as hydrocephalics that were decompressed with ventriculoperitoneal (VP) shunts at 6-13 days post-kaolin. Significant (p less than 0.05) decreases in CBF were detected in cortical areas 4 (61% below control), 22 (54%) and 17 (55%), as well as the thalamus (46%), midbrain/pons (54%) and caudate nucleus (64%); non-significant decreases occurred in the hippocampus (41%) and cerebellum (40%). Since mean CBF never fell below 23 ml/min/100g in any area, ischemia may not play a major role during hydrocephalus. Hydrocephalics exhibited pre-shunt increases in the SSER cortical peak latency, which was not reveresed immediately by VP shunting. Latency did return to normal by 7 days post-shunt. This early recovery suggests that axons are not structurally impaired during hydrocephalus.

500.8

NEURONAL DAMAGE IN THE SUBSTANTIA NIGRA RETICULATA IS DELAYED AFTER REPETITIVE FOREBRAIN ISCHEMIA. A. Shuaib, S. Ijaz,*J. Kalra,*W. Code, S. Mantha,*T. McConnell,* W. Howlett,*S. Lim.*

Neuronal damage after repetitive cerebral ischemia is more severe compared to a similar degree of exposure to a single ischemic insult. In the gerbil model of forebrain ischemia, in addition to damage in the striatum, cortex and the hippocampus, frequent damage is also seen in the medial geniculate nucleus, substantia nigra reticulata (SNr) and inferior colliculus, regions not supplied via the carotid arteries. We postulated that damage in the SNr was a result of loss of inhibitory input from the striatum and was therefore delayed in onset. Three, three minute transient forebrain ischemic insults were produced with one hour of reperfusion interval between episodes. Animals were sacrificed 2 days and 7 days after the insult and silver staining was used to assess neuronal damage. Beginning within two days of the insult, there was extensive damage in the hippocampus, striatum and thalamus. Damage in the SNr was apparent after 3 days and was maximum in the 7 day survival animals. This delay in the onset of damage in the SNr offers a window of 48 hours where proper intervention may prevent neuronal damage.

500.9

BLOOD FLOW THRESHOLD FOR ISCHEMIC DEPOLARIZATION OF RAT NEOCORTEX Y. Takeda*, M. Jacewicz and W. Pulsinelli. Dept. of Neurology and Neuroscience, Cornell University Medical College, New York, NY

To determine the ischemic blood flow threshold below which rat neocortex depolarizes, we implanted a DC potential electrode (DCPE) in the neocortex of halothane-anesthetized Spontaneously Hypertensive rats (n=27) and occluded their middle cerebral (MCA) and ipsilateral common carotid (CCA) arteries. The DCPE was inserted 800 μm below the cortical surface at 2 mm posterior and 3 mm lateral to bregma. This location represents a previously-determined transition zone between moderate and mild ischemia. To assure the second-to-second stability of CBF in the vicinity of the DCPE, a laser-Doppler flow probe was positioned within 1 mm of the DCPE. Approximately 10 minutes after MCA/CCA occlusion ^{14}C-iodoantipyrine (^{14}C-IAP) was injected intravenously and the animals were decapitated 5-6 seconds later. CBF was measured at the site of the DCPE using autoradiographies of frozen, dried coronal brain sections and image analysis (Quantimet 970). The DCPE site was located by injecting 0.15 μl of fluorescein (0.9 mg/ml) through the DCPE just prior to injecting ^{14}C-IAP. Statistical analysis of 27 rats (13 with depolarization, 14 without) using logistic regression and the jack-knife method indicated that 10% of rats depolarized at a CBF of 72.7 ± 5.3, 50% at 47.8 ± 1.5 and 90% at 22.9 ± 3.0 ml/100 gm/min. The results are consistent with our observations that the CBF in the border zone of focal cerebral infarction is approximately 45-50 ml/100g/min (Jacewicz et al, Soc. Neurosci. Abst., 16:1278, 1990). We conclude that the threshold for ischemic depolarization in the SHR and therefore the threshold for ischemic brain injury in this species lies at approximately 50 ml/100g/min.

500.10

ISCHEMIA IN GERBIL FOREBRAIN: POST-ISCHEMIC TREATMENT OF DI- CALCIPHOR PREVENTS CALCIUM-MEDIATED DEGRADATION OF SPECTRIN AND MAP2. **D. F. Matesic, T.M.Devlin*, A.Kalenak*, R. J. McKenzie* D.K.J.E.von Lubitz*, and R.C.S.Lin, Depts.of Physiol.& Biophys., and Biochem. Hahnemann University, Philadelphia, PA 19102**

To elucidate the mechanism of selective cell death in the ischemic forebrain, degradation of spectrin and MAP2, which is known to be mediated by the calcium-activated protease calpain I was studied using both immunohistochemical and immunoblotting techniques. Previously, we have shown that Di-Calciphor has calcium ionophoretic activity and that post-ischemic treatment reduces mortality. Therefore, the effects of this drug on the breakdown of spectrin, MAP2 and other structural proteins after ischemia were studied.

Immunoblot analyses of proteins from both dorsal hippocampus (DHP) and lateral striatum (LStr) in gerbils subjected to a 20 min. occlusion vs controls revealed 2 major spectrin degradation products (150-160 kD; Seubert, BR:492,1989) in day 1 and up to day 4 post-ischemia, with a corresponding decrease in spectrin (240 kD). These 2 bands were indetectable in control animals. Decrease of MAP2 on immunoblots in the DHP and LStr was observed 1 day and 4 days post-ischemia which correlated with the loss of immunohistochemical staining of MAP2. Levels of actin showed no major differences in control vs ischemic tissues, suggesting that breakdown of calpain I substrates preceeds breakdown of other structural proteins.

Degradation of both spectrin and MAP2 in DHP and LStr was prevented in animals treated with Di-Calciphor (10 mg/kg at 30 min. and 24 hrs after ischemia). These results provide evidence that Di-Calciphor may be a useful tool in studying calcium- mediated mechanisms of neuronal damage. **(supported by ONR grant N00014-89-J-1738 and Harvard Apparatus).**

500.11

CEREBRAL ISCHEMIA IN GERBILS: THERAPEUTIC EFFECTS OF DELAYED TREATMENT WITH CALCIPHOR (DIMER OF 16'16-DIMETHYL PROSTAGLANDIN B1). Dag K.J.E.von Lubitz*, T.M.Devlin*, A.Kalenak*, R.C.S.Lin, D.F. Matesic, R.J.McKenzie*.Depts.of Biochem.and Physiol. and Biophys., Hahnemann Univ. Sch. Med., Philadelphia, PA 19102

We demonstrated previously that immediately postischemic application of Di-Calciphor resulted in a pronounced improvement of survival in gerbils, and in enhanced quality of neurological recovery. We now describe the actions of Di-Calciphor on normal animals and the resuscitative effect of the drug following a delayed postischemic application.

Bilateral carotid occlusion (20 min) in female gerbils was followed by Di-Calciphor given I.P. at 5 or 10 mg/kg at 30, 60 or 180 min and 24 hrs postischemia (N=25/group). Ischemic controls (N=25) were injected with the vehicle. A group of nonischemic gerbils (N=25) was injected with 10 mg/kg Di-Calciphor. Rectal and epicranial temperature (RT,ET), mean arterial blood pressure (MABP), and cardiac rate (BPM) were noninvasively monitored in all animals. Epicranial platinum electrodes were used to record EEG. Postinjection/postischemic RT, RE, MABP, BPM and EEG were monitored at 60 min intervals for 6 hrs. Postischemic locomotor activity was monitored for 56 hrs and postischemic survival for 14 days.

Minimum postischemic life expectancy was 9 days in the animals treated at 5 mg/kg at 60 or 180 min postischemia, or 10 days at 10 mg/kg at 180 min, and 5 days in the controls ($p<0.05$). Postischemic recovery of the treated animals was characterized by EEG slow-down, reduction of the severity and frequency of seizures, and a reduction of the hyperactive period. Neuronal preservation was significantly improved in the hippocampus and striatum ($p<0.05$). Following injections of the drug, the postischemic/ postinjection RT, RE, MABP and BPM did not differ from the preischemic level.

Our experiments indicate that, although its mechanism of action is not fully known, Di-Calciphor is a compound of interest as a potential agent in treatment of cerebral ischemia. (supported by ONR grant N00014-89-J-1738, assistance of Harvard Apparatus is also acknowledged)

500.12

EFFECTS OF ACUTE VERSUS CHRONIC PRE-ISCHEMIC HYPERGLYCEMIA ON POSTISCHEMIC MORTALITY IN THE RAT. TX Gionet*, DS Warner, MM Todd. Department of Anesthesia, University of Iowa, Iowa City, IA 52242

Acute hyperglycemia (i.e., i.v. glucose infusion) prior to global ischemia often results in brain edema, seizures and death. To determine if chronic pre-ischemic hyperglycemia has similar effects on mortality, 3 groups of male Sprague-Dawley rats were subjected to 10 mins of bilateral carotid artery occlusion combined with systemic hypotension (MAP = 30±5 mmHg) and allowed 5 days recovery. The control group (n = 5) were healthy rats fasted from food for 12-18 hrs prior to ischemia. The acute hyperglycemic group (n = 7) were similarly fasted but also administered 1.5 ml of 25% glucose in saline, i.v., over 30 mins immediately prior to ischemia. The chronic hyperglycemia group were given 45 mg/kg streptozotocin subcutaneously 5-7 days prior to ischemia. These rats were fasted 6-8 hrs prior to ischemia and further studied if plasma glucose was between 300-400 mg/dL after surgical preparation (n = 9). Postischemic mortality rates were compared by Chi-square analysis. Blood gases, pericranial temp. and blood pressure were similar between groups. Plasma glucose values at onset of ischemia were 129±28, 379±66, and 358±33 mg/dL for the control, acute and chronic hyperglycemic groups respectively. There were no deaths in the control group, and one death in the chronic hyperglycemia group (11%), while 4 of 7 (57%) acute hyperglycemia rats died ($p<.03$). These results are consistent with human observations made by Woo et al. (Arch Neurol 47; 1990, 1174-7) and suggest a possible effect of duration of pre-ischemic hyperglycemia on relevance of this variable to postischemic outcome.

500.13

TRANSIENT FOREBRAIN ISCHEMIA IN THE CFW MOUSE.

R.N. Willette, R.K. Clark, E.V. Lee* and F.C. Barone, Division of Pharmacology, SmithKline Beecham Pharmaceuticals, King of Prussia, PA 19406.

The effects of bilateral common carotid artery occlusion on local cortical perfusion, EEG power and forebrain histopathology were examined in CFW mice. Under chloral hydrate anesthesia transient bilateral occlusion of the common carotid arteries elicited an immediate and sustained reduction in local cortical perfusion. The maximal reduction in perfusion (85±2%) was reached within 1 min and was maintained throughout the occlusion period (5 to 10 min). Similarly, the concomitant deterioration of the EEG power reached a minimum (-82±7%) within 1 min and remained depressed during ischemia. A histopathologic evaluation of the forebrain performed 7 days following 5 and 10 min periods of bilateral common carotid artery occlusion revealed significant neuronal damage in the cortex, dentate, and CA1 and CA3 fields of the hippocampus. The degree of damage in these areas were related to the duration of the ischemia. A carbon black study of the posterior communicating arteries in 16 mice indicated that 25% had patent arteries bilaterally, 37.5% had only one patent artery and 37.5% had no patent arteries. We conclude that the posterior cerebral circulation in the CFW mouse provides inadequate forebrain perfusion following bilateral common carotid artery occlusion. Hence, the CFW mouse may provide a useful model for the study of transient forebrain ischemia.

500.14

CALPAIN INHIBITION AND NEUROPROTECTION. L. Kent*, C. Zobre*, M.S. Miller, Neurosciences Dept., Sterling Drug, Rensselaer, NY 12144.

Excitatory amino acids (EAAs) released by neurons during ischemia may interact with **NMDA** receptors of nearby cells to induce Ca^{++} influx. Increased cytoplasmic Ca^{++} can activate calpain (**CAL**) which can hydrolyze cytoskeletal proteins, such as fodrin. Degradation of the cytoskeleton may contribute to ischemic and EAA-induced neuronal injury. This study determined if **NMDA**-stimulated **CAL** activation in cultured neurons precedes neuronal injury and whether inhibition of **CAL** attenuates injury. Appearance of a 155 kD fragment of fodrin in cells was a marker of **CAL** activity. Release of **LDH** was a measure of neuronal injury. Exposure of cultured mouse cortical neurons to 500 uM **NMDA** for 30 min induced **CAL** activity which was minimally elevated 30 min after **NMDA** exposure and maximally increased (10-20 fold) by 2 hrs. Maximal **LDH** release was observed 6-8 hr after **NMDA** exposure. Leupeptin (100 uM) inhibited both **CAL** activity and **LDH** release when measured 4-8 hr after **NMDA** exposure, but not at 24 hours. Thus, a temporal correlation existed between **CAL** inhibition and neuronal protection. Inhibition of **CAL** may reduce excitotoxic neuronal injury.

500.15

CALPAIN INHIBITORS AS UNIQUE THERAPEUTICS FOR ISCHEMIC BRAIN DAMAGE. [1]R.T. Bartus, [1]R.L. Dean, [1]D.D. Eveleth, [1]D.L. Lutz, [1]M.E. Harris*, [2]J. Powers. [1]Cortex Pharmaceuticals, Irvine, CA 92718, [2]Georgia Institute of Technology, Atlanta, GA 30332.

Abnormal elevations in intracellular calcium has been strongly implicated in the neurodegeneration that follows brain ischemia. Moreover, disassembly of the neurons' cytoskeleton has recently been shown to be an early event in their neuronal death, while activation of calcium-mediated proteases (calpains) appears to represent a final common pathway for the neurons' destruction.

For these reasons, inhibition of calpain provides a novel and potentially powerful means of reducing neuronal death due to ischemia and other neurotoxic perturbations.

Several novel inhibitors have been identified which are more potent, selective and efficacious than standard compounds. We evaluated the ability of these compounds to protect hippocampal neurons from transient global ischemic damage induced in the rat 4 vessel occlusion model. These studies demonstrated the superior efficacy of the novel, non-aldehyde inhibitors, providing more effective protection of pyramidal cells. They therefore provide the clearest evidence, to date, that despite the likely involvement of other events in the pathogenic cascade, inhibition of calpain is sufficient to protect a large number of vulnerable hippocampal neurons from the damaging effects of ischemia. Moreover, these data provide the first evidence that complete protection of certain hippocampal neuronal populations can be achieved, even when the inhibitors are administered well after the ischemic event is over.

500.16

NOVEL CALPAIN INHIBITORS OFFER GREATER HYPOXIC PROTECTION OF SYNAPTIC TRANSMISSION IN HIPPOCAMPAL SLICES. [1]H.T. Ton*, [2]A. Arai*, [1,2]D. Eveleth, [1,2]G.S. Lynch and [1,2]R.T. Bartus. [1]Cortex Pharmaceuticals, Irvine, CA 92718, [2]Univ. of California, Irvine, CA 92717.

Activation of the calcium dependent cysteine protease calpain has been implicated in long-term potentiation, excitatory amino acid-induced neurodegeneration and ischemic brain damage. This study was conducted to evaluate the efficacy and membrane permeability of four recently developed non-aldehyde calpain inhibitors (CX216, CX217, CX218 and CX233), versus the standard aldehyde inhibitors, Calpain Inhibitor 1 (CI1) and Leupeptin (LEU) in protecting synaptic transmission of hippocampal slices from hypoxic insult.

Hippocampal slices (400 um) were maintained in chambers perfused with artificial CSF and 95% O^2 : 5% CO^2. Hypoxic exposure involved substituting nitrogen for oxygen (i.e., 95% N^2 : 5% CO^2). Evoked field potentials were measured by stimulating the Schaeffer collaterals and recording from the apical dendritic region of CA1. Survival rate following 10 minutes hypoxia and recovery of synaptic transmission from hypoxia were measured.

While the standard aldehyde calpain inhibitors were only modestly effective in protecting hippocampal slices from hypoxia, all four novel non-aldehyde inhibitors were found to be more potent, efficacious and membrane permeant. For example, 100 micromolar of all four CX compounds, preincubated for either 30 minutes or 1 hour, produced a 40% to 80% survival rate, respectively. CX218 (at 100 uM) and 1 hour preincubation improved the recovery of synaptic transmission to 62% following hypoxia. In contrast, CI1 and LEU were ineffective under similar conditions. CI1 required 200 uM with a 2 hour preincubation period, while LEU required 1 mM with a 3 hour preincubation period to provide even modest protective effects. These results provide clear evidence that inhibition of calpain can reliably ameliorate functional damage in hippocampal slices induced by hypoxia. Moreover, recently developed non-aldehyde inhibitors are much more effective than the commercially available aldehyde inhibitors.

500.17

ADENOSINE AGONISTS AND NEUROPROTECTION; ISSUES RELATED TO BLOOD-BRAIN BARRIER PERMEANCE. [1]R.L. Dean, [1]D.L. Lutz, [1]M.E. Harris*, [1]J.E. Foreman*, [2]K.S. Lee, [3]K.A. Jacobson, [1]D.D. Eveleth and [1]R.T. Bartus. [1]Cortex Pharmaceuticals, Irvine, CA 92718. [2]Univ. of Virginia, Charlottesville, VA 22908, [3]National Institutes of Health, Bethesda, MD 20892.

Adenosine has been characterized as a natural modulator of glutamate neurotransmission and adenosine analogs have therefore been explored as possible neuroprotective agents for excitatory amino acid toxicity. However, attempts to develop effective peripherally administered adenosine agonists for neuroprotection have been confounded by two key issues. The first is that at efficacious doses, moderate side effects (i.e., hypothermia, sedation, hypotension) are seen. These side effects, alone or in combination with effects on synaptic transmission, can protect neurons from damage in several animal models of global ischemia. The second issue is whether adenosine analogs even penetrate the blood-brain barrier (BBB). It has been suggested that the neuroprotection attributed to adenosine agonists is due primarily to their peripheral side effects rather than a direct effect on central neurons.

In a series of experiments, we addressed a number of issues related to these points. We have shown that: 1) when non-BBB penetrating adenosine analogs are administered centrally (ICV), hypothermia and sedation are still observed, demonstrating the likelihood of both PNS and CNS mediated side effects; 2) brain levels of certain adenosine analogs can be detected following IP and IV administration using an HPLC method, demonstrating penetrance, albeit modest, through the BBB; and 3) direct infusions of A1 agonists into the CA1 region of the hippocampus following global ischemia (4 vessel occlusion procedure in rats) protected hippocampal neurons, demonstrating centrally mediated neuroprotective effects of adenosine analogs in the absence of confounding side effects.

500.18

INTRACELLULAR EVENTS RELATED TO THE DELAYED NEURONAL DEATH FOLLOWING TRANSIENT GLOBAL ISCHEMIA. D.L. Lutz, R.L. Dean, M.E. Harris*, J.A. Miotke, H.T. Ton*, B.K. Miyazaki*, D.D. Eveleth and R. T. Bartus. Cortex Pharmaceuticals, Irvine, CA 92718.

Delayed neuronal death has been reported in the gerbil two vessel occlusion model and in the rat 4 vessel occlusion model of global ischemia. To better understand this phenomena, the temporal damage of hippocampal neurons following transient global ischemia in the 4 vessel occlusion rat model was studied using biochemical, histological and electrophysiological markers.

Male Sprague-Dawley rats were sacrificed at various time periods following the occlusion (6 hours - 7 days). The brains were removed and 400 um sections of hippocampus were harvested for *in vitro* electrophysiological characterization of CA1 field potentials. A portion of the same hippocampus was immersion-fixed and stained with cresyl violet (Nissl stain) or silver (for degenerating neurons), while the remainder of fresh tissue was used in a Western assay to quantitate spectrin breakdown.

Ischemic-induced degeneration was first observed with the Nissl stain at 48 hours post-occlusion and peaked at 7 days. For silver stain, evidence of degeneration was first seen at 24 hours and peaked at 4 days. Breakdown of spectrin, a major substrate for the calcium stimulated protease, calpain, occurred very early in the neurotoxic cascade, preceding histological evidence of neuronal death by days. Electrophysiological changes also preceded the evidence of histological cell death. These observations suggest that a number of interdependent intracellular events participate in the delayed cell death phenomenon which follows transient global ischemia and add further support for the idea that calpain-mediated spectrin breakdown may play a causal role in this neurodegenerative cascade.

500.19

ELECTRON MICROSCOPIC STUDY OF THE CEREBRAL CORTEX AFTER TRANSIENT CEREBRAL ISCHEMIA IN GERBILS.
H. Tomimoto* and T. Yanagihara, Dept. of Neurology, Mayo Clinic, Rochester, MN 55905

Prompt dendritic damage and its central propagation have been observed in the periphery of dendrites in the gerbil hippocampus after transient cerebral ischemia. We examined the frontoparietal cortex of gerbils after bilateral carotid occlusion for 20 min and reperfusion for 72 hrs. During ischemia without reperfusion, progressive swelling of the periphery of ascending dendrites with swelling of mitochondria, disintegration of microtubules and formation of microvacuoles occurred in layer I and less notably in layers II and III. After reperfusion for 3 hrs, swelling of dendritic terminals and mitochondria receded but disintegration of microtubules and formation of microvacuoles propagated to deeper layers. Swelling of glial processes also became apparent in many layers after 3 hrs, causing indentation in dendrites. Neuronal perikarya showed disaggregation of polyribosomes and occasional fragmentation of rough endoplasmic reticulum after reperfusion for 3 hrs. Disintegration of mitochondrial cristae and formation of microvacuoles occurred after 6 hrs, particularly in layers III and Vb, and some neurons became clumped and electron-dense. While the number of these degenerated neurons increased progressively, surviving neurons with reaggregation of polyribosomes also increased beyond reperfusion for 12 hrs. The present study showed central propagation of dendritic damage and progressive structural disintegration of neuronal perikarya before "delayed neuronal death" became apparent with light microscopy.

ISCHEMIA: EXCITOTOXICITY

501.1

7-CHLOROKYNURENIC ACID REDUCES CA1 CELL LOSS IN A RAT MODEL OF CEREBRAL ISCHEMIA IN VIVO.
E.R.Wood, T.J.Bussey* & A.G.Phillips. Dept. Psychology, Univ. British Columbia, Vancouver, B.C., Canada, V6T 1Z4.

Transient cerebral ischemia results in loss of selectively vulnerable neurons, including hippocampal CA1 pyramidal neurons. A prominent hypothesis is that ischemic damage is mediated in part by the action of glutamate at NMDA receptors which causes pathological Ca^{++} influx, and cell death by subsequent Ca^{++}-mediated processes. However, although extracellular glutamate levels increase during ischemia, there is a close correlation between the areas of the brain which suffer cell loss and those in which glycine (and not glutamate) is elevated during ischemia and reperfusion. Binding of glycine at the strychnine-insensitive glycine binding site associated with the NMDA receptor-channel complex is required in combination with glutamate binding for channel opening. Prevention of glycine binding might therefore be expected to attenuate the cell loss associated with ischemia.

Preliminary results show that bilateral intraventricular administration of 2ul 500uM 7-chlorokynurenic acid--a potent competitive antagonist at the strychnine-insensitive glycine binding site--immediately before the induction of ischemia significantly reduces cell loss in CA1. Rats were subjected either to ischemia induced by 20 minutes bilateral carotid occlusion combined with hemorrhagic hypotension, or to sham surgery. Half of the rats in each group received 7-chlorokynurenic acid immediately before carotid clamping while the other half received the same volume of saline. 6 weeks following surgery there were significantly fewer viable CA1 pyramidal neurons in the ischemia with saline group than in all other groups. The ischemia with drug group did not differ from either of the sham surgery groups. These data suggest that elevated levels of glycine contribute to ischemic damage, and that antagonism of the glycine site associated with the NMDA receptor provides one means of attenuating cell loss.

501.2

NECESSITY OF CONTINUED INFUSION FOR NEUROPROTECTIVE EFFECT OF NMDA ANTAGONISTS IN CEREBRAL ISCHEMIA

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While both competitive and noncompetitive NMDA antagonists have been shown to protect against focal cerebral ischemia, it is not clear whether a single dose or continued maintenance infusion of these drugs is necessary to achieve neuroprotection. We studied this issue using dextromethorphan (DM), dextrorphan (DX), and MK801 in a rabbit model of transient focal ischemia. Thirty rabbits underwent two hour occlusion of the left internal carotid, anterior cerebral, and middle cerebral arteries followed by four hours of reperfusion. Ten minutes after occlusion, the rabbits were treated with DM 20 mg/kg or 20 mg/kg followed by 10 mg/kg/h; DX 16.7 mg/kg or 16.7 mg/kg followed by 8.35 mg/kg/h; MK801 1 mg/kg or 1 mg/kg followed by 0.75 mg/kg/h or normal saline. The loading dose was given over 30 minutes. Ischemic injury was assessed using somatosensory evoked potentials, magnetic resonance imaging and histopathology. For DM and DX, maintenance infusions were necessary to achieve neuroprotection, whereas a loading dose only was protective for MK801. Maintenance infusion of MK801 tended to worsen ischemic injury, possibly by lowering blood pressure. The need for continued dosing of NMDA antagonists may depend on their serum and brain half lives.

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501.3

POSTISCHEMIC ADMINISTRATION OF AMPA- BUT NOT NMDA-RECEPTOR BLOCKERS DECREASES NEURONAL DAMAGE FOLLOWING TRANSIENT CEREBRAL ISCHEMIA IN THE RAT.
T. Wieloch* and B. Nellgård Laboratory for Experimental Brain Research, Lund University, S-221 85 Lund, Sweden.

Glutamatergic transmission is an important factor in the development of neuronal death following transient cerebral ischemia. The effect of NMDA and non-NMDA receptor antagonists on neuronal damage were studied in rats exposed to 10 min of transient cerebral ischemia induced by bilateral common carotid occlusion combined with hypotension. Blockade of the AMPA-receptor by 2.3-dihydroxy-6-nitro-7-sulmamoyl-benzo (F) quinoxaline (NBQX), given post-ischemia as an i.p. bolus dose of 30 $mg \cdot kg^{-1}$ followed by an i.v. infusion of 75 $\mu g \cdot min^{-1}$ for 6 h, decreased neuronal damage in the hippocampal CA1 area by 44-69%, with the largest relative decrease in the temporal part of hippocampus. Neuronal damage in neocortex also decreased. Blockade of NMDA-receptors by either the non-competitive receptor antagonist dizocilipine given 1 $mg \cdot kg^{-1}$ i.p. at recirculation and 3 hours post-ischemia, or the competitive receptor antagonist DL-(E)-2-amino-4-methyl-5-phosphono-3-pentenoic acid (CGP 40116), 5 $mg \cdot kg^{-1}$, given i.p. at recirculation. did not provide any protection.

Our data demonstrate that AMPA- but not NMDA-receptor antagonists decrease neuronal damage following transient severe cerebral ischemia in the rat, and that the protection by NBQX may be dependent on the severity of the ischemic insult.

501.4

EXTRACELLULAR pH AND K^+ GRADIENTS IN FOCAL ISCHEMIA OF RAT CORTEX.
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Decreases in extracellular pH (pHo) below 6.6 abolish NMDA-gated currents (1). Thus, in ischemia, the behavior of pHo may determine the extent of excitotoxic injury. We studied pHo in rat cortex following middle cerebral artery (MCA) occlusion. Non-fasted, female, Long Evans-Hooded rats were anesthetized with pentobarbital, paralyzed and ventilated on O2-supplemented air. pH microelectrodes were inserted through a 300 μm hole in a silicone sheet, covering a 2 mm parietal craniotomy. Control pHo was 7.31 ± 0.02 with no cortical gradient. Mean arterial pH, pCO2 and O2 (torr) were 7.43/39/130 (pre-occl.) and 7.40/33/82 (post-occl.) respectively. Within 15 min. of MCA occl., minimum pHo (6.62 ± 0.05, n=5) consistently occured at cortical depths of 1000-1500 μm. At 250 μm, mean pHo was 6.87 ± 0.09, which decreased by 0.20 ± 0.05 pH units/mm depth. Below the point of maximum acidity, pHo increased by 0.47 ± 0.13 pH units/mm, with mean pHo of 6.94 ± 0.19 at 2000 μm. Decreases and increases in pHo were accompanied by neg. and pos. shifts in DC potential respectively. Preliminary measurements of $[K^+]o$ revealed similar U-shaped gradients, with troughs at upper and lower depths.

These data indicate that in non-fasted rats, MCA occl. can cause a pHo fall sufficient to protect against NMDA receptor-mediated injury. Vulnerability of superficial neurons may be related to higher pHo. Proximity of CSF, preservation of blood flow and CO2 egress might account for these gradients, however, their cause remains to be determined. (1) Tang, C.M. *et al.* (1990). *PNAS*, 87(16):6445. Supported by NINDS Grants NS10164 and NS27011

501.5

PROTECTION FROM OXYGEN-GLUCOSE DEPRIVATION INJURY IN CORTICAL CULTURES BY GLUTAMATE ANTAGONISTS COMBINED WITH EXTRACELLULAR ACIDITY. D.A. Kaku, R.G. Giffard and D.W. Choi. Dept. of Neurology, Stanford Univ. Med. Sch., Stanford, CA 94305.

NMDA antagonists reduce neuronal vulnerability to hypoxic neuronal injury both in vivo and in vitro. However, prior in vitro studies have taken place at normal pH, whereas hypoxia-ischemia in vivo is associated with extracellular acidity, a perturbation that attenuates NMDA receptor activation, glutamate neurotoxicity, and hypoxic injury in vitro. We investigated whether acid pH would abolish the value of glutamate antagonists against a combined oxygen- and glucose-deprivation insult in murine cortical cell cultures.

Near-complete protection from insults lasting up to 60 min was produced by dropping extracellular pH to 6.4. The neuronal death produced by longer insults (80-120 min) was still reduced by the addition of the NMDA antagonists MK-801 or CGS-19755. Even further injury reduction was produced by addition of CNQX to saturating concentrations of MK-801, consistent with earlier reports of synergy at normal pH. Thus whereas brief exposure to extracellular acidity was neuroprotective, additional protective effects were produced by glutamate antagonists. When both NMDA and AMPA/kainate receptors were maximally blocked, dropping pH to 6.4 produced additional injury reduction. Extracellular acidity may have neuroprotective effects in hypoxic neuronal injury independent of glutamate neurotoxicity.

501.6

PROTECTION FROM EXCITOTOXIC BRAIN DAMAGE BY DRUG-INDUCED METABOLIC SLOWING AFTER STROKE. B.E. Morton and D.T.Shirae, Dept. Biochem-Biophys, Univ. Hawaii Sch. of Med., Honolulu, 96822.

In Mongolian gerbils, ten minutes of bilateral carotid artery clamping, followed by reperfusion, causes forebrain ischemia, glutamate release, and permanent injury to NMDA receptor-bearing brain areas after a 24 hour window of vulnerability. During this time, use of NMDA receptor antagonists, or blockers of the NMDA-regulated calcium channel, such as MK-801 which binds to PCP receptors within these channels, markedly reduces extent of brain damage present one week later.

Based upon the idea that sigma opiate ligands might also block the calcium channel, we tested the sigma ligand, progesterone, whose level increases greatly by birth, a time fraught with threat of anoxia, for its ability to reduce brain damage due to ischemic insult. We also tested another sigma opiate ligand, haloperidol. Both were injected, i.p., one hour after the above ischemic insult.

In gerbils injected with high concentrations of either progesterone (30 mg/kg) or haloperidol (30 mg/kg), there was almost complete absence of necrotic brain damage in the hippocampus and caudate, compared to saline injected controls one week after carotid artery clamping. Each of these animals had shown marked sedation from its drug, suggesting that their protection from the excitotoxic injury of anoxic insult was not directly due to sigma opiate receptor binding. Animals readily recovered from haloperidol, but not progesterone treatments.

As a test of an alternate hypothesis, that metabolic inhibition of the brain by pharmacologic means, could, like hypothermia, reduce ischemic brain damage, Δ^9-tetrahydrocannabinol was administered, 5 mg/kg, i.v., one hour after ischemic insult. This dose of THC, which had been reported to greatly reduce global brain metabolism in rats, potently prevented any visible brain damage from 10 minutes of bilateral carotid artery clamping when assessed one week later. Thus, certain of these and other compounds which reduce global brain activity appear promising for use in minimizing excitotoxic injury due to anoxic brain insult.

501.7

COMBINED TREATMENT WITH MK-801 AND A D1/D2 DOPAMINE ANTAGONIST REDUCES CEREBRAL ISCHEMIC DAMAGE. S. M Nurse*, D. Corbett and S.J. Evans*. Basic Medical Sciences, Fac. of Medicine, Memorial Univ., St. John's, NF, CANADA, A1B 3V6.

During ischemia extracellular levels of both dopamine(DA) and glutamate rise substantially, an event which may contribute to ischemic injury. This study examined the therapeutic efficacy of DA antagonists alone and in combination with MK-801.

Global ischemia was induced in gerbils by 5 min carotid artery occlusion, at 38°C. MK-801, 5 mg/kg, was given 1 hr prior to surgery; cis-flupenthixol, 0.4 and 1.2 mg/kg, 3 hr prior to surgery; and SCH-23390, 1 mg/kg, 0.5 hr prior to surgery. MK-801 but not. cis-flupenthixol reduced CA1 cell loss. Together these drugs were slightly more effective than MK-801 alone.SCH-23390 was ineffective.

Combined drug therapy with D_2 DA and NMDA antagonists may be a useful treatment strategy in cerebral ischemia.

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501.8

DELAYED TREATMENT WITH NBQX ATTENUATES NEOCORTICAL INFARCTION. D. Xue*, Z.G. Huang*, K.E. Smith*, H. Lesiuk and A.M. Buchan. Neuroscience Unit, Ottawa Civic Hospital, Ottawa, Canada, K1Y 4E9.

NBQX, a non-NMDA glutamate receptor antagonist protects against neuronal injury following forebrain ischemia. This study was done to determine whether NBQX attenuated neocortical infarction in a well characterized rodent model of transient MCA occlusion.

METHODS: 19 male SHR rats were subjected to 2 hours of reversible ischemia by permanent right common carotid artery and transient right middle cerebral artery occlusion, followed by reperfusion. 90 minutes following the onset of ischemia. Saline or NBQX (30 mg/kg) was administered i.p. Subsequent doses were given at the time of reperfusion (RP)(120 mins) and 30 minutes following RP. Animals were kept normothermic throughout ischemia and RP, and sacrificed at 24 hours. A priori power analysis predicted 7 animals per group would be needed to detect a 30% infarct reduction (α=0.05, β=0.2). Cortical infarct volume was calculated and expressed in mm^3. A Student-T test was used.

RESULTS:

Group	Mean volume of cortical infarction ± S.D.
Saline (n=10)	181 ± 31
NBQX (n=9)	125 ± 19*

* p<0.001

CONCLUSION: Treatment with MBQX reduces infarct size by 33% even when administered 90 minutes after the onset of ischemia.

501.9

ISCHAEMIA INDUCED RELEASE OF GLUTAMATE, ASPARTATE AND GABA FROM RAT RETINA IN VITRO: POSSIBLE INVOLVEMENT OF FREE-RADICALS. M.J. Neal and J.R. Cunningham.* Dept Pharmacology, St Thomas's Hospital, London, SE1 7EH, UK

We have examined the effects of "ischaemia" on the release of amino acids from the rat retina *in vitro*. Isolated retinas (dark-adapted or exposed to room light) were incubated at 32°C in Krebs bicarbonate medium gassed with 95% O_2/5% CO_2 (controls) or in glucose-free medium gassed with 95%N_2/5% CO_2 (ischaemia). Retinas were incubated for 10-90 min and the amino acids in the medium measured by HPLC.

When control retinas were incubated in the light for periods up to 90 min there was a steady release of glutamate (GLU) aspartate (ASP) and GABA (1.5±0.2; 0.19±0.026; 2.1±0.15 nmol/retina/30 min respectively, mean ±SEM n=5). Release was similar from control dark-adapted retinas. The release of GLU, ASP and GABA from retinas incubated in the light under ischaemic conditions for 90 min was increased 14.0, 19.1 and 9.5 fold respectively. The release of GLU and especially ASP from dark-adapted retinas incubated under ischaemic conditions was significantly higher than that from retinas in the light.

Remarkably, the ischaemia-induced release of GLU, ASP and GABA from retinas incubated in the light was not Ca-dependent, whilst the release from dark-adapted retinas was reduced by approximately 60% in high-Mg/low-Ca medium. The NMDA antagonist MK801 totally prevented the ischaemia-induced release of GLU and ASP at 30 min and reduced the increase in GABA release by about 75%. This inhibitory effect on release was less striking after 60 min of ischaemia and not significant after 90 min.

When control retinas were incubated for 30 min in medium containing xanthine and xanthine oxidase, the release of GLU, ASP and GABA was increased to levels similar to those caused by ischaemia, suggesting that free-radical formation might be involved in the ischaemia-induced release of amino acids.

501.10

THE EXCITOTOXICITY OF QUINOLINIC ACID IN HYPOXIC RAT HIPPOCAMPAL SLICES. A. Schurr, C.A. West* and B.M. Rigor, Dept. of Anesthesiology, Univ. of Louisville Sch. of Med., Louisville, KY 40292.

Quinolinic acid (2,3-pyridine dicarboxylic acid, QA), an NMDA receptor agonist, has been shown to induce excitatory, neurotoxic, and convulsant effects in both mice and rats. It is the most potent endogenous excitotoxin known to date.

The aims of this study were to explore the structural essentials of QA agonistic properties and to define certain conditions that potentiate its excitotoxicity. Orthodromically evoked CA1 population spikes were recorded from rat hippocampal slices. QA's ability to enhance hypoxic neuronal damage (lack of recovery of evoked response after 30-min reoxygenation) was compared with that of different QA derivatives. In addition, the effect of changes in the concentration of several ions in the perfusion medium on the excitotoxicity of QA was tested.

QA enhanced hypoxic neuronal damage in dose-dependent fashion, an effect that was blocked by the NMDA antagonist APV. None of the five other pyridine dicarboxylic acids tested could enhance hypoxic damage. Doubling of $[Mg^{2+}]$ or depletion of Ca^{2+} from the perfusion medium blocked the effect of QA.

QA is a weaker agonist than NMDA in enhancing hypoxic damage despite the structural resemblance between the two. Other structurally related compounds were innocuous. Both Ca^{2+} and Mg^{2+} play a role in QA excitotoxicity.

501.11

CYSTEIC ACID ENHANCES HYPOXIC AND HYPOGLYCEMIC NEURONAL DAMAGE IN VITRO. C.A. West*, A. Schurr and B.M. Rigor, Department of Anesthesiology, University of Louisville School of Medicine, Louisville, KY 40292.

L-Cysteic acid (CYS) is classified as a relatively weak analogue of NMDA in terms of its excitatory potency.

We have selected the rat hippocampal slice preparation and its electrophysiology as the in vitro system most suitable for studies on the involvement of excitotoxins in the mechanism of hypoxic and hypoglycemic neuronal damage. The combined effects of CYS and compromised energy metabolism on the degree of neuronal damage is the focus of this investigation.

Under normoxic and normoglycemic conditions (95% O_2, 10 mM glucose) CYS was harmless at 1 mM. However, this concentration of the agonist reduced the recovery rate of neuronal function (evoked, CA1 orthodromic population spike) after either 10-min hypoxia (95% N_2) and 30-min reoxygenation or 75-min hypoglycemia (0 mM glucose) and 30 min glucose repletion in a dose-dependent fashion. At 0.2 mM, the NMDA antagonist APV did not block the effect of CYS on hypoxic slices.

The present results indicate that CYS is as potent as glutamic and aspartic acid in enhancing either hypoxic or hypoglycemic neuronal damage but 10- to 100-fold weaker than quinolinic acid or NMDA, respectively.

501.12

PROTECTIVE EFFECT OF CCK-8 AND CERULETIDE AGAINST GLUTAMATE AND ISCHEMIA-INDUCED NEURONAL DAMAGE. G. Katsuura, S. Shinohara*, M. Eigyo*, H. Shintaku*, T. Shiomi* and A. Matsushita*. Shionogi Res. Labs., Osaka 553, Japan

Glutamate is now recognized to play a pivotal role in learning/memory processes and neuronal damage. Recently, there is increasing evidence suggesting the interaction between glutamate and neuropeptides. In the present study we examined the effect of CCK-8 and a potent analogue, ceruletide(CLT), on glutamate-induced neuronal death in neuron cultures and neuronal damage in the hippocampus of ischemic gerbils in vivo. By means of LDH efflux, CCK-8 ($>10^{-9}$M) and CLT ($>10^{-10}$M) markedly prevented glutamate (5×10^{-4}M)-induced neuronal cell death in neuron cultures. Gastrin-I ($>10^{-9}$M) showed the same effect. The response of CLT was completely blocked by an antagonist for CCK-B receptors, (+)L-365,260. These peptides significantly suppressed the increase in cytosolic free Ca^{2+} levels induced by glutamate. In ischemic gerbils, CLT(30 and 100μg/kg: sc) significantly attenuated the ischemia-induced neuronal damage in the hippocampus and amnesia, when injected 30 min before and immediately after ischemia. These findings clearly indicate that these peptides may be useful in the therapeutic treatment of neuronal deficits caused by cerebral ischemia.

501.13

BASIC FIBROBLAST GROWTH FACTOR (bFGF) PROTECTS AGAINST ISCHEMIC NEURONAL DEATH IN VIVO. D.J. Berlove, C.G. Caday*, M.A. Moskowitz and S.P. Finklestein. Massachusetts General Hospital, Boston, MA 02114.

Basic fibroblast growth factor (bFGF) promotes neuronal survival and blocks excitatory amino acid (EAA) toxicity in CNS neurons in vitro. EAA toxicity is thought to play a major role in delayed neuronal death after cerebral ischemia in vivo. We tested the neuroprotective effects of bFGF after temporary (3 min.) global forebrain ischemia in the Mongolian gerbil. Core temperature was kept constant during and after surgery. Animals were untreated, or received vehicle (artificial CSF) alone, or vehicle plus bFGF (1.2 ugm/day) through continuous infusion via osmotic pump into the right lateral ventricle for 3 days before and 7 days after ischemia. The degree of neuronal damage in the CA-1 field of hippocampus was scored on a 0-3 scale (0=no damage, 3=severe damage).

	Right Hippocampus	Left Hippocampus
Untreated (N=6)	3.0 ± 0.0	3.0 ± 0.0
Vehicle-treated (N=4)	3.0 ± 0.0	2.5 ± 1.0
bFGF-treated (N=6)	0.8 ± 1.2*	1.2 ± 1.3*

(Data are means ± SD; * = values differ from untreated by $p<0.01$) These data suggest that pretreatment with bFGF protects against ischemic cell death in vivo. Whether this represents a direct neuronal effect or is mediated through other cellular processes (e.g., glial activation, or angiogenesis) requires further study.

501.14

PHENYTOIN ATTENUATES HYPOXIA-INDUCED CHANGES IN GLUTAMATE AND ACETYLCHOLINE RELEASE FROM RAT HIPPOCAMPAL SLICES P.E. Potter[1], P. Detwiler[2]* and J.R. Moskal[3] Depts. Anesthesiology[1] and Neurosurgery[2], Albert Einstein College of Medicine, Montefiore Medical Center, Bronx, NY 10467, and Chicago Inst. Neuroscience and Neuroresearch[3], Chicago, IL

The effects of the anticonvulsant drug diphenylhydantoin (phenytoin) on hypoxia-induced changes in release of ^{3}H-glutamate and ^{3}H-acetylcholine were studied in superfused rat hippocampal slices. Hypoxia (25 min perfusion with Krebs buffer gassed with 95% O_2:5% CO_2) caused a large release of glutamate which lasted for 15-20 minutes. Re-oxygenation caused a second large release of glutamate, which persisted for at least 20 minutes. Both the hypoxia and re-oxygenation induced release of glutamate were decreased significantly by adding 20 μM phenytoin before or after hypoxia. The evoked (electrical stimulation, 4Hz, 2 min) release of ^{3}H-acetylcholine was halved by hypoxia; this decrease was reversed by 20 μM phenytoin. These results indicate that phenytoin reduces hypoxia-induced release of the excitatory amino acid glutamate, while allowing cholinergic neurons to remain functional. Thus, phenytoin may protect neurons from stroke-induced toxicity and may also reduce re-perfusion injury and delayed neuronal damage. Supported by the Health Foundation.

501.15

THE EFFECTS OF DIFFERING LEVELS OF HYPOGLYCEMIA ON HYPOXIC NEURONAL INJURY OF CEROBROCORTICAL CELL CULTURES. G.J. Birrell*, A.W. Probert, and F.W. Marcoux. Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, MI 48105.

Calcium accumulation and neuronal injury of rat cortical cell cultures in vitro were examined following temporal exposure to hypoxia under conditions of normal and low glucose. Two-hour hypoxic (89% N_2/10% CO_2/1% O_2) exposures were performed on mixed glial cultures in a test medium of HBSS containing 4.0, 0.3, 0.1 and 0 g/l (22.2 - 0 mM) D-glucose. Normoxic (70% N_2/10% CO_2/ 20% O_2) controls were incubated at the same temperature, humidity and pH (7.2-7.6).

Measurements of $^{45}Ca^{2+}$ uptake and lactate dehydrogenase (LDH) release were measured immediately following and at 12 and 24 hours after hypoxic exposure. Normoxic cultures or hypoxic cultures exposed to 4g/l D-glucose displayed only small increases in Ca^{2+} uptake and LDH release with time. In hypoxic cultures exposure to low glucose (0.3 or 0.1 g/l) resulted in a delayed (12 hours) increase in Ca^{2+} accumulation associated with a rise in LDH release at 24 hours. Although those cultures experiencing hypoxia combined with zero glucose displayed both immediate (2 hours) and delayed increases in Ca^{2+} accumulation, little associated rise in LDH release at 24 hours was observed.

These results suggest that while low glucose potentiated hypoxic neuronal damage, severe hypoglycemia was surprisingly neuroprotective under the conditions of the present study.

501.16

HIPPOCAMPAL GLUTAMATE RELEASE DURING 'IN VITRO ISCHEMIA' IS CALCIUM-INDEPENDENT AND TTX-SENSITIVE. S.P. Burke and C.P. Taylor. Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, Ann Arbor, MI 48105.

Excess neurotransmitter release may cause neurodegeneration following CNS ischemia. The mechanism(s) of such release may differ from classical exocytosis. We examined overflow of amino acids from slices of rat hippocampal CA1 area (Nadler et al., 1990) during superfusion with media lacking glucose and gassed with nitrogen ('in vitro ischemia', IVI). Slices were held at 37°C and fractions of superfusate were frozen for analysis by HPLC.

Within 10', IVI evoked a large overflow of glutamate, as well as aspartate and GABA, which was maximal at 20' and returned to baseline 5' after the reintroduction of normal media. Taurine levels increased more gradually but remained elevated after IVI. Glutamine steadily decreased during IVI. Increases in amino acid levels were comparable to those evoked by 50mM KCl. Substitution of calcium by magnesium had little effect upon IVI release, but virtually eliminated KCl evoked release. Reduction of the bath temperature to 31°C reduced IVI release by half.

TTX (1μM) completely blocked IVI release for the first 15'. With continued TTX, overflow eventually rose to control IVI levels. Our results agree with in vivo studies (Yamasaki et al., 1991) showing focal TTX in rat hippocampus to mitigate neuronal damage from cerebral ischemia. The calcium-independence and TTX-sensitivity of glutamate release from IVI suggest that it may be driven by a reversal of plasma membrane transporters subsequent to cellular sodium loading.

501.17

THE EFFECT OF NMDA ANTAGONISTS ON EARLY ULTRASTRUCTURAL CHANGES IN NEURONAL CULTURE AFTER HYPOXIA. M.L. Weber, F.W. Marcoux, A.W. Probert and M.A. Dominick, Departments of Pharmacology and Pathology and Experimental Toxicology, Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, Ann Arbor, MI, 48105.

Mixed cortical neuronal-glial cultures isolated from fetal rat brain were exposed to hypoxia for 5 hours. Some plates were treated with the competitive NMDA antagonist 4-(3-phosphonopropyl)-2-piperazinecarboxylic acid (CPP) prior to hypoxic exposure. Cultures were fixed with 1% glutaraldehyde immediately after hypoxia and then processed for transmission electron microscopy. Early ultrastructural changes were evident in neurons immediately after hypoxia at a time when only slight neuronal swelling was seen at the light microscopic level. Glial cells showed no apparent damage at 5 hours. Swollen mitochondria with disrupted cristae and vacuolization of the neuronal cytoplasm were seen at 5 hours. Control cultures subjected to 5 hours normoxia showed none of these changes. These changes paralleled early changes reported in vivo shortly after ischemia. Neurons treated with CPP showed no swelling grossly and ultrastructurally were identical to normoxic neurons. These results demonstrate NMDA antagonists ability to prevent the early intracellular changes in neurons consequent to hypoxia.

501.18

ISCHEMIC RAT SPINAL CORD INJURY INDUCED BY DYNORPHIN A INVOLVES ELEVATIONS IN EXTRACELLULAR EXCITATORY AMINO ACIDS. M.A. Oleshansky and J.B. Long. Dept. of Med. Neurosci., Walter Reed Army Inst. Res., Wash. D.C. 20307.

Lumbar subarachnoid injection of dynorphin A (DYN) causes dose-dependent ischemia, neuronal degeneration, and persistent hindlimb paralysis in rats. Excitatory amino acids (EAAs) have been implicated as mediators of DYN-induced spinal cord injury in this model due to the protective effects of selective antagonists for the NMDA receptor complex, as well as 2-chloroadenosine (2-CA), an inhibitor of glutamate release. To further characterize the involvement of EAAs in DYN-induced rat spinal cord injury, extracellular amino acid levels were measured in spinal cord CSF samples removed after injection of paralytic doses (20 and 40 nmoles) of DYN. DYN was injected through 30 ga needles inserted between the L4 and L5 vertebrae into the spinal subarachnoid space of rats transiently anesthetized with halothane. CSF was collected through similarly placed needles at 15 and 45 minutes postinjection. Amino acids were separated by reverse-phase HPLC and quantitatively detected by fluorometry after OPA derivatization. By 15 minutes postinjection, DYN selectively elicited 5-6 fold elevations in the EAAs glutamate and aspartate that were substantially reduced by 45 minutes postinjection. Coinjection of protective doses of 2-CA failed to alter the DYN-induced increases in the EAAs. These results indicate that DYN-induced ischemic spinal cord injury is associated with an endogenous release of glutamate and aspartate at potentially neurotoxic levels which does not appear to be altered by 2-CA.

501.19

IN VIVO ACTIVATION OF NMDA RECEPTORS DURING AND AFTER ISCHEMIA MEASURED BY *IN VIVO* ^{3}H-MK-801 BINDING. P.H. Schwartz and C.G. Wasterlain. Epil. Res. Lab., VAMC, Sepulveda, CA 91343.

Several lines of evidence implicate activation of NMDA receptors as a mechanism of ischemic neuronal necrosis and of delayed neuronal death. While it has been shown that post-ischemic antagonism of NMDA receptors confers neuroprotection, *in vivo* microdialysis experiments indicate that extracellular levels of glutamate return to normal after the end of ischemia. In this study, 15 min after a hypoxic-ischemic insult (bilateral coratid ligation and exposure to 6% O_2 for 1hr) in the 7-day-old rat, pups were injected s.c. with [3-^{3}H]-MK-801, with or without a prior injection of cold MK-801, and were sacrificed 10, 30, or 60 min later. The brains were rapidly removed and the cortices were frozen. Binding was determined by the filtration technique. Three additional groups were examined: untreated controls and pups decapitated 10 min after ^{3}H-MK-801 injection with the heads kept at either 37 or 0°C for up to 60 min before dissection. Both total brain concentrations and non-specific binding were linearly related to dose. In control pups specific ^{3}H-MK-801 binding was 0.251 ± 0.044 pmol/g wet weight after 10 min and rose to 0.395 ± 0.042 pmol/g after 60 min. Decapitation ischemia at 37°C induced a rapid increase in binding to 1.685 ± 0.092 pmol/g by 30 min ($p<0.001$ vs controls) while binding during decapitation ischemia at 0°C was similar to that of controls. Binding in the post-hypoxic/ischemic brains was 1.737 ± 0.060 pmol/g after 30 min ($p<0.001$ vs controls). These results indicate that ischemia induces a dramatic activation of NMDA receptors and that this activation persists in the post-ischemic state. This may account for the efficacy of post-ischemic neuroprotection by NMDA antagonists and for delayed neuronal necrosis.

501.20

EFFECT OF QUISQUALATE AND HYPOXIA ON NEURONAL CELL INJURY. J. A. Kelleher and P. H. Chan University of California CNS Injury and Edema Research Center, Dept. of Neurology, M-794, Box 0114 San Francisco, California 94143 U.S.A.

Excitatory amino acid receptors are coupled to both ionic channels and signal transducing mechanisms. Excessive presynaptic release of glutamate (Glu), coupled with inhibition of reuptake in astrocytes, following ischemia is implicated in neurotoxicity. The non-NMDA receptor quisqualate/AMPA (Quis) is coupled to both Na channels and phosphatidylinositide (PPI) turnover. We have studied the response of the Quis receptor on hypoxic and normoxic neuronal cultures. At 7-8 days in vitro the production of inositol phosphate induced by Quis treatment is only observed with hypoxia. The PPI turnover is correlated with cell swelling (^{14}C-urea space) and cell death (fluorescein diacetate staining). Hypoxia followed by Quis (250uM) caused cell death at two hours post-treatment, which was not observed with hypoxia or Quis (normoxia) alone. At similar post-treatment time periods, a reduction in cell volume was observed for hypoxia/Quis neurons compared to hypoxic treatment alone. Increased inositol phosphate production, cell death and decreased swelling at this later time period due to Quis plus hypoxic treatment could be reversed by preincubating the cells with the AMPA-insensitive, quisqualate antagonist d,l-2-amino-3-phosphonopropionate (AP3). Immediately following the Quis exposure (zero time), hypoxic cells exhibited greater cell volume than normal cells. This swelling could be inhibited by addition of either the non-NMDA receptor antagonist DNQX or AP3. Our data suggest swelling and injury due to Quis and hypoxia in combination is associated with the inositol phosphate production.
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501.21

SYNAPTIC STIMULATION EXACERBATES HYPOXIC INJURY IN PENUMBRAL NEURONS. P.R. Trueblood, R.A. Wallis, K.L. Panizzon and C.G. Wasterlain . Sepulveda VAMC, Depts. of Neurology and Kinesiology, UCLA Sch. of Medicine, and Sepulveda, CA 91355.

Early physical therapy is recommended in cerebral ischemia, yet the effect of the resulting synaptic firing on survival of neurons in the ischemic penumbra has never been studied. We examined the effects of synaptic stimulation on hypoxic CA1 neurons unable to generate an evoked potential upon Schaffer collateral stimulation. After hypoxic disappearance of evoked responses, paired slices were stimulated at 2 or 20 Hz (for an average duration of 2.2 min. ± 0.3 SE) until the appearance of the hypoxic injury potential (HIP) in the 2 Hz slice. With the disappearance of the HIP in the 2 Hz slice, oxygenation was restored. 20 Hz slices showed significantly reduced recovery of mean population spike (PS) amplitude (9% ± 5.3) compared to 2 Hz slices which received less stimulation during hypoxia (64% ± 14.8). In a similar paradigm, slices receiving orthodromic stimulation showed a significant decrease in the mean percent CA1 PS amplitude recovery (11% ± 3.4) compared to slices receiving antidromic stimulation (83% ± 17.0), presumably because the latter releases less neurotransmitter. In low calcium medium, increased stimulation did not increase hypoxic injury significantly. These results suggest that synaptic stimulation can be detrimental to hypoxic penumbra neurons and that this damage is mediated by transmitter release.

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502.1

ROLE OF INTRACELLULAR CALCIUM IN HYPOXIC/ISCHEMIC INJURY IN CULTURED ASTROCYTES. R. S. Goldman, Department of Neurology, Medical College of Wisconsin, Milwaukee, WI 53226

A substantial body of evidence suggests that an elevated level of intracellular calcium is associated with the neuronal response to hypoxia. I have investigated the role of intracellular calcium changes in the astrocytic response to hypoxia. Primary cultures of rat astrocytes were exposed to sodium cyanide (CN; 1mM), an inhibitor of oxidative metabolism, and to iodoacetic acid (IAA; 0.25mM), an inhibitor of anaerobic metabolism, for various lengths of time. The cells were then washed, and incubated overnight. Cell viability was determined by a two color fluorescence assay. Cell survival was related to duration of exposure to CN and IAA; all cells were killed by a 16 minute exposure. Survival was increased by similar exposure in the absence of extracellular calcium. Additionally, astrocytes were preincubated with the intracellular calcium chelator BAPTA-AM (50uM). This should cause a significant blunting of any hypoxia-induced intracellular calcium increase. This also resulted in increased cell survival.

Relative changes in the intracellular calcium concentration of cultured astrocytes were assessed using digital fluorescence microscopy, and the calcium indicator dye, fluo-3. A 10 minute incubation with CN and IAA produced a rapid rise in intracellular calcium. Calcium levels did not return to baseline following washout. The rise in calcium was significantly abated by incubation in low calcium buffer. Cells preincubated with BAPTA-AM showed little change in intracellular calcium levels during the first 5 minutes. However, with continued exposure, calcium levels did increase.

These results indicate that elevated levels of intracellular calcium are correlated with hypoxic injury in cultured astrocytes. Some of the proposed therapies for stroke based upon limiting the hypoxia-induced rise in intracellular calcium in neurons may also have a beneficial effect upon astrocyte survival.

502.2

HYPOXIC DEPOLARIZATION IN DIFFERENTIALLY VULNERABLE HIPPOCAMPAL PYRAMIDAL CELLS. K.S. Lee, T. Lowenkopf*, P. Brooks* and L. Arlinghaus. Dept. of Neurological Surgery, University of Virginia, Charlottesville, VA 22908 and Jefferson Med. Col. Philadelphia, PA 19107.

A precipitous depolarization during ischemia and hypoxia marks the severe compromise of membrane polarization and widespread entry of calcium into neurons. It has been suggested that the latency to onset of hypoxic depolarization (HD) is a critical determinant of the fate of a neuron. In support of this concept, vulnerable CA1 neurons exhibit a shorter latency to HD than relatively resistant dentate gyrus granule cells (Balestrino et al., Brain Res. 497, 1989) and, treatments that delay the onset of HD reduce post-hypoxic pathophysiology. We examined whether variations in HD latency characterize the responses of differentially vulnerable hippocampal pyramidal cells.

In vitro hippocampal slices were prepared from adult gerbils. In each experiment, simultaneous recordings were made in two of the following regions: CA1a, CA1b and CA3. Slices were maintained at interface with the atmosphere of the recording chamber and hypoxic treatment consisted of substituting 99% N_2 for the normal 95% O_2 in the atmosphere. To verify viability of the slices, evoked synaptic responses were tested prior to and following the hypoxic event in each region. The average delay to HD was virtually identical in areas CA3 and CA1b. Experiments in which DC potentials were monitored simultaneously in these two regions showed that one area usually underwent HD slightly before the other with CA3 being first in 46% of the cases and CA1b first in 54% of the cases (n=50 slices). The CA1a region consistently exhibited longer delays to HD than CA3 or CA1b (n=33). The relative, regional latencies to HD (CA1b=CA3<CA1a) do not parallel the relative vulnerabilities of these regions (CA1a>CA1b>CA3, from most to least sensitive). It therefore appears that regional variations in the latency to HD do not explain the differential vulnerability of subpopulations of hippocampal pyramidal cells. (Supported by NINDS grant NS24782).

502.3

MEASUREMENT OF INTRACELLULAR CALCIUM IN RAT CEREBRAL CORTICAL TISSUE DURING HYPOXIA AND AGLYCEMIA Keith J. Brooks & Philip I. Aaronson*. Departments of Pharmacology and Medicine, UMDS, St Thomas's Campus, London SE1 7EH, U.K.

Intracellular Ca ($[Ca]_i$) has been implicated as a probable mediator of neuronal damage during cerebral ischemia. In order to study the effects of ischemia on $[Ca]_i$ in rat cortex, we loaded cortical slices (350 µm thickness) with fura 2 AM (10 µM + 0.1% pluronic F-127 for 1 hour at room temperature). Tissues were subsequently superfused with Krebs solution, gassed with 95%O_2, 5%CO_2, at 37 °C. Tissues were judged viable if depolarisation with 40 mM K solution caused a substantial increase in $[Ca]_i$. When glucose was removed from the solution, there was a delayed rise in $[Ca]_i$; if hypoxia was induced by gassing with N_2 instead of O_2 there was little change in [Ca]i. If, however, tissues were made both hypoxic and aglycemic, $[Ca]_i$ rose rapidly and markedly. This result suggests that it is necessary to induce both of these insults simultaneously in order to cause immediate disruption of the $[Ca]_i$ regulating mechanisms in cortical tissue.

502.4

The Contribution Of Excitatory Amino Acid Transmission In The Activation Of Inward Currents During Hypoxia: A Patch Clamp Study. S. Veregge and N. Hershkowitz. Dept. Neurol., Georgetown Univ., Wash., DC 20007

Excitatory amino acid (EAA) transmission has been demonstrated to contribute to the neuronal injury that occurs as a result of hypoxia. We investigated the effects of alterations in EAA transmission on the resting leak current measured from CA1 pyramidal neurons during hypoxia. In vitro hippocampal slices were made hypoxic by exposing them to a gas mixture containing 95% N_2/5% CO_2. Whole-cell patch-clamp recordings were made from CA1 neurons with K-gluconate electrodes (V_H=-60 mV). In control neurons (n=11), hypoxia resulted in the development of a slowly increasing inward current within 60 sec of exposure to nitrogen gas. This current was interrupted by a rapidly developing, high-amplitude inward current (RIC) (mean latency=157+/-35 sec), which was typically associated with a negative shift in the field potential. Selective inhibition of non-NMDA glutamate receptors with 5 uM CNQX resulted in a moderate increase in the latency of the RIC (n=10, 522+/-38 sec), as did blocking propagated synaptic transmission with 1 uM TTX (n=5, 391+/-69 sec) or cutting the Schaffer collaterals (n=7, 485+/-48 sec). Selective inhibition of NMDA receptors with 5 uM CPP (n=8) or non-selective blockade of both NMDA and non-NMDA glutamate receptors with 5 mM kynurenic acid (n=5) resulted in a more marked increase in the latency of the RIC (752+/-135 sec and 787+/-118 sec, respectively). These data are consistent with previous studies in which hypoxia was evaluated with extracellular and current clamp techniques. In addition, they suggest that both non-NMDA- and NMDA-mediated events may be involved in hypoxia-induced cell injury. (supported by NINDS grant NSO1460-01).

502.5

Effects Of Hypoxia On Spontaneous And Evoked Synaptic Currents In The Hippocampus. N. Hershkowitz and S. Veregge, Dept. of Neurology, Georgetown Univ., Wash. DC 20007.

Little is known about the influence of hypoxia on synaptic transmission. We examined the effects of hypoxia on synaptic activity in CA1 neurons of the hippocampal slice using patch-clamp techniques (V_H=-60 mV, K-gluconate electrodes). A stimulating electrode placed in the stratum radiatum was used to elicit orthodromic synaptic responses. Slices were made hypoxic by exposing them to a gas mixture containing 95% N_2/5% CO_2. Orthodromic stimulation elicited an inward current (EPSC) followed by an outward current (IPSC). In addition to evoked currents, smaller spontaneous inward currents were also observed. Hypoxia resulted in a gradual decline in the evoked EPSC amplitude and a rapid decline in the evoked IPSC amplitude. Thus, after approximately 80 sec the IPSC was 15 ± 11% (mean ± S.E.) of control whereas the EPSC was 86 ± 7% of control (n=5). A pure NMDA response could be eliceited in two experiments carried out in the presence of CNQX (5 uM). Under these conditions, the orthodromically elicited inward current was rapidly inhibited with complete suppression occurring within 80 sec. Hypoxia also resulted in an increase in the frequency of spontaneous inward currents in many of the cells examined. Cutting the Schaffer collaterals did not abolish this increase, but kynurenic acid (5 mM, n=5) and TTX (1 uM, n=5) suppressed these spontaneous currents, identifying them as glutamate-mediated EPSCs. These results suggest that glutamate release during hypoxia may in part result from an increase in spontaneous EPSCs. In addition, the differential sensitivity of the IPSC and EPSC may represent the mechanism of hypoxia-induced seizures. (Supported by NINDS grant NSO1460-01).

502.6

METABOLIC ADAPTATIONS TO ANOXIA IN THE ISOLATED TURTLE CEREBELLUM. M. Pérez-Pinzón, J. Bedford, M. Rosenthal, P. L. Lutz and T.J. Sick. University of Miami Schools of Medicine and Marine and Atmospheric Sciences, Miami, FL.

Turtle brain can survive prolonged anoxia during which ion homeostasis (K^+_o) is maintained, evoked potential activity is depressed but not suppressed, and ATP levels are unchanged from control. Strategies for such anoxia tolerance likely include decreased ATP demand and increased anaerobic ATP production. Present goals were to account more precisely for such metabolic activity and to seek mechanisms controlling anaerobiosis during and subsequent to the transition to anoxia. Studies were conducted in isolated turtle cerebellum which demonstrates similar anoxia tolerance to that of the turtle brain *in situ*. Unlike in mammalian brain or mitochondria which are O_2 regulators (i.e. O_2 consumption was independent of O_2 availability over a broad range), O_2 consumption in turtle cerebellum varied sensitively with declines in bath O_2 tension from saturation. Parallel studies of heat output in turtle cerebellum by flow-through microcalorimetry demonstrated that ATP production decreased approx 50% during 2 hours of anoxia. Calculations from O_2 consumption and heat production indicated that oxidative metabolism provided approx 55% of normoxia ATP production and that anaerobic ATP production was increased within 30 min ('Pasteur effect') to account for energy demands not significantly altered by the transition to anoxia. After this time, however, anaerobic ATP production declined to level characteristic of a markedly hypometabolic state. These studies indicate that the turtle brain is an O_2 conformer and suggest sensitive endogenous regulation of anaerobiosis and ATP-consuming physiological activities such as by an O_2 sensor.

502.7

ELECTROPHYSIOLOGICAL ANALYSIS OF POST-ISCHEMIC CA1 NEURONS USING IN VITRO HIPPOCAMPAL SLICES. E. F. Cregan, E. W. Harris and G. C. Palmer, Biology Department, Fisons Pharmaceuticals, Rochester, NY 14623, USA.

Transient forebrain ischemia causes a loss of neurons in hippocampal area CA1 after a delay of 1-3 days. A leading candidate mechanism is the "excitotoxic hypothesis": CA1 excitatory afferent hyperactivity, which develops after ischemia, causes CA1 cell death. We have examined synaptic transmission along the major excitatory afferent to CA1 (the commissural/associational, or C/A, pathway) at different times after transient ischemia in rats (similar studies have been carried out in gerbil; Urban et al., Stroke 21: II-23, 1989).

Transient forebrain ischemia was induced using the "4-Vessel Occlusion" (4VO) model (Pulsinelli & Brierly, Stroke 10: 267, 1979). At select times after ischemia, slices of left & right hippocampus were prepared, and allowed recover semisubmerged at 36.5°C for 90 min. Two slices from septal and mid-septotemporal hippocampus from each side were examined. The C/A pathway was stimulated near CA2 at 8 fixed intensities, and the amplitude of synaptic field potentials and evoked cell firing were measured in CA1b. Trains of near maximal stimuli (20 stimuli at 2 Hz and 5 Hz) were used to test the ability to induce repetitive CA1 cell firing.

502.8

MECHANISMS OF ANOXIA-INDUCED DEPOLARIZATION IN BRAINSTEM NEURONS: IN-VITRO INTRACELLULAR STUDIES.

G. G. Haddad and C. Jiang, Dept. of Pediatrics, Sect. Respiratory Medicine, Yale Univ. Sch. of Medicine, New Haven, CT. 06510

We have previously shown that hypoglossal (HYP) and vagal (DMNX) motoneurons depolarize in a major way when exposed to short periods of low O_2 environment in adult rat slices. In addition, we have also demonstrated that extracellular Ca^{++} and Na^{+} decrease during anoxia. We hypothesized then that, under our experimental conditions, the anoxia-induced brainstem neuronal depolarization and ionic flux is due to increased extracellular levels of glutamate, data we recently obtained by microdialysis. We performed therefore blockade experiments in the adult rat slice preparation in which we measured the anoxia-induced depolarization before and after NMDA and non-NMDA receptor antagonists. During 4-5 min of anoxia, HYP or DMNX neurons depolarized by 35-50 mV and recovered with reinstitution of O_2 over 10-15 min. Neither MK801 (10-20 μM, n=3), Kynurenate (0.5-1.0 mM, n=3) nor CNQX (10-20 μM, n=2) changed the trajectory of the depolarization or the recovery from it. We then asked whether this depolarization is due to failure of energy-requiring processes. In order to answer this directly, we studied the anoxia-induced depolarization profile before and after iontophoresing ATP salt solution intracellularly for 20-30 min through the recording electrode. We found that after ATP iontophoresis, 6/7 HYP cells recovered totally from periods of anoxia that were 2 to 3x longer (up to 12 min in PO_2=0 Torr). In addition, the depolarization magnitude was $\leq$ than that before ATP, also in 6/7 HYP neurons. We conclude that: 1) NMDA and non-NMDA mechanisms are not essential in inducing the anoxic depolarization in brainstem neurons and 2) ATP-dependent mechanisms play a major role in altering membrane potential during O_2 deprivation.

502.9

MILD HYPOTHERMIA PROTECTS MAMMALIAN CENTRAL WHITE MATTER AGAINST ANOXIC INJURY. P.K. Stys, S.G. Waxman, B.R. Ransom, Dept. of Neurology, Yale Univ., New Haven, CT 06510 and Neuroscience Research Center, VA Hospital, West Haven, CT 06516.

Strokes cause disability by involving both gray matter and white matter (WM) structures of the CNS, and spinal cord injury can result in severe neurological disability by disrupting myelinated tracts. Extracellular Ca2+ ions play a central role in the production of irreversible anoxic injury in both gray and white matter. The mode of entry of Ca2+ into cells in WM, however, is different from that in gray matter; a large portion of the damaging Ca2+ influx occurs via reverse operation of the Na+-Ca2+ exchanger. Given the substatial temperature dependence of the Na+-Ca2+ exchanger, it could be expected that the degree of anoxic injury in WM would be very susceptible to temperature. To test the extent of temperature dependence of anoxic injury in WM, we used the in vitro rat optic nerve (RON) preparation, and studied the degree of injury produced by a 60 min anoxic insult at temperatures above and below the physiological range. At 37°C, the area under the compound action potential of the RON recovered to 35.4 ± 7.2% of control, following 60 min of anoxia and a 60 min period of re-oxygenation. Modest cooling to 34.5°C during anoxia increased recovery of CAP area substantially, to 64.6 ± 15.3% (SD, P<0.00001), and at 32°C CAP area recovered fully (100.5 ± 13.8%). Conversely, higher temperatures had a deleterious effect; at 39.5°C, recovery of CAP area was reduced to 22.3 ± 13.8, and at 42°C even more injury occurred (8.5 ± 7.0%). Our results indicate that even modest reduction in temperature during the anoxic insult can very significantly improve functional recovery of CNS WM from anoxia, and that the degree of injury increases substantially with increasing temperature above the physiological range. This mechanism likely reflects the temperature dependence of ion channel, transporter and enzyme kinetics.

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502.10

ANOXIC INJURY AND EARLY POST-ANOXIC RECOVERY IN RAT OPTIC NERVE: ULTRASTRUCTURAL CORRELATES. S.G. Waxman, J.A. Black, P.K. Stys, B.R. Ransom, Dept. of Neurology, Yale University, New Haven, CT 06510 and Neuroscience Research Center, VA Hosptial, West Haven, CT 06516.

We have developed a reproducible, quantitative model of anoxic white matter injury using the rat optic nerve *in vitro*. In this model, action potential conduction is rapidly abolished by anoxia, and is restored with decreased conduction velocity in about 35% of axons, following a 60-min anoxic period. We have now examined the ultrastructural correlates of anoxic injury and early post-anoxic recovery in this model. Optic nerves examined following 60 min of anoxia, displayed large, distended myelin sheaths associated with empty zones located adjacent to the axon. Myelin remained compact and retained its periodicity. In some regions, the extracellular space was enlarged. There was mitochondrial swelling with loss of normal cristae and loss of microtubules and, to a smaller degree, of filaments in large-diameter axons. Nodes of Ranvier in anoxic optic nerves displayed detachment of terminal oligodendroglial loops and, in more severely affected fibers, retraction of the myelin from the node. In optic nerves that had been permitted to recover for 60 min in oxygenated Ringers, ultrastructural changes were less pronounced. Although some axons appeared swollen, empty zones were only rarely observed within myelin sheaths. Most nodes of Ranvier appeared normal, and only rare terminal oligodendroglial processes remained detached from the axon membrane. The axoplasm of large fibers continued to show loss of microtubules and filaments, as well as mitochondrial swelling. These findings provide support for the idea that, during anoxia, myelinated axons are damaged, likely by an influx of calcium. These results suggest that damage to paranodes leads to conduction block in the anoxic optic nerve, and that repair of paranodal axon-oligodendroglial terminal loops may provide a morphological basis for the early recovery of conduction that occurs after re-oxygenation.

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502.11

Anoxic changes in rat CA1 pyramidal layer cells recorded with patch electrodes

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Recordings (with seals > 1GΩ) were made in 500 μm thick, partly submerged slices with electrodes containing 150 mM K gluconate, 2 mM $MgCl_2$, 0.2 mM Tris GTP and 10 mM HEPES (pH 7.25). Hypoxia was produced by switching to 0_2-free aerating gas and superfusing solution, typically for 4 min.

The overall resting potential (V_m) was -61.4 (S.E.1.44) mV and input resistance (R_N) 86.3 (±5.07) MΩ (for n=60): mean R_N was thus nearly 80% greater than in recordings with conventional microelectrodes.The presence or absence of 1 mM EGTA inside the electrodes made no significant difference to V_m (-1.0 ± 1.02 mV) or R_N (3.1 ± 7.82 MΩ); but in the presence of 2 mM ATP, V_m was significantly less negative (-59.8 ± 1.14 mV) and R_N higher (101.8 ±6.40 MΩ) than in its absence (V_m -63.1 ± 0.77 mV; R_N 70.7 ± 2.74 MΩ). These data indicate a substantial involvement of ATP in the regulation of V_m and R_N.

In general, anoxic changes in V_m and R_N were much smaller than previously observed with conventional intracellular electrodes: overall (n=68) mean ΔV_m was +2.4 ± 0.783 mV and ΔR_N -11.6 ± 2.42%. The presence (n=24) or absence (n=44) of ATP in the electrodes made no significant difference to anoxic ΔV_m and ΔR_N. However, a direct involvement of Ca^{2+} is suggested by the greater anoxic reduction in R_N in the absence of EGTA (-14.3 ± 3.78%, n=28) than in its presence (-8.1 ± 3.29%, n=30): when recording with ATP-containing electrodes, the magnitude of ΔR_N was significantly correlated with the absence of EGTA (r=0.38, p=0.05, n=24).

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502.12

THE EFFECT OF FORSKOLIN ON CYCLOHEXYLADENOSINE INHIBITION OF COMBINED HYPOXIC / HYPOGLYCEMIC-INDUCED 45Ca UPTAKE BY NEOCORTICAL CULTURES. A.W. Probert and F.W. Marcoux. Parke-Davis Pharm. Research Division, Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, MI 48105.

Treatment of rat neocortical cultures with cyclohexyladenosine (CHA) inhibits ^{45}Ca uptake resulting from exposure to a combined hypoxic / hypoglycemic insult. CHA's potency (IC_{50}=60μM), however, differs markedly from its low nanomolar affinity for the adenosine A1 receptor site. This discrepancy in potency verses A1 receptor affinity may reflect activity at the low affinity, intracellular P site. Since activation of this site, as well as that for A1, has been reported to down regulate adenylate cyclase activity, we examined the adenylate cyclase activator, forskolin, for its ability to reduce CHA's inhibitory effects.

Cultures were exposed to 150 min of combined hypoxia (1% O_2) / hypoglycemia (0.2g/L D-glucose) in the presence of 1000μM CHA, 50μM FORSK, 50μM of the nonactivating FORSK analog, 1,9-dideoxyforskolin (dFORSK), or 100μM CPP. Inhibition of ^{45}Ca uptake by CHA, FORSK, dFORSK, or CPP were 81, -3, 64, and 59 percent, respectively. Combined treatment with FORSK reduced CHA inhibition to 10 percent while having little effect on CPP efficacy (63 percent inhibition). Inhibition was additive when dFORSK was combined with either CHA or CPP (99 and 102 percent, respectively).

These results suggest CHA's inhibitory activity is exerted through down regulation of adenylate cyclase activity, possibly by an action at an intracellular P site.

502.13

A CRITICAL TEST OF CHANNEL ARREST. C. J. Doll, P. W. Hochachka* and P. B. Reiner. Depts. of Zoology and Psychiatry, Univ. of British Columbia, Vancouver, B.C. V6T 2A9.

One possible mechanism of anoxia tolerance is the "channel arrest" hypothesis which predicts that ion leakage across the plasma membrane is reduced during an anoxic episode. The reduction of ion leakage would reduce demand on ATP utilizing ion pumps, thereby conserving metabolic energy. We used the turtle (*Chrysemys picta*) as an anoxia tolerant model system, as this species has been shown to survive anoxia for over 6 months. To monitor changes in ion leakage, whole cell input resistance and specific membrane resistivity were measured under control and anoxic conditions using intracellular recording in cortical slices incubated at 25°C. There were no significant changes in measured parameters indicating that ion leakage was not reduced. These results do not support channel arrest as a mechanism of energy conservation during short term anoxia ($\leq$ 120 mins.).

502.14

EFFECTS OF COBALT AND NICKEL IONS ON SPREADING DEPRESSION IN RAT HIPPOCAMPAL SLICES. J. Jing*, P.G. Aitken and G.G. Somjen, Dept. Cell Biology, Duke Univ. Med. Center, Durham, NC 27710.

During spreading depression (SD) and hypoxic SD-like depolarization, interstitial calcium concentration ($[Ca^{2+}]_o$) decreases precipitously, presumably by influx into cells. The mechanism of the influx is not known. We used ion selective microelectrodes to test the effect, in the CA1 region, of ions which block voltage-gated Ca^{2+} channels on the drop of $[Ca^{2+}]_o$ as well as on the change in extracellular potential (V_o) and $[K^+]_o$. SD was induced by application of 1-2 µl 3M KCl solution to the slice surface, or by withdrawing oxygen. Co^{2+} and Ni^{2+} (2mM in the bathing medium) had similar effects; these effects were essentially identical in K^+-induced and hypoxia-induced SD. The decrease of $[Ca^{2+}]_o$, the increase of $[K^+]_o$, and the negative shift of V_o were curtailed but not abolished, more so in st. pyramidale than in st. radiatum. For example, in K^+ induced SD the average effect of Co^{2+} or Ni^{2+} was to reduce $\Delta[Ca^{2+}]_o$ to 72% of control value in st. pyramidale and to 94% of control value in st. radiatum. At the site of high K^+ application SD-related changes were only moderately depressed by Co^{2+} or Ni^{2+}, but at some distance from the focus the propagation of SD was powerfully suppressed. We conclude that (1) During SD some but not all Ca^{2+} enters cells through voltage-gated channels; (2) SD-related membrane processes in neuron somata can vary independently from those in the dendrites; (3) The mechanism of SD initiation may be different from that of SD propagation. (Supported by NS18670, 17771, 06233.)

502.15

SUPPRESSION OF PRESYNAPTIC CALCIUM CURRENTS BY HYPOXIA IN HIPPOCAMPAL TISSUE SLICES. J.N. Young*, G.G. Somjen, Dept. Cell Biol. and Div. Neurosurgery, Duke Medical Ctr., Durham, NC 27710.

During hypoxia of CNS synaptic transmission fails rapidly and, initially, reversibly. We tested the hypothesis that voltage gated calcium channels in the presynaptic terminals are inactivated by hypoxia, leading to failure of transmitter release. Rat hippocampal slices were studied in a dual-well interface chamber at 34.5°C. Interstitial calcium concentration ($[Ca^{2+}]_o$) was recorded with ion selective microelectrodes in st. radiatum of CA1 and sometimes also in st. pyramidale. Ortho- and antidromic stimulus trains (5s, 20Hz) were applied to evoke changes in $[Ca^{2+}]_o$. In order to record $[Ca^{2+}]_o$ responses due to Ca-influx into presynaptic axon terminals, postsynaptic responses were blocked by 10 µM 6,7-dinitroquinoxaline-2,3-dione (DNQX) and 20 µM 3-((±)-2-caboxypiparazine-4-yl)-propyl-1-phosphonic acid (CPP), or, in other experiments, by lowering bath Ca^{2+} to 0.12 mM. Oxygen was withdrawn until signs of spreading depression (SD)-like depolarization occurred. During hypoxia orthodromic (presynaptic) $[Ca^{2+}]_o$ responses in st. radiatum were extinguished before SD began; antidromically evoked $[Ca^{2+}]_o$ responses in st. pyramidale failed only at the moment of SD. "Resting" baseline of $[Ca^{2+}]_o$ declined first gradually, then, with the onset of SD, precipitously. In the presence of DNQX and CPP hypoxic SD occurred later, and during SD $[Ca^{2+}]_o$ decreased less, than in slices bathed in control solution. The findings are consistent with a failure of voltage gated Ca^{2+} channels in presynaptic terminals during hypoxia, possibly due to inactivation caused by a rise of $[Ca^{2+}]_i$. During hypoxic SD some but not all the Ca^{2+} appears to enter cells through glutamate-controlled channels. (Supported by grants NS 18670 and NS 06233)

502.16

CONSEQUENCES OF TRANSIENT ASPHYXIA IN CORTICAL AND HIPPOCAMPAL NEURONS IN CULTURE. J.L. Daval, F. Nicolas* and V. Koziel*, INSERM U.272, 30 rue Lionnois, 54013 NANCY, FRANCE.

Following a brief episode of oxygen deprivation, some neural cells, especially in cortex and hippocampus develop a so-called "delayed neuronal death", and a critical role for postanoxic release of excitatory amino acids has been proposed. The present study was designed in order to develop a reproducible model to study hypoxic damage in primary cultures of neurons. Functional response of cultured neurons from rat cortex and hippocampus to transient hypoxia/asphyxia was studied by the measurement of [^{3}H]2-deoxyglucose (2DG) specific uptake. Cell morphology, lactate dehydrogenase (LDH) efflux and protein contents were also examined. Hypoxia was induced by incubating the cells either in a 5% CO_2 -95% N_2 gas mixture or in 1 mM NaCN-containing culture medium for various periods of time. Twenty-four hours following hypoxia (6 to 8 h in an anaerobic atmosphere or 90 min in the presence of NaCN), 2DG transport was significantly enhanced, with a concomitant increase in LDH efflux, whereas no major morphological alteration could be noticed. Cell injury was more evident 3 days post-hypoxia, with a reduced rate of cellular metabolism and cell degeneration. The transient stimulation of 2DG uptake by the neuronal cells in culture indirectly suggests the participation of amino acid excitotoxicity.

502.17

Hypoglycemic injury in organotypic hippocampal culture is similar to NMDA neurotoxicity and is prevented by delayed MK-801. R. C. Tasker, J. J. Vornov and J. T. Coyle Depts. Anesthesiology, Neurology, Neuroscience and Psychiatry, The Johns Hopkins School of Medicine, Baltimore, MD 21205

In animal models of hypoglycemia, neurotoxicity is rapid and selectively involves CA1 and the dentate gyrus. Injury is attenuated by concomitant NMDA receptor antagonists. In organotypic hippocampal culture, which preserves the regional differentiation of the hippocampus, we have examined the time course of neuronal injury following *in vitro* "hypoglycemia" using propidium iodide fluorescence microscopy to observe directly the regional distribution of membrane injury in real time. Increasing duration of exposure to glucose-free media, for up to 3 hours, was associated with an increasing severity of injury. The pattern of propidium staining following 2 hours of hypoglycemia was the same as that observed following a 30 minute exposure to 10 µM NMDA: simultaneous staining of CA1 and the dentate gyrus with sparing of CA3, beginning after 4-6 hours of recovery. To facilitate further examination, a briefer "hypoglycemic" insult was achieved with the addition of 2-deoxyglucose (2-DG) to inhibit glycolysis. A 30 minute exposure to 5 mM 2-DG also produced propidium staining simultaneously in CA1 and the dentate gyrus after 4-6 hours of recovery. MK-801, during or up to 30 minutes after hypoglycemia, prevented propidium staining in CA1. At 24 hours, histologic evaluation confirmed the protective effect of MK-801 in CA1 and the dentate gyrus. Hypoglycemic injury appears to be mediated by acute, toxic NMDA receptor activation, which continues for at least 30 minutes into the recovery period in organotypic hippocampal culture.

502.18

EFFECT OF ANOXIA ON EXCITATORY AMINO ACIDS IN BRAIN SLICES OF RATS AND TURTLES: *IN VITRO* MICRODIALYSIS STUDY. R.S.K. Young, M.J. During, G.G. Haddad, D.F. Donnelly, W.J. Aquila*, V.L. Perry*. Yale University School of Medicine; New Haven, CT.

The purpose of this study was to utilize *in vitro* microdialysis to test the hypothesis that anoxia has a differential effect upon the concentrations of excitatory and inhibitory amino acids released from brain slices of rats compared to those of turtles. Ten minutes of anoxia (95% N_2 / 5%CO_2) produced signficiant elevation of glutamate (from 0.39±0.03 to 0.90±0.18 µM/L dialysate, Mean ± SE, $p < 0.05$, Anal. of Variance), aspartate (from 0.28±0.12 to 1.20±0.49, $p < 0.05$), glycine and alanine in the rat. During reoxygenation, alanine and glycine returned toward baseline values, whereas aspartate and glutamate did not. In contrast, prolonged anoxia (60 minutes) produced only a slight increase in aspartate (from 0.06±0.01 to 0.09±0.02, $p < 0.05$) and a decrease in glutamate (from 0.50±0.11 to 0.33±0.09, $p < 0.05$) in the turtle. Levels of glycine and alanine did not change. We speculate that the mechanism of anoxia-resistance in the turtle is at least partly due to the lack of increase in excitatory amino acids in response to severe oxygen limitation.

502.19

PROTECTION OF BRAIN SLICES FROM ISCHEMIC INJURY DURING PREPARATION BY HYPOTHERMIA, PHENYTOIN AND PENTOBARBITAL.
H. Qi, F. E. Hospod, K. Grundmann and G. C. Newman. Dept. of Neurology, SUNY at Stony Brook, Stony Brook, NY, 11777 and Northport VAMC.

Our goals are: 1) to test the hypothesis that ischemia due to decapitation is the primary cause of brain slice injury and 2) to improve slice quality.

We have studied histology of 500μ hippocampal slices incubated for 5 hours in K-R with 4 mM glucose by determining the number of normal appearing neurons in CA1 and the dentate. Male Sprague-Dawley rats (300g) were sacrificed without anesthesia (Control), after cooling to 31°C. with sedation using pentobarbital or a mixture of 1.5% halothane/NO_2 (Hypothermia), or after phenytoin (200mg/kg; PHT) or pentobarbital (60mg/kg; PB). The histologic effects of varying slice thickness from 350μ to 500μ or incubating at temperatures from 30°C. to 37°C. were also evaluated.

Approximately 20% of CA1 pyramidal cells appear normal in Control slices but hypothermia prior to sacrifice results in the normal appearance of virtually all CA1 neurons. Pre-treatment with PHT or PB increases the number of normals to over 50%. Incubation at 30°C. improved slice histology less than hypothermia prior to sacrifice while incubation at 35°C. produced no benefit. CA1 histology was improved only minimally by reducing slice thickness.

Dentate of Control 500μ slices show a zone of severe core injury beginning 90μ from the slice surface. This injury disappears completely in 350μ slices. Hypothermia shifted the onset of injury to a depth of 150μ. Pre-treatment with drugs or incubating in the cold had relatively little effect on dentate histology.

Hypothermia before sacrifice produces slices with near normal histology after 5 hours *in vitro* without introducing drugs or requiring changes in incubation temperature.

502.20

PROTECTIVE EFFECTS OF PHENYTOIN AND PENTOBARBITAL IN A THICK SLICE MODEL OF ISCHEMIA.
K. Grundmann, H. Qi, F. E. Hospod and G. C. Newman. Dept. of Neurology, SUNY at Stony Brook, Stony Brook, NY, 11777.

Thick brain slices (1000μ) differ from thin slices because they retain substances released into the extracellular space and thus create a more realistic model of ischemia. In this study we combine histology and brain slice glucose utilization (SGU) to further define the model and assess the effects of phenytoin (PHT, 10μM) and pentobarbital (PB, 100μM).

Hippocampal brain slices (500μ and 1000μ) were prepared from male Sprague-Dawley rats (250g). Histology and SGU were studied at multiple slice depths after 2 or 5 hours *in vitro*. CA1 histology was assessed by counting the percentage of neurons that were normal, shrunken, swollen or lysed. SGU was measured in the dendritic fields of CA1, CA3 and dentate.

In 500μ Control slices, normal cells in CA1 decreased from 32% at 2 hr to 20% after 5 hr and cell lysis increased from 6 to over 12%. SGU varied from 25 μmole/100g/min (U) in dentate to 38 U in CA1 str. radiatum after 2 hr and declined by about 20% after 5 hrs. PHT and PB had virtually no effect on 500μ slice histology but increased SGU in CA3 by 50%. In contrast, in 1000μ control slices, only 5% of CA1 neurons were normal after 2 hr and 1 % by 5 hrs while cell lysis increased from 8% to 28% after 5 hrs. SGU of 1000μ control slices was much higher than in thin slices, ranging from 35 U in dentate to 84 U in CA1 at 2 hr with a 50% decline after 5 hrs. Both PHT and PB increased the percentage of normal neurons slightly but significantly decreased cell lysis, especially after 5 hours *in vitro*. PHT and PB significantly increased SGU in all regions and reduced the relative decline after 5 hrs.

Both PHT and PB improve CA1 histology in this thick brain slice model of ischemia. This effect correlates with increased glucose utilization rather than the reduced metabolic rate expected from studies with non-ischemic tissue.

INFECTIOUS DISEASES

503.1

DIFFERENT MODES OF HIV-1 TRANSCRIPTION ACTIVATION IN GLIAL, AND IN PHORBOL ESTER (PMA)- AND RETRINOIC ACID (RA)-TREATED HUMAN NEURONAL CELLS.
D. Mondal* and O. Prakash. Dept. of Molecular Oncology, Alton Ochsner Medical Foundation, New Orleans, LA 70121.

A variety of neurological dysfunctions, collectively known as AIDS dementia complex, are observed in AIDS patients. Previously, it was considered that macrophages or microglial cells were the only cell types which carried the virus in the brain. However, recently it has been shown that HIV can also infect neuronal and glial cells. We carried out transient transfection experiments in order to assess the transcriptional activity of HIV-LTR in neuronal and glial cell lines.

HIV LTR expression was significantly higher in RA-treated compared to PMA-treated neuroblastoma (SHSY5Y) cells. The NF-kB responsive element did not appear to play a major role in this activation. In addition, the LTR expression was refractory to tat-mediated transactivation and induction by TNF-α, insulin and morphine. On the other hand, HIV-LTR expression in PMA-treated cells was inducible with tat, TNF-α, insulin and morphine. In the astrocytoma cell line U-373 MG, the LTR expression was inducible with TNF-α and tat but not with insulin and morphine. These results suggest that neuronal and glial cells have the capacity to induce cellular factors which can activate HIV expression and can contribute to neurological dysfunctions. Studies are underway in our laboratory to characterize these factors.

503.2

Recombinant gp120 causes astrocyte alteration.
L. Pulliam, D. West*, N. Haigwood*, R. A. Swanson Depts. of Laboratory Medicine and Neurology, VA Medical Center and Univ of CA, San Francisco, 94121 and Chiron Corp., Emeryville,CA

The gp120 of HIV has been reported to be neurotoxic to rat neurons; however, these results were not consistent with pathologic changes observed in patients with AIDS. In an effort to further characterize AIDS dementia and to define the role of gp120, we studied the effects of 2 recombinant gp120 preparations (SF2 and 3B) and 2 gp120 SF2 deletion mutant preparations on human brain cell aggregates. The 4 glycosylated recombinant gp120 molecules were used at concentrations of 1 pM or 1nM. The SF2 molecule binds to a CD4 receptor and 3B molecule does not. Using flow cytometry, we did not see a significant decrease in the neuron population nor was gp120 binding observed; however, there was a disruption of cellular matrix by histological exam. Using flow cytometry, there was a decrease in GFAP-staining cells treated with SF2 in 2 out of 4 brain preparations. By ultra-structure examination, cells consistent with astrocyte morphology but lacking cytoplasmic fibrils could be seen. To explore the possibility that altered astrocyte function may influence neuron viability, we measured glutamate uptake of gp120 treated human astrocyte cultures and did not find a significant decrease.

503.3

EVIDENCE FOR THE CELL-TO-CELL SPREAD OF HSV WITHIN THE TRIGEMINAL GANGLION AND ROOT AFTER CORNEAL INOCULATION.
J. H. LaVail and W. E. Johnson*. Dept. Anatomy and Neuroscience Program, UCSF, San Francisco, CA 94143.

We earlier used various antibody probes to characterize the classes of neurons in the V_1 division of the mouse trigeminal ganglion (TG) that become actively infected with Herpes simplex virus (Type 1) (HSV) 3 days after corneal infection (Margolis et al. 1989). Unspecified, however, were which classes of neurons in the TG became primarily infected following retrograde transport of virus from the cornea and which were infected secondarily, either after spread between cells within the ganglion or following transport to synaptic endings and reuptake by neighboring terminals. We now have identified neuron classes that innervate the cornea, based on their ability to transport FluoroGold (FG) from axon endings in the cornea back to cell somata in the TG and on their labeling with antibodies against Substance P (SP), Calcitonin gene-related peptide (CGRP) or monoclonal antibodies LD2 and SSEA3, that are directed against specific neuronal glycoconjugates. About 230 TG neurons innervate the central cornea of the mouse. About 44% of these are immunoreactive for SSEA3; about 45% contain CGRP and of these about half also contain SP. None of the FG+ cells label with LD2 antibody, although LD2+ neurons represent about 36% of the total neurons and 18% of HSV infected neurons in the V_1 division of the ganglion. We found in an earlier study that anterograde transported HSV does not appear in the trigeminal complex of the brainstem until 3 days after inoculation. Thus LD2+ ganglion cells observed on day 3 must have been infected with HSV following spread from nearby cells in the ganglion or proximal trigeminal root rather than by release at synaptic ending and reuptake by nearby terminals of other TG neurons.

503.4

DETECTION OF TAU-2 EPITOPES IN AIDS TEMPORAL LOBE.
S.X.Zhang*, L.C.Stanley, W.S.Metzer and W.S.T.Griffin. Departments of Anatomy, Neurology, and Pediatrics, UAMS, Little Rock, AR 72205

Tau proteins are low molecular weight microtubule-associated proteins found in the protease-resistant paired helical filaments of neurofibrillary tangles in neurons in Alzheimer disease (AD). Approximately 66% of all AIDS patients suffer from clinical dementia. The loss of cognitive function in AIDS is similar to that characteristic of AD dementia. We hypothesized that neuropathological changes similar to those in AD occur in AIDS, including the aberrant expression of Tau-2 epitopes. Using a specific monoclonal antibody, Tau-2 (gift from L.I.Binder), we examined formalin-fixed, paraffin-embedded, 10 μm thick, temporal lobe sections taken postmortem from AIDS, age-matched controls (AMC), and AD (positive control). We found a greater number of Tau-2 immunoreactive astrocytes, microglia, macrophages, neurons, and fibers in AIDS than in AMC. These immunoreactive cells and fibers were not as prevalent as in AD but had a similar appearance to those in AD. We conclude that the prominence of the immunohistochemical labeling is indicative of excessive expression of Tau-2 epitopes in AIDS temporal lobe. Our results provide evidence that a common cascade of neuropathological events occurs in AIDS as we postulate in AD. This work was supported in part by NS27414.

503.5

S100β IS ELEVATED IN BRAIN CELLS OF AIDS PATIENTS. W.S.T.Griffin, L.C.Stanley, R.E.Mrak, and L.J.Perrot*, Departments of Anatomy, Pathology, and Pediatrics, UAMS, Little Rock, AR 72205

We have recently reported an elevation of IL-1 in microglia in AIDS temporal lobe. Because IL-1 has been shown to stimulate astrogliosis in rats (Guilian et al., 1988), we proposed that elevated IL-1 induces astrogliosis, including synthesis of S100, in Alzheimer Disease (AD), Down Syndrome, and other neurodegenerative disorders, including AIDS, where gliosis is a characteristic neuropathology (Griffin et al., 1989). We have recently shown an elevation of the level and neurotrophic activity of S100β in AD and suggested that this elevation contributes to the overgrowth of neurites in senile plaques (Marshak et al., 1991). In order to determine if reactive astrocytes, containing elevated levels of S100β, are present in AIDS temporal lobe along with the elevated levels of microglia-derived IL-1, formalin-fixed, paraffin-embedded temporal lobe sections from HIV infected individuals (AIDS, n=8) and age-matched controls (AMC, n=6) were examined, using a specific antibody to S100β (gift from D. Marshak). The number of S100β immunoreactive astrocytes in AIDS was approximately 2 times that in AMC in both grey and white matter. Furthermore, these cells were enlarged and had prominent processes. We conclude that astrogliosis with elevated expression of S100β is associated with HIV infection and elevation of microglia-derived IL-1 in AIDS. If this elevation of S100β in AIDS is reflective of neurotrophic activity, as in AD, it could lead to the formation of dystrophic neurites and neuronal dysfunction. This work was supported in part by NS27414.

503.6

IL-1α AND IL-1β ARE ELEVATED IN BRAIN CELLS OF AIDS PATIENTS. L.C.Stanley, R.E.Mrak, L.J. Perrot*, and W.S.T.Griffin, Departments of Anatomy, Pathology, and Pediatrics, UAMS, Little Rock, AR 72205

The neuropathogenesis of an Alzheimer Disease-type dementia in AIDS is unknown but is likely due to HIV infection of brain cells (Navia et al., 1986). Numerous reactive microglia are infected with HIV in AIDS brain (Stoler et al., 1986; Michaels et al., 1988), and HIV preferentially infects and replicates in microglia rather than in other glia or in neurons (Watkins et al., 1990). We have previously shown an elevation of IL-1 in reactive microglia in Alzheimer Disease and Down Syndrome (Griffin et al., 1989). We hypothesized that numerous reactive microglia, containing elevated levels of IL-1, would be present in AIDS temporal lobe in response to HIV infection. We examined formalin-fixed, paraffin-embedded temporal lobe sections from HIV infected individuals (AIDS, n=8) and age-matched controls (AMC, n=6), using specific antibodies to IL-1α and IL-1β. The number of IL-1α immunoreactive cells in AIDS was approximately 5 times that in AMC. These cells were identified as reactive microglia based on their morphology and double labeling for IL-1α and GFAP which excluded the majority of these cells from the astrocyte population. The number of IL-1β immunoreactive cells in AIDS was approximately 9 times that in AMC but was only about 33% of the number of IL-1α+ microglia. Cells containing IL-1β immunoreactive product were identified as reactive astrocytes. We conclude that elevated expression of brain-derived IL-1 is associated with HIV infection and possibly contributes to the neuropathogenesis of dementia in AIDS. This work was supported in part by NS27414.

503.7

IDENTIFICATION OF UBIQUITIN-CONTAINING CELLS IN THE BRAIN FROM AIDS PATIENTS. H. Astle and F.J. Denaro, Dept. of Neurology, Texas Tech University Health Sciences Center, Lubbock, TX 79430.

Ubiquitin belongs to a class of proteins produced by cells in response to physiological stress. Of central importance is ubiquitin's role in the formation of ubiquitin-protein complexes. These complexes are a signal for the degradation of atypical and short-lived proteins. To date, ubiquitin has been identified in neurofibrillary tangles, senile plaques, Lewy bodies, and cellular inclusions in other tissues. The brain, therefore, is capable of producing ubiquitin and this production appears to occur in association with various neuropathological states. Neurological problems and neuropathological lesions are associated with HIV infection. Work has been undertaken to clarify the role of HIV and opportunistic infections in the production of the neuropathology which is found in AIDS. In the present study, by immunocytochemistry we examined postmortem CNS tissue from AIDS patients. By this approach we have been able to identify ubiquitin in neurons in these brains. Opportunistic infections or HIV may be responsible for the production of ubiquitin in these cells. If so, the mechanism involved is not understood. For example, is the production of ubiquitin a consequence of direct neuronal infection by virus or is it an indirect result of the systemic effects of HIV or other opportunistic infections? Work is under way to examine the role of viral infections in the production of ubiquitin in the CNS.

503.8

NEUROTROPISM OF BORNA DISEASE VIRUS IS LINKED TO THE PRODUCTION OF NERVE GROWTH FACTOR AND OTHER SOLUBLE FACTORS BY NEURAL CELLS, K. M. Carbone, S. A. Rubin*, S. W. Park*, A. M. Sierra-Honigman*, R. W. Waltrip, II, W. I. Lipkin and R.H. Yolken*, Div. of Infectious Diseases, Depts. of Medicine & Pediatrics, Johns Hopkins Univ. Sch. of Med., Balto., MD 21205.

Borna disease virus (BDV) is an RNA virus which causes immunopathological neurobehavioral disease. We examined the preferential replication of BDV in neural cells, assumed to be due to a receptor unique to these cells. We identified no increased binding of BDV to C6 cells, an astrocytoma cell line, as compared to the MDCK cells, a canine kidney cell line. C6 cells were extremely permissive to BDV replication, as 100% of the cells expressed viral antigens after BDV inoculation (C6-BDV) vs 2% of the MDCK cells. C6 conditioned medium (C6-CM) or NGF alone, when added to PC12 cells, a rat pheochromocytoma cell line, or to SHEP and SY5Y, human neuroblastoma cell lines, *after* inoculation with BDV, produced a 3 to 10-fold increase in BDV-infected cells and expression of BDV proteins, as compared to cells inoculated with BDV without NGF or C6-CM treatments. C6-CM or NGF treatment prior to inoculation with BDV had no effect. A comparison of C6-BDV and MDCK-BDV cell lines using immunoprecipitation and Northern Blot analysis revealed that the C6-BDV cell line produced more viral RNA and protein than the MDCK-BDV cell line. Thus, the neurotropism of BDV may be associated with the production of soluble factors by neural cells.

503.9

PCR AMPLIFICATION OF BORNA DISEASE VIRUS (BDV) SPECIFIC SEQUENCES IN NON NEURAL TISSUES AND BODY FLUIDS IN NEWBORN, CHRONIC AND ACUTELY-INFECTED RATS. A. M. Sierra-Honigmann*, W. I. Lipkin, J. W. Griffin, R. W. Waltrip, II, R. H. Yolken*, and K. M. Carbone.
Divisions of Infectious Diseases, Depts. of Pediatrics and Medicine, Dept. of Neurology, The Johns Hopkins University, School of Medicine, Baltimore, MD 21205.

Borna disease virus (BDV) is a neurotropic RNA virus that infects a wide range of species, including birds and mammals. BDV-infected cells can be identified immunohistochemically using serum from infected animals or by in situ hybridization with RNA probes from BDV cDNA clones. Previous studies in neonatally-infected rats (NB) indicated that viral antigen and infectious virus were found in neural and non-neural organs. In contrast, in adult infected rats, BDV was found to be restricted to the nervous system. We have developed a quantitative polymerization chain reaction assay (PCR) to measure BDV-specific sequences in NB, chronic (CH) and acutely (AC) infected rats. We amplified BDV RNA in brain and peripheral white blood cells as well as in CSF, saliva, nasal secretions, urine, and stool. BDV-specific sequences were not recovered from normal rats, nor from plasma from BDV-infected rats. Identification of BDV in body secretions in rodents suggests a possible mechanism for transmission of BDV in nature.

503.10

NUCLEIC ACID SEQUENCE FOR THE ORF ENCODING THE 38 kD PROTEIN OF THE BORNA DISEASE AGENT. Gus Ray*, W. Ian Lipkin, Neurology; Anatomy & Neurobiology; Microbiology & Molecular Genetics, University of California, Irvine, CA 92717.

The Borna disease agent (BDA) is an RNA virus characterized by tropism for pyramidal neurons of hippocampus and a wide vertebrate host range. Serologic data suggest that BDA or a related virus may cause neuropsychiatric disease in man.

BDA RNAs are transcribed in the nucleus and then transported to the cytoplasm. Histochemistry indicates that BDA antigens are primarily nuclear in location. Here we report nucleic acid sequence for the ORF encoding the major immunogenic protein of BDA. Predicted amino sequence for this 38 kD protein includes a putative nuclear import signal motif.

503.11

VIRAL GLYCOPROTEINS MEDIATE NEURONAL INFECTION BY PSEUDORABIES VIRUS. J.P. Card, M.E. Whealy*, A.K. Robbins* & L.W. Enquist*. Viral Diseases Group, DuPont Merck Pharmaceutical Co., Wilmington, Delaware 19880.

We have previously demonstrated that wild type pseudorabies virus (PRV-Be; Becker strain) and an attenuated strain of PRV (PRV-Ba; Bartha) differentially infect rodent visual circuits. Intraocular injection of PRV-Be results in two temporally separated waves of infection which ultimately target all known retinorecipient neurons. In contrast, PRV-Ba only infects a functionally distinct subset of these neurons. These data suggest that the wild type virus recognizes two receptors which are differentially distributed upon projection specific populations of retinal ganglion cells and that the mutations present in PRV-Ba prevent it from interacting with one of those receptors. In the present analysis we have examined the effects of defined glycoprotein mutations upon the pattern of central infection induced by PRV. PRV-Ba contains a deletion which removes two glycoprotein genes (gI and gp63) in the unique short (Us) region of the genome and also produces a defective gIII glycoprotein. Two strains of PRV lacking the gene for glycoprotein gI were examined; PRV-91 contains a single deletion of the gI gene on a Becker background while PRV-D is the Bartha strain with a rescued Us segment and a gI deletion. These strains of PRV exhibited reduced virulence and also produced a pattern of central transport essentially identical to that observed following injection of PRV-Ba. These data indicate that gI is necessary for infection of a functionally distinct subset of retinal ganglion cells and supports the contention that invasion of the central nervous system by alpha herpesviruses is the product of a complex interaction of multiple viral envelope glycoproteins and cellular receptors.

503.12

NEUROACTIVE KYNURENINES AFTER INTRASPINAL INOCULATION OF MACAQUES WITH LIVE POLIOVIRUS M.P. Heyes*, K. Saito*, S.P. Markey* and J.H. Vickers[2] Section on Analytical Biochemistry[1], NIMH and Pathobiology and Primatology Laboratory[2], FDA, Bethesda MD 20892.

Immune stimulation increases the concentrations of quinolinic acid (QUIN), an excitotoxic kynurenine pathway metabolite (*J. Neurochem.* 51 1946, 1988; *Ann Neurol.* 29 202, 1991). In the present study, 96 rhesus macaques received an intraspinal injection of poliovirus (PV) as part of a neurovirulence test for vaccine safety and compared to 6 sham and 6 uninjected controls. CSF QUIN concentrations increased between 3- to 550-fold on day 17 and were highly correlated ($r > 0.70$) to increases in indoleamine-2,3-dioxygenase (IDO) activity and QUIN levels in the spinal cord. CSF L-kynurenine (L-KYN), anthranilic acid and kynurenic acid levels were also increased and correlated with CSF QUIN levels and spinal cord IDO activity ($r > 0.70$). Smaller increases in IDO activity and QUIN levels were found in motor cortex (a PV target area) without changes in frontal cortex. The largest metabolic and histopathologic changes occurred in macaques with lower limb paralysis. Quantitative measures of neurologic damage and inflammatory responses in the spinal cord correlated with the magnitude of each of these metabolic responses in CSF and spinal cord ($r>0.78$). Serum QUIN and L-KYN levels were unchanged in the PV-inoculated macaques. We conclude that PV-inoculated macaques offer a primate model to determine the effects of intracerebral immune stimulation on kynurenine pathway metabolism and to investigate whether endogenous production of neuroactive kynurenines *in vivo* are associated with functional or neuropathologic consequences.

503.13

HISTOPATHOLOGICAL CHANGES IN THE PITUITARY OF FEMALE HAMSTERS INFECTED WITH THE 139H STRAIN OF SCRAPIE. X. Ye,[1] R.I. Carp,*[2] S. Callahan,*[2] J. Shek[2]. [1]CSI/IBR Center for Developmental Neurosciences, [2]New York State Institute for Basic Research for Developmental Disabilities, 1050 Forest Hill Road, Staten Island, New York, 10314.

Previous studies showed that the 139H strain of scrapie injected intracerebrally in hamsters caused obesity, a marked hypertrophy of the islets of Langerhans in the pancreas, generalized endocrinopathy and marked hypoglycemia-hyperinsulinemia. The extremely low scrapie infectivity levels in pancreas suggested that the changes noted in that organ were of neuroendocrine origin. In the current study, female, weanling, Syrian hamsters, strain LVG/LAK, were injected intracerebrally with scrapie strain 139H or with normal hamster brain. Coronal sections of the pituitary were stained with hematoxylin and eosin, and were examined with light microscopy. The scrapie strain 139H infected hamsters showed extensive vacuolization in the pituitary. Most vacuoles were located on the ventral and/or ventrolateral parts of the pars distalis. The pituitaries of scrapie infected hamsters also showed cellular hypertrophy, cellular atrophy, and cytoplasmic vesicles. There were nuclear pathological changes such as swelling, vesicular changes, chromatin increase, pyknosis and karyorrhexis. The cellular and nuclear pathological changes were most pronounced in the regions with vacuolation. We will also report on immunocytochemical studies of pituitary cells from these animals. These studies will help establish the relationship between the changes in the central nervous system and the pathological findings seen in the pancreas and other endocrine organs in 139H infected hamsters.

503.14

BRAIN LEVELS OF NEUROPEPTIDE Y IN EXPERIMENTAL PNEUMOCOCCAL MENINGITIS IN RABBITS. M.G. Täuber*, S.L. Kennedy*, R.A. Sheldon*, L. Guerra-Romero* and D.M. Ferriero. Departments of Medicine and Neurology, Univ. Calif. San Francisco, San Francisco, CA 94143.

Alterations of cerebral blood flow (CBF) have been documented in humans and experimental animals with pneumococcal meningitis (PM). Both the loss of CBF autoregulation and vascular inflammation resulting in spasm and thrombosis are likely to affect CBF, but little is known about other factors that affect regulation of vascular tone and CBF during meningitis. Neuropeptide Y (NPY), which is found in high concentrations in nerve fibres surrounding cerebral vessels, has been proposed to play a role in regulating CBF in CNS diseases. In the present study we examined whether NPY concentrations change in various regions of the brain during experimental PM. Rabbits were infected intracisternally with S. pneumoniae. At various time points after infection, levels of NPY were determined by RIA in the brain regions indicated (expressed as ng/g of protein):

	Cortex	Medulla	Caudate	Hippocampus
0 hr (n=4)	6.5±4.1	3.8±1.3	7.8±1.3	25.0±18.7
24 hrs (n=5)	17.6±15.2	3.8±0.8	20.4±19.5	51.2±36.1
48 hrs (n=7)	9.3±4.2	12.9±5.6*	13.9±16.5	27.6±37.0

While all brain regions showed increasing NPY concentrations, typically after 24 hrs of infection, changes were most pronounced in the medulla (*$p<0.001$), where NPY levels peaked after 48 hrs of infection. Immunocytochemistry confirmed these findings, showing markedly increased NPY-immunoreactivity in nerve fibres surrounding small vessels in the medulla in animals after 48 hrs of PM compared to controls. These results suggest that NPY may play a role in CBF alterations occuring during PM.

NEUROTOXICITY: MPTP

504.1

REDUCTION IN GLUCOSE LEVELS ENHANCES 2'ET-MPTP-INDUCED TOXICITY IN PC12 CELLS. A.N.Basma*, R.E. Heikkila, E.T. Browning[1], M.S. Saporito, H.M. Geller[1] and W.J. Nicklas. Depts. of Neurology & [1]Pharmacology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854.

The active agent in MPTP neurotoxicity is thought to be its metabolite, MPP^+. MPP^+ and analogs such as 2'Et-MPP^+, are actively accumulated by mitochondrial preparations *in vitro* and block NADH dehydrogenase of complex I. This blockade results in biochemical sequelae which are cytotoxic. In certain model systems, the blockade of complex I is accompanied by an increase in glycolysis and a resultant stimulation in lactate formation. In this study, the catecholamine-containing PC12 cell line was used to investigate the effects of 2'Et-MPTP, 2'Et-MPP^+ and the complex 1 inhibitor, rotenone, on toxicity as well as the rates of glucose utilization and lactate production. Untreated PC12 cells utilized glucose and concomitantly produced lactate in a time-dependent manner at all concentrations of glucose studied. Treatment with 50μM 2'Et-MPP^+ or 50nM rotenone increased both rates significantly. Cell death caused by the toxins was also time-dependent and markedly attenuated by the availability of glucose in the medium. Iodoacetate (5μM), an inhibitor of glycolysis, was toxic to the cells suggesting that the glycolytic pathway is necessary for cell survival. The data further support the hypothesis that the ability of MPTP or its analogs to induce toxicity is dependent on the relative contributions of glycolysis and mitochondrial oxidation to the energy needs of a particular cell or model system.

504.2

INTRACELLULAR CALCIUM RESPONSES TO GLUTAMATE AND MPP^+ IN CULTURED CEREBELLAR GRANULE CELLS. A.M. Marini, Y. Ueda*, and C.H. June*. CNB, NINDS, NIH, and Naval Medical Research Institute, Bethesda, Maryland 20892.

The effects of glutamate on the intracellular calcium concentration were tested in cultured rat cerebellar granule cells loaded with fura-2 using digital image microscopy. During early culture times, glutamate (500 micromolar) elicited striking but transient increases in intracellular calcium levels. The response was rapid in onset (<10 sec) and the pattern of response heterogeneous in that only a subset of cells responded to glutamate. At later culture times (12-30 days in culture), glutamate caused no or only modest increases in intracellular calcium. The N-methyl-D-aspartate (NMDA) receptor antagonist, MK-801, caused a partial inhibition of the glutamate-induced calcium response; single cell analysis shows that the effects of MK-801 are complete on some cells and ineffective on other cells, suggesting that subsets of the cultured cells exist based on sensitivity to glutamate-induced calcium mobilization and the NMDA receptor antagonist.

We have shown previously that cultured cerebellar granule cells are susceptible to the neurotoxic effects of MPP^+. Exposure of cultured neurons to MPP^+ (50 micromolar) results in cell death by 72 to 96 hours. No significant increase in calcium was observed in MPP^+-treated cells for up to 48 hours, suggesting that signal transduction by calcium is unlikely to play a role in the initiation of MPP^+-induced neurotoxicity.

504.3

SENSITIVITY OF AN IMMORTALIZED DOPAMINERGIC CELL LINE TO MPP+ CYTOTOXICITY. H.K. Choi, L. Won, P.C. Hoffmann, and A. Heller
Dept. of Pharmacological and Physiological Sciences, The University of Chicago, Chicago, IL 60637

1-methyl-4-phenylpyridinium ion (MPP+) produces toxic effects on dopaminergic neurons of the substantia nigra in human, non-human primate, and mouse resulting in lesions similar to those found in human parkinsonism. The study of biochemical mechanisms underlying MPP+ toxicity is complicated by the heterogeneity of the cells obtained from dissection of the mesencephalon since dopaminergic neurons constitute less than 1% of the cells. Cell lines have simplified studies on diverse aspects of the nervous system. We have generated hybrid cell lines by fusion of cells from the mouse mesencephalic tegmentum at embryonic day 14 with a neuroblastoma cell line (N18TG2) (Choi et al., *Brain Res.*, in press). One of the monoclonal hybrid cell lines (MN9D) contains a high level of dopamine (DA). This line showed a marked sensitivity to the DA-depleting effect of MPP+ in contrast to a PC12 cell line. This report concerns the sensitivity of MN9D hybrid cells to the cytotoxicity of MPP+. Fifty thousand MN9D hybrid cells were plated per well in 48-well plastic culture plates with 500 ul of Eagle's basal medium containing 10 % fetal bovine serum and increasing concentrations of MPP+ from 1 uM to 1mM. Forty-eight hours later, the cells were trypsinized and counted. MN9D hybrid cells responded in a dose-dependent manner to the cytotoxicity of MPP+ with 1 uM MPP+ reducing the cell number to 65.9 ± 18.5 % of control. Ten uM MPP+ produced a reduction to 30.3 ± 12.2 % of control. Many of the cells detached from the plate and appeared non-viable. In contrast, the parental N18TG2 cells required an MPP+ concentration 2 orders of magnitude higher (100 uM) for a comparable level of cytotoxicity. The MN9D hybrid cell line therefore provides a useful model system for studying the mechanisms of toxicity of MPP+ as well as other cytotoxic substances affecting dopaminergic cells. Supported by MH28942 and GM07151.

504.4

ASSESSMENT OF THE NEUROTOXICITY OF A HALOPERIDOL PYRIDINIUM METABOLITE. H. Rollema, B. Subramanyam*, P. De Boer*, J. d'Engelbronner* and N. Castagnoli Jr*.
Dept. Medicinal Chemistry, University Groningen, 9713 AW The Netherlands and Dept. Chemistry, Virginia Tech, Blacksburg, VA 24061 USA.

The neurotoxic profile of the pyridinium metabolite derived from haloperidol, HALP+ (Subramanyam et al., BBRC, 166:238, 1990; Chem.Res.Toxicol., 4:123, 1991), was further characterized by intracerebral microdialysis studies of dopamine, serotonin and acetylcholine in rats. The extent of irreversible nerve damage was established by a standard MPP+ challenge perfusion one day after the intrastriatal infusion with HALP+. The almost similar toxic effects of HALP+ on serotonin (cortex) and acetylcholine (striatum) as on dopamine (striatum) suggest that HALP+ is less selective for dopaminergic neurons than MPP+. In vitro studies showed that HALP+ is a more potent inhibitor of respiration in intact mice liver mitochondria (RCR=12) than MPP+, the IC50 values being 35 uM and 113 uM respectively. Results of immuno-histochemical studies on the effects of unilateral intrastriatal infusions with HALP+, MPP+ and with the non-toxic N-methyl-pyridinium ion, via the dialysis probe, on the TH-ase activity in the ipsi- and contralateral substantia nigra will be presented. Until now our results show that HALP+ is a neurotoxin which may affect dopaminergic, serotonergic and cholinergic systems and that it, like MPP+, can inhibit mitochondrial respiration. Supported by NS23866, NATO-CRG890573 and the Peters Center, Virginia Tech.

504.5

ENHANCED MPTP TOXICITY IN THE RAT USING INTRANASAL ADMINISTRATION. C.C. Stahlbaum*[1], A. Giovanni, L. Manzino, R.E. Heikkila, and R.L. Doty*[1]. [1]Smell and Taste Center, Hosp. Univ. of Penn., Philadelphia, PA 19104 and Dept. of Neurology, UMDNJ-RWJ Medical School, Piscataway, NJ 08854.

Systemic administration of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induces a lesion of the dopaminergic nigrostriatal pathway in several species, including man, monkeys, mice and rats. Of these species, the rat is least suspectable to these neurotoxic effects, being relatively unaffected by a dose of MPTP which would produce an 80-90% loss of dopamine in the neostriata of mice. The present experiments compared the effects of MPTP administered intranasally or subcutaneously to rats. The acute behavioral effects associated with MPTP dosing appeared more quickly and at a lower dose under the intranasal condition. Additionally, the intranasal administration of MPTP results in an enhancement of dopaminergic neurotoxicity. For example, lower doses of MPTP can be used to produce similar decrements in striatal dopamine content. These data indicate intranasal administration of MPTP is a more effective means of inducing lesions of the dopaminergic nigrostriatal pathway than subcutaneous administration.

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504.6

CHRONIC PARAQUAT ADMINISTRATION DECREASES DOPAMINE TURNOVER IN THE MOUSE STRIATUM R.J. Zavodny* K.A. Young and P.B. Hicks. Department of Psychiatry, Scott & White Hospital/Foundation; Departments of Medical Pharmacology and Medical Anatomy and Neurobiology, Texas A&M College of Medicine, Temple, TX, 76508.

The close structural resemblance of the herbicide paraquat to the neurotoxin MPTP prompted us to investigate the putative neurotoxicity of paraquat. We exposed mature pigmented mice (8 mo.-old C57BL) to paraquat injections for 5 and 28 days to determine effects on striatal monoamine pathways. After a 5-day exposure to various doses of analytical grade paraquat (0.05-10 mg/kg SC), DA turnover (DOPAC+HVA/DA) in the striatum was decreased dose-dependently. The highest dose decreased DA turnover by 46% (ANOVA $p<.01$), although at that dose animals also displayed weight loss and respiratory distress. Mice injected SC with 1 mg/kg paraquat for 28 days maintained normal weight and displayed normal spontaneous locomotor behavior after 28 days treatment. There were no histological signs of toxicity to the lungs, liver or kidney. DA, norepinephrine, serotonin (5-HT) levels and DA D2 receptor density in the striatum were not significantly affected by paraquat treatment. Levels of the DA metabolites DOPAC and HVA, however, were significantly ($p<.05$) decreased by 26% and 12%, respectively, in the striatum of the paraquat-treated group, and DA turnover was significantly ($p<.05$) decreased by 18%. The turnover of serotonin was significantly ($p<.05$) decreased by 9%. Chronic treatment with 1 mg/kg commercial grade paraquat product (Ortho) produced very similar effects on striatal neurotransmitters. Paraquat binds with high affinity to melanin which is found in high concentration in nigrostriatal DA neurons, a factor that may contribute to the development of changes in neurotransmitter function in these neurons. Thus, compared to earlier studies which found no effect of paraquat treatment on monoamine function in the striatum of younger mice, our findings suggest that age and length of exposure may be factors in the development of paraquat effects on brain monoaminergic pathways.

504.7

CALBINDIN-D_{28k} IS LOCATED IN THE MIDBRAIN DOPAMINERGIC NEURONS WHICH ARE RESISTANT TO MPTP-INDUCED DEGENERATION. K.F. Manaye, P. K. Sonsalla, B.A. Brooks, and D.C. German. Dept. of Psychiat., UT Southwestern Med. Cntr., Dallas, TX.; Dept. of Neurol., UMDNJ-Robert Wood Johnson School of Med., Piscataway, N.J.; Dept. of Physiol., UT Health Sci. Cntr. San Antonio, TX.

The calcium-binding protein calbindin-D_{28k} (CaBP) is localized within the same population of midbrain dopaminergic (DA) neurons that is spared by Parkinson's disease (German et al., Neurosci. Abstr. 16:696, 1990). The present experiment sought to determine whether CaBP-containing cells are spared in MPTP-treated Macaca fascicularis monkeys (4-32 mg/kg, i.v.), and C57BL/6 mice (4 X 20 mg/kg, i.p.). Sections through the midbrain were stained with antibodies against tyrosine hydroxylase and CaBP, forebrain dopamine was measured, and computer imaging techniques were used to map the cell locations. In both species, regions containing many CaBP cells exhibited minimal DA cell loss, and regions containing few CaBP cells exhibited extensive DA cell loss (>75%). These data support the hypothesis that CaBP protects midbrain DA cells from MPTP-induced degeneration. Supported by American Parkinson Disease Association, and AG-08013.

504.8

EFFECTS OF GANGLIOSIDE GD1a ON MPTP NEUROTOXICITY IN MICE. M. Gupta, X.L. Chen and F.J. Roisen. Dept. of Anatomical Sciences & Neurobiology, Univ. of Louisville Sch. Med., Louisville, KY 40292.

Previous studies from this laboratory have shown that intraventricular injections of a ganglioside mixture (BBG) prior to MPTP treatment reduces MPTP toxicity on tyrosine hydroxylase (TH)-positive neurons in the susbtantia nigra (SN). The present studies were undertaken to investigate if individual gangliosides (that were present in the BBG mixture), when injected prior to MPTP treatment, would have a similar effect on the dopaminergic neurons of the SN. Young adult male C57BL/6 mice were anesthetized and injected with ganglioside GD1a (that comprised 43% of the ganglioside mixture, 2µl in each lateral ventricle, conc. 200mg/ml in 0.1M PO4). Control animals received a similar volume of the vehicle injections. 17-20 hrs later, one half of the control and GD1a-treated mice received multiple injections of MPTP (total dose 90mg/kg) and were sacrificed three days later under anesthezia. Brains were processed for TH immunocytochemistry and the number of TH-positive neurons were quantitated in the SN. The results show that GD1a alone does not alter the number of TH-immunoreactive neurons in the SN. However, when given prior to MPTP treatment, it reduces MPTP-induced loss of dopaminergic neurons in the SN. These data suggest that GD1a alone might play a role in preventing neuronal degeneration. Supported by USPHS grant NS24291 and the Parkinson's Disease Foundation.

504.9

Species-specific action of gangliosides on MPTP toxicity in vitro. F.J. Roisen, J. Sosnowski, G. Yorke and M. Gupta. Dept. of Anatomical Sciences and Neurobiology, University of Louisville School of Medicine, Louisville, KY 40292.

Previously, we have shown that MPTP has a dose-dependent effect on the survival of PC12 cells and that pretreatment of these cells with a mixture of bovine brain gangliosides (BBG) can partially prevent MPTP toxicity. In these studies we examined the efficacy of individual gangliosides in the mixture to reduce MPTP cytotoxicity. PC12 cells were maintained in RPMI 1640 medium supplemented with 10% horse serum and 5% fetal bovine serum in the presence of Nerve Growth Factor (NGF, 40 ng/ml) and the test agents for 6 hr prior to incorporation of MPTP into the medium. Alternatively, the cells were maintained in the medium supplemented with NGF and GM1 or GM3 (not present in BBG) for 4 days prior to MPTP exposure and continued for 4 days. Cytotoxicity was examined morphologically on the basis of neurite development, confluency and an index of dying cells. In the undifferentiated model, GM1 increased MPTP cytotoxicity. In contrast, GM3 surprisingly reduced MPTP-mediated degeneration. Furthermore, in the presence of NGF and MPTP, GM3 (but not GM1) had a significant effect in preventing MPTP toxicity. Similar results were obtained with differentiated cultures. The neuritogenic potential of GM3 and the action of released ganglioside oligosaccharides is being assessed. GM3 was more effective in potentiating NGF-mediated neuritogenesis than GM1. These studies demonstrate an apparent species-related effect of gangliosides in promoting neuritogenic activity and in preventing MPTP cytotoxicity on PC12 cells. Supported by NIH NS24524 and the Parkinson's Disease Foundation.

509.10

EFFECTS OF AN MPTP ANALOG ON DIFFERENT COMPARTMENTS OF THE SUBSTANTIA NIGRA. X.L. Chen and M.Gupta. Dept. of Anatomical Sciences & Neurobiology, Univ. of Louisville Sch. Med., Louisville, KY 40292.

It has been shown that the caudal (lateral) portions of the substantia nigra (SN) is more severly afffected than the rostral (and medial) SN in idiopathic Parkinson's disease, resulting in an uneven dopamine loss in the striatum. We have recently shown that MPTP analog, 2'-CH_3-MPTP, produces a significant decrease in the number of tyrosine hydroxylase (TH)-positive neurons in the SN. The present studies were undertaken to investigate if there is compartmental (medial to lateral) effect of 2'-CH_3-MPTP on TH-positive neurons in the SN and gliosis in the striatum. Young adult male C57BL/6 were treated with 2'-CH_3-MPTP (total dose 45-60mg/kg, i.p.) over a two day period, anesthetized and perfused with the fixative three days after MPTP and adjacent 40µm thick serial sections through the brain were immunostained for TH and Glial Acidic Fibrillary Protein (GFAP). The SN was divided into a medial and lateral compartment and the number of TH-positive neurons were quantitated. The number of GFAP-positive astrocytes were quantitated in medial, lateral, dorsal and ventral compartments of the striatum. The results show that 2'-CH_3-MPTP produces a dose-dependent decrease of TH-positive neurons in both the compartments of SN and and an increase in GFAP-immunoreactive astrocytes in the striatum. The following will be discussed: Is the reduction in TH-positive neurons more significant in any one of the compartments of SN; Is there a compartmental effect on gliosis in the striatum. Supported by USPHS grant NS24291 to MG.

509.11

CATECHOLAMINES INHIBIT MITOCHONDRIAL COMPLEX I ACTIVITY IN RAT BRAIN. **V. Jackson-Lewis, A. Naini, S. Przedborski, S. Simonetti*, S. Fahn & J.L. Cadet. Columbia University, Dept. of Neurology, New York, N.Y. 10032**

Complex I of the electron transport chain is one of the two main sites of superoxide radical formation in the mitochondria (Feher, 1985). 1-Methyl-4-phenylpyridinium (MPP^+) which is toxic to the dopamine (DA) system blocks the rotenone-sensitive site of Complex I in mammalian mitochondria. Recently, it was reported that the substantia nigra of Parkinson's Disease (PD) patients has abnormalities of Complex I. These changes might be related to the production of either endogenous or exogenous toxins. We, thus, assessed possible inhibitory effects of other DA-related compounds on mitochondrial rotenone-sensitive Complex I activity in rat brain. Mitochondria were prepared according to the methods of Clark and Nicklas (1970) and preincubated with various concentrations (0.1-10uM) of dopamine (DA), 6-hydroxydopamine (6-OHDA), norepinephrine (NE) and 6-hydroxydopa (6-OHDOPA). All four compounds caused significant inhibition of Complex I activity except for the lowest concentrations of DA and 6-OHDA. Inhibition occurred in the following order: 6-OHDOPA> NE> 6-OHDA> DA. The DA receptor antagonist, fluphenazine (FLU), also blocked the activity of Complex I. Since these compounds generate oxygen-based radicals during their metabolism, it is possible that these oxygen-based radicals are toxic to some component of the Complex I. These preliminary results also suggest that the reported abnormalities in Complex I activity in the substantia nigra of PD might be related to inhibition by endogenous dopamine.

509.12

PUTATIVE VESICULAR LOCATION OF ^{3}H-MPP^+ BINDING SITES IN MOUSE STRIATUM. M.Del Zompo, M.P.Piccardi*, S.Ruiu*, A.Vaccari*, G.U.Corsini**. Dept. Neuroscience, Univ. Cagliari, I-09124 Cagliari, and *Inst.Pharmacology, Med.School, Univ. of Pisa, I-56100 Pisa, ITALY

MPP^+ is actively transported into dopaminergic neurons by the catecholamine uptake system, where its accumulation seems required for MPTP neurotoxicity. The site of long-term MPP^+ storage in brain of sensitive species is unknown. A specific binding was obtained in brain with an apparent KD of 20 ± 2 nM and a Bmax of 1800 ± 197 fmol/mg protein. Almost all density of binding sites was found in the synaptosomal fraction. The regional distribution showed the highest density of binding sites in the striatum. The binding of ^{3}H-MPP^+ (0.12-4 nM) to mouse striatal membranes was saturable, specific and of high affinity (KD 1.4 ± 0.4 nM; Bmax 168 ± 15 fmol/mg of protein). Dopamine, tyramine and d-amphetamine were found to potently compete with ^{3}H-MPP^+ for its sites. (-)-Norepinephrine, reserpine and tetrabenazine were slightly weaker, though potent, displacers. 6-OHDA lesion significantly reduced (60-70%) the number of ^{3}H-MPP^+ sites. Our data are consistent with a vesicular location of high affinity MPP^+ binding sites in dopaminergic nigrostriatal terminals. Any possible involvement of this site on MPP^+ neurotoxicity deserves further investigation.

NEUROTOXICITY: BIOGENIC AMINES

505.1

ORAL ADMINISTRATION OF MDMA PRODUCES SELECTIVE SEROTONERGIC NEUROTOXICITY IN THE NONHUMAN PRIMATE. S.F. Ali, G.D. Newport*, A.C. Scallet, Z. Binienda*, S.A. Ferguson*, J.R. Bailey*, M.G. Paule and W. Slikker, Jr*. Div. of Reprod. and Developmental Toxicology, National Center for Toxicological Research, Jefferson, AR 72079.

MDMA (3,4-methylenedioxymethamphetamine) is a recreational drug known as "Ecstasy" and also has been used as an adjunct to psychotherapy. Several reports indicated that MDMA produces serotonergic neurotoxicity in primates at doses as low as 2.5 mg/kg (1-2 times the human dose). This study was designed to evaluate whether this and a lower dose of MDMA causes any selective serotonergic neurochemical alterations in primates. Adult female rhesus monkeys (n=4/group) were dosed orally with 0, 1.25, 2.5 or 20 mg/kg MDMA twice daily for 4 days. One month after the last dose, animals were sacrificed and brains were dissected into several regions for neurochemical analysis. Biogenic amines and their metabolites were analyzed by HPLC/EC. MDMA produced no statistically significant (ANOVA) alterations in 5-HT or 5-HIAA concentrations in different brain regions after 1.25 or 2.5 mg/kg, although hippocampal 5-HT concentrations were only 68% of controls after 2.5 mg/kg. At 20 mg/kg, however, MDMA produced significant and marked decreases in 5-HT and 5-HIAA concentrations in several cortical and midbrain structures. Concentrations of 5-HT and 5-HIAA were, however, not changed significantly in brain stem. When combined with earlier data, these results indicate a selective dose-response relationship for MDMA-induced neurotoxicity in the nonhuman primate.

505.2

THE INTERACTION OF AMBIENT TEMPERATURE WITH REPEATED METHAMPHETAMINE (METH) EXPOSURE: EFFECTS ON REGIONAL BRAIN MONOAMINE LEVELS IN THE RAT. R.R. Holson, S.F. Ali, G.D. Newport*, W. Slikker, Jr.* and J.F. Bowyer. Division of Reproductive and Developmental Toxicology, National Center for Toxicological Research, Jefferson, AR 72079.

Adult male rats were exposed to four ip METH or saline injections at 2-hr intervals. Some animals were kept at room temperature (23°C), others were placed in a cold room (4°C) during the 8 hrs of METH exposure. Animals were killed 1 day, 3 days or 2 weeks after exposure. Monoamine levels in caudate nucleus (CN), olfactory tubercle (OT) and hippocampus (HC) were measured by HPLC-EC. The effect of 5.0 mg/kg METH 4X at room temperature was similar in all three brain regions. Levels of dopamine (DA) and serotonin (5HT) were depressed at 3 days and showed substantial or full recovery by 2 weeks. Major metabolites of 5HT and DA responded similarly. DA and 5HT depletion was least in OT. DA depletion was greatest in CN, and 5HT depletion was greatest in HC. Cold exposure protected completely against these METH effects at 5.0 or 7.5 mg/kg, but only partially at 10 mg/kg METH levels. There was no effect of cold exposure per se on DA or 5HT levels in any brain region, and again this pattern was seen across regions for both 5HT and DA. It is concluded that cold can protect completely against METH toxicity at moderate but not high doses, that METH is equally toxic for 5HT and DA, and that METH toxicity does not appear to depend upon regional DA or 5HT concentrations.

505.3

METHAMPHETAMINE-INDUCED DEPLETION OF STRIATAL DOPAMINE: IMPACT OF INDOMETHACIN. T.G. Hastings and M.J. Zigmond
Dept. Behavioral Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260

The mechanism whereby methamphetamine causes the long-term depletion of striatal dopamine (DA) is unknown. However, there are parallels between methamphetamine- and ischemia-induced DA depletions. For example, both insults are accompanied by a large increase in extracellular DA, and the loss of tissue DA can be prevented by prior inhibition of DA synthesis. Thus, we hypothesized that other parallels might also exist and provide insights as to a shared mechanism for the neurotoxic events. Ischemic insult is known to increase free arachidonic acid as well as other fatty acids in whole brain and in striatum. This is accompanied by an elevation in arachidonic acid metabolites, such as prostaglandins and lipoxygenase products, and some of these products may contribute to the neurotoxic process. This proposal is supported by the observation that inhibition of arachidonic acid metabolism attenuates ischemic brain damage. To examine the role of arachidonic acid metabolism in methamphetamine-induced DA depletion, we examined the impact of pretreatment with an inhibitor of the cyclooxygenase component of prostaglandin synthase, indomethacin. Methamphetamine (3.25 - 25.0 mg/kg, s.c) was administered to male Sprague-Dawley rats (290-330 g) every 2 h for a total of 4 injections. When striatal tissue was analyzed by HPLC for DA content 7 days later, methamphetamine had caused a dose-dependent decrease in DA levels with a maximal effect observed at 12.5 mg/kg. When the drug effect was examined over time, DA depletion was maximal by 3 days and constant for at least 14 days. Indomethacin (5 mg/kg, s.c.) was then given 1 h before and 4 h after methamphetamine (4 x 12.5 mg/kg) and DA levels were measured 1 week later. Methamphetamine alone caused a 57% depletion of DA (saline, 157.3 ± 4.6 ng/mg protein; methamphetamine, 66.8 ± 18.6 ng/mg protein). This effect was not attenuated by indomethacin (methamphetamine + indomethacin, 38.4 ± 13.6 ng/mg protein). Thus, our initial results do not support the hypothesis that methamphetamine acts to deplete striatal DA via the cyclooxygenase component of arachidonic acid metabolism.

505.4

CALCIUM CHANNEL BLOCKERS ATTENUATE SEROTONIN NEUROTOXICITY INDUCED BY (±) 3,4-METHYLENEDIOXY-METHAMPHETAMINE (MDMA). M. Martello, A. Martello and G. Ricaurte. Department of Neurology, Francis Scott Key Med. Ctr., Johns Hopkins School of Medicine, Baltimore, MD 21224.

The finding that the N-methyl-D-aspartate (NMDA) receptor blocker, dextrorphan, prevents the serotonin (5-HT)-depleting effects of (±)3,4-methylenedioxymethamphetamine (MDMA) in rats (Finnegan et al., Neurosci. Lett. 105:300-306) has implicated excitatory amino acids (EAAs) in the neurotoxic action of MDMA. EAAs have been postulated to mediate neuronal injury by triggering excessive influx of calcium (Ca^{++}) into cells. The purpose of the present study was to test the hypothesis that Ca^{++} is involved in the neurotoxic action of MDMA. Rats were treated with MDMA alone and in combination with various doses of Ca^{++} channel blocking agents (flunarizine, nimodipine, verapamil). All of the Ca^{++} channel blockers examined afforded either partial or complete protection, depending on the dose of the blocker used and the regimen of MDMA employed. As none of the Ca^{++} channel blockers interfered with synaptosomal 5-HT uptake, it is unlikely that their neuroprotective effect is related to an interaction with the 5-HT transporter. These results suggest that Ca^{++} is involved in the neurotoxic action of MDMA. Further, they lend support to the view that the neurotoxicity of MDMA and related drugs may be mediated by endogenous EAAs.

505.5

PERSISTENT NEUROTOXIC EFFECTS OF METHAMPHETAMINE ON DEVELOPING DOPAMINE (DA) AND SEROTONIN (5-HT) NEURONS IN REAGGREGATE TISSUE CULTURE. L. Won, P.C. Hoffmann and A. Heller. Dept. of Pharmacological and Physiological Sciences, University of Chicago, Chicago, IL 60637.

Methamphetamine (Meth) produces long-term reductions in brain DA and 5-HT levels in adult animals (Ricaurte et al., Brain Res. 193: 153-163, 1980). In order to study whether there is a similar persistence of Meth's effect on developing neurons, we have used reaggregate tissue cultures containing neurons of mesencephalic tegmentum and corpus striatum from embryonic mice. Reaggregates were exposed for an acute (4 hrs of treatment on day 22 of culture) or chronic period (7 days of treatment, between 15-22 days of culture) to 10^{-4} M Meth and then allowed to recover (7 days for acute; 20 days for chronic exposure) in drug-free media. During the recovery period, samples of drug-treated, as well as control reaggregates were collected for analysis of monoamine levels. At the end of the acute or chronic drug treatment period, DA and 5-HT levels in Meth exposed reaggregates were markedly depressed (DA and 5-HT levels were 39% and 64% of control, respectively, for the acute treatment; 28% and 15% of control for the chronic exposure). During the recovery period, DA and 5-HT levels increased in both acute and chronically-treated reaggregates, as well as in control cultures. Despite increases in the treated groups, neither DA or 5-HT attained the levels observed in control reaggregates by the end of the recovery period. As with adult neurons, it is clear that the effects of Meth on developing neurons are marked and persistent. Supported by MH42134 and NIDA#271-90-7404.

505.6

PRESYNAPTIC GLUTAMATE(GLU)-EVOKED DOPAMINE (DA) RELEASE IS TRANSIENTLY INHIBITED BY METHAMPHETAMINE (METH) PRETREATMENT. J.F. Bowyer[1], A.W. Tank[2], G.D. Newport*[1], S.F. Ali[1], W. Slikker Jr*.[1], and R.R. Holson[1] [1]Div. of Reprod. & Dev. Tox., Natl. Ctr. for Tox. Res., Jefferson, AR 72079 and [2]Dept. of Pharmacol., Univ. of Rochester Med. Ctr., Rochester, NY 14642.

Exposure to toxic doses of METH is often associated with decreased striatal levels of tyrosine hydroxylase and DA. These experiments were designed to determine post METH changes in the presynaptic regulation of DA release and uptake using superfused striatal slices from adult male rats. DA levels in the striatum contralateral to that used for *in vitro* release were determined to be 88% of saline control 1 day post, 38% of control 3 days post, and 70% of control 14 days post METH (4 doses [1 dose/2 hrs] of 5 mg/kg). There were no changes in either the 15 mM K^+-and 1 μM METH-evoked [^{3}H]DA release or the amount of [^{3}H]DA accumulated in striatal slices of rats pre-treated with METH compared to control at any time point. These results indicate that, although METH decreased striatal DA levels, DA terminals may not have been destroyed. A 30% decrease (relative to saline control) in the 100 μM GLU-evoked [^{3}H]DA release (no Mg^{2+} present) was observed in striatal slices from 1 day post METH but not from 14 day post METH rats. Therefore, a transient down-regulation in GLU/NMDA receptors may occur 1 day or less post METH. Since environmental temperatures of 4°C during METH exposure did not prevent a decrease in GLU-evoked DA release from striatal slices 1 day post METH but significantly blocked striatal DA level decrease 3 days post METH, the relationship between the initial changes in GLU-evoked DA release and METH toxicity is unclear.

505.7

EFFECTS OF AMBIENT TEMPERATURE AND REPEATED INJECTIONS OF METHAMPHETAMINE ON CAUDATE MONOAMINES AS MEASURED BY CEREBRAL MICRODIALYSIS. B. Gough, J. Bowyer, S.F. Ali, W. Slikker, Jr. and R. Holson. Division of Reproductive and Developmental Toxicology, National Center for Toxicological Research, Jefferson, AR 72079.

Female Sprague-Dawley rats were injected four times at 2-hr intervals with vehicle or 5 mg/kg methamphetamine (METH). Some rats were caged individually at room temperature (23°C), others were held individually in a cold room (4°C). Extracellular levels of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), 5-hydroxy indole-acetic acid (5-HIAA), serotonin (5-HT) and homovanillic acid (HVA) were assayed in the caudate of these freely-moving rats using microdialysis and HPLC-EC. Dialysates were collected at 15 min intervals over the 8-hr course of dosing. METH reduced the classical effect on caudate monoamines. DA and 5-HT release was enhanced, while extracellular levels of their major metabolites (DOPAC and 5-HIAA) were reduced. Cold depressed extracellular levels of DOPAC, 5-HIAA and HVA, with or without METH exposure. The inhibitory effects of METH and cold on these metabolites was additive. This cold-induced depression of caudate monoamine metabolites may be related to the ability of cold to block toxic METH effects on caudate 5-HT and DA content.

505.8

PROTEIN SYNTHESIS INHIBITION BLOCKS THE NEUROTOXIC EFFECTS OF METHAMPHETAMINE IN RATS. KT Finnegan and R Karler. Depts. of Psychiatry and Pharmacology, Univ. of Utah Sch. Med. and the Salt Lake City VAMC, Salt Lake City, UT 84148.

Programmed cell death is a striking feature of normal embryonic growth and development. The CNS is a particularly common site, with an estimated one half of all neurons present during embryogenesis dying before adulthood. The survival of neurons appears to depend upon the continuous secretion of various tropic factors by target tissues. Historically, neurotropic factors have been thought to promote neuronal survivability by acting to sustain critical intracellular metabolic processes. Recently, however, it has been shown that inhibitors of protein synthesis prevent neuronal death induced by tropic factor deprivation. Cell death during embryogenesis thus appears to be an active process, involving the synthesis of new ("killer") proteins. Based on these findings, we examined whether protein synthesis might also be involved in the neuronal damaging effects of methamphetamine (METH) in the mature rodent CNS.

Male CF-1 mice were injected with METH, either alone or in combination with the protein synthesis inhibitors cycloheximide or anisomycin, and then killed one week later. Neostriatal DA, DOPAC, and HVA were assayed by HPLC-EC. METH alone induced a 50-60% depletion of neostriatal DA and its metabolites. Pretreatment with either cycloheximide or anisomycin, however, completely blocked the neurotoxic effects of METH. In other experiments, cycloheximide failed to alter either amphetamine-induced stereotypy or the threshold for convulsions induced by N-methyl-D-aspartate. The latter findings indicate that the neuroprotective effects of cycloheximide are unlikely to be explained by an antagonistic action at either DA or NMDA receptors. The findings suggest that programmed cell death during embryogenesis and METH-induced neuronal cell damage in the mature CNS involve similar biochemical mechanisms.

505.9

MK-801 PROTECTS AGAINST STRIATAL DOPAMINE TERMINAL DAMAGE AND REDUCES EXTRACELLULAR DOPAMINE OVERFLOW DURING A NEUROTOXIC REGIMEN OF METHAMPHETAMINE. F.B. Weihmuller, S.J. O'Dell and J.F. Marshall. Dept. of Psychobiology, University of California, Irvine, CA 92717.

Although the NMDA antagonist MK-801 prevents methamphetamine (m-AMPH)-induced damage to striatal dopamine (DA) terminals, the protective mechanism remains unknown. Using microdialysis in awake rats we show that a neurotoxic schedule of m-AMPH injections (4 x 4 mg m-AMPH/kg, sc, every 2 hrs) produced a massive overflow of striatal DA, peaking after the fourth injection. Also, total DA overflow was negatively related (r = -0.74) to remaining DA levels in striatal tissue measured one week later. Several pretreatment schedules with MK-801 (0.5 mg/kg, ip) provide protection against m-AMPH toxicity: 15 min before each m-AMPH treatment, before each of the last two m-AMPH injections, or before the first m-AMPH injection only. Each schedule of MK-801 pretreatment attenuated both the striatal DA overflow and the striatal tissue DA loss as measured one week later. In contrast, a single injection of MK-801 15 min before the fourth m-AMPH treatment produced only a modest decrease in total DA overflow and did not attenuate m-AMPH-induced injury to striatal DA terminals. These findings suggest: 1) that the large increase in striatal DA overflow is correlated with terminal damage and 2) that MK-801 might prevent this damage by reducing the amount of DA released.

505.10

DOPAMINE RECEPTOR ANTAGONISTS ATTENUATE METHAMPHETAMINE-INDUCED STRIATAL DOPAMINE OVERFLOW AND TERMINAL DAMAGE. S.J. O'Dell, F.B. Weihmuller and J.F. Marshall. Dept. of Psychobiology, Univ. of California, Irvine, CA. 92717

Four injections of methamphetamine (m-AMPH, 4 mg/kg, sc, at 2 hr intervals) produce a massive efflux of striatal dopamine (DA), peaking after the fourth injection and result in damage to striatal DA terminals. Moreover, the amount of DA overflow is positively correlated with the degree of subsequent DA depletion. We used microdialysis to examine m-AMPH-induced striatal DA efflux in rats treated concomitantly with the neuroprotective agents SCH 23390 (a D1 receptor antagonist) or eticlopride (a D2 receptor antagonist). Four injections of SCH 23390 or eticlopride alone induced modest increases in striatal DA overflow (reaching a maximum of 2-3 fold of pre-treatment values). However, when given 15 min prior to each m-AMPH treatment, either eticlopride or SCH 23390 reduced total DA overflow when compared to animals treated with m-AMPH alone. Additionally, pretreatment with eticlopride completely protected against m-AMPH-induced dopaminergic damage while SCH 23390 pretreatment afforded substantial, but not total protection. One mechanism by which DA receptor antagonists may attenuate m-AMPH-induced neurotoxicity is by reducing stimulant-induced increases in DA overflow.

505.11

EFFECT OF PARGYLINE PRETREATMENT ON P-CHLORO-AMPHETAMINE (PCA)-INDUCED NEUROTOXICITY TO SEROTONIN NEURONS IN RAT BRAIN ASSESSED USING [3H]CYANOIMIPRAMINE AUTORADIOGRAPHY. Saloua Benmansour* and David J. Brunswick, Dept. of Psychiatry, Univ. of Pa. Sch. of Med. and Dept. Vet. Affairs Med. Ctr., Phila., PA 19104.

A number of drugs with an amphetamine-like structure can produce long-term neurotoxic effects on serotonergic neurons in brain. It has been suggested that the weak monoamine oxidase (MAO) inhibitory properties of these amphetamines contribute to their neurotoxic effects. If so, then more complete inhibition of MAO might potentiate their neurotoxicity. In the present study, the effect of pretreatment of rats with the MAO inhibitor pargyline on pCA-induced neurotoxicity was examined by quantitative autoradiography using [3H]cyanoimipramine. This radioligand binds with high affinity and specificity to serotonin uptake sites. There were three groups of rats (n=6/group): 1) saline four hours prior to PCA (4mg/kg, s.c.); 2) pargyline (50mg/kg, s.c.) four hours prior to PCA; 3) saline four hours prior to saline. Rats were killed 7 days after drug administration. PCA treatment alone caused significant reductions (30-70%) in [3H]cyanoimipramine binding in all terminal fields receiving serotonergic innervation but not in cell body areas (dorsal and median raphe nuclei). Pargyline pretreatment significantly potentiated the neurotoxic effect of pCA in nine of the terminal field areas and produced a trend for greater toxicity than that seen with PCA alone in the other terminal areas. These results show that increasing the inhibition of MAO potentiates the neurotoxicity of pCA. (Research funds from the Dept. of Vet. Affairs & USPHS grant DA 05317).

505.12

DOPAMINERGIC NEUROTOXICITY IN RAT STRIATUM? F. Filloux and J.J. Townsend. Depts. Neurol., Pediatr., Psychiat. and Pathol., Univ. Utah Sch. Med., Salt Lake City, UT 84132.

Considerable evidence indicates that dopamine (DA) may play a neurotoxic role in brain under certain pathologic circumstances. To investigate this issue, a high concentration of DA (1000 nmoles/μl) was injected into the striatum of anesthetized 200 gm Sprague-Dawley rats. Control animals received injections of GABA or NaCl at identical concentrations (diluted in dH_2O, pH 7.0-7.7, as reported by others: Olney & Gubareff, Nature 271:557, 1978). Brains were removed 9 days later, frozen and sectioned (10μm) for histologic and autoradiographic analysis. DA injection resulted in a small volume (~3mm^3) lesion in comparison to control GABA and NaCl injections which produced only a needle track of < 0.6 mm^3 in volume ($p < 0.01$). Within the DA lesion, marked neuronal loss, macrophage invasion and glial proliferation was present. Acetylcholinesterase staining and D1 receptor binding were markedly reduced as well. [^{3}H] R05-4864 binding (to peripheral benzodiazepine receptors on astrocytes) was increased. The findings support the contention that dopamine may act as a low potency neurotoxin.

505.13

INDUCTION OF c-*fos* mRNA BY STIMULATION OF D_1-DOPAMINE RECEPTORS IN NS20Y NEUROBLASTOMA CELLS: EFFECTS OF MANGANESE CHLORIDE. S. Peña*, D.J. Minnema*, and G.P. Cooper. Dept. Environ. Hlth., University of Cincinnati, Cincinnati, OH 45267-0056.

D_1-dopamine receptors are coupled to stimulatory G-proteins that activate adenylate cyclase. Recently, it has been shown that selective D_1 dopamine agonists can induce the expression of c-*fos* mRNA in the rat striatum. However, the dose-dependency and time-course of this induction have not been characterized. For this purpose we are using NS20Y neuroblastoma cells, which express D_1 receptors. Cells were synchronized by serum depravation for 72 hours prior to treatments and were exposed to dopamine and/or manganese for 30 minutes. The effect of dopamine on c-*fos* mRNA expression is dose-dependent and ranges from 1 nM to 100 μM. The dopamine concentration giving maximal induction is ~1 μM. This effect on c-*fos* mRNA expression is specific, as shown by its inhibition by the selective D_1 antagonist SCH 23390. Induction of c-*fos* mRNA by manganese occurs at a concentration of 30 μM. Ongoing experiments involve determination of the effects of manganese on the dopamine dose-effect curve and time-course of induction. The effects of lead (Pb^{2+}) on c-*fos* expression are also being investigated.

505.14

DEVELOPMENTAL ONSET OF SEROTONIN ALTERATIONS IN THE CHRONICALLY HYPERAMMONEMIC SPARSE FUR MOUSE. S. Djali, B.A. McLaughlin, M.L. Batshaw and M.B. Robinson. Children's Seashore House; Depts. Ped. and Pharm., Univ. of PA; Philadelphia, PA, 19104.

Due to an X-linked genetic deficiency, the congenitally hyperammonemic sparse fur mouse (*spf*/Y), has only 5-20% of normal ornithine carbamoyltransferase activity. In the cortex of adult *spf*/Y mice, we previously demonstrated two-fold increases in the levels of the serotonin metabolite 5-hydroxyindole acetic acid (5-HIAA). The purpose of this study was to evaluate the development of serotonin alterations in the *spf*/Y mouse. Brain glutamine (Gln) levels were used as an index of hyperammonemia. Cortical levels of tryptophan, serotonin, 5-HIAA, norepinephrine, homovanillic acid, γ-aminobutryric acid, glutamate, and glutamine were measured in one- and three-week old *spf*/Y mice and littermate controls (B6). In one-week-old *spf*/Y mice, there were no alterations in 5-HIAA levels (*spf*/Y, 1.97 ± 0.97; B6, 2.26 ± 0.60 pmol/mg, $p > 0.5$, mean ± SD, n=6), or Gln levels (*spf*/Y, 9.80 ± 2.35; B6, 8.95± 2.28 nmol/mg, $p > 0.5$). Glutamate levels were significantly decreased (*spf*/Y, 6.53 ± 0.69; B6, 7.53 ± 0.47 nmol/mg, $p < 0.01$). At three weeks of age, Gln levels were increased two-fold (*spf*/Y, 10.4 ± 4.50; B6, 5.35 ± 2.51, $p < 0.04$), and there was a trend towards an increase in 5-HIAA (*spf*/Y, 3.68 ± 0.54; B6, 3.09 ± 0.56 pmol/mg, $p < 0.09$) in preliminary studies. There were no alterations in the other neurotransmitters or their metabolites at either age. The finding that Gln correlates with 5-HIAA in three-week-old animals ($r = 0.758$, $n = 12$, $p < 0.01$) supports the hypothesis that the increase in 5-HIAA found in hyperammonemia may be due to increased brain Gln levels.

505.15

COCAINE INDUCED HYPOXIA *IN UTERO* RESULTS IN A CNS "AT RISK" FOR INJURY: GM1 REDUCES POSTNATAL DYSFUNCTIONS AND INCREASED VULNERABILITY TO CNS DAMAGE.

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Since GM1 (monosialoganglioside) treatment reduces CNS ischemic injury, this study was undertaken to assess if this neuroprotective agent might decrease CNS damage associated with *in utero* hypoxia that results from the vasoconstrictive effects of cocaine exposure. Pregnant rats (Wistar) were orally dosed (prenatal days 7-20) with cocaine hydrochloride (60mg/kg) and either GM1 (10mg/kg sub.cu.) or saline. Pups born from cocaine exposed dames [CEDs] had reduced ultrasonic vocalization rates & nipple attachment latencies, and increased activity levels. Pups born from GM1 treated CEDs did not show these dysfunctions, and were not different from vehicle controls. Primary neuronal cell cultures (cortical neurons harvested at pre-natal day 15 from CED fetuses) had reduced thresholds to glutamate toxicity as evidenced by associated increases in urea & Ca^{++} uptake. This increased vulnerability to injury was not seen in cells from GM1 treated CED fetuses. This increased vulnerability to CNS damage was also evidenced when cortical focal ischemia was induced in mature rats. In rats born of CEDs there were higher levels of edema found in primary & peri-ischemic cortices. These increased levels of injury were reduced in rats born from CEDs treated with GM1.

GM1's neuroprotective effects can occur *in utero* as evidenced by the reduced postnatal dysfunctions. We hypothesize that cocaine abuse during pregnancy may result in a CNS cell population whose injury threshold is markedly lower, resulting in a mature CNS that is "at risk" to injury (e.g. trauma, fever, neurotoxins etc.) for its entire life-span.

This work was supported in part by a grant from the FIDIA Research Foundation and a BRSG grant.

507

SYMPOSIUM. THE CANNABINOID RECEPTOR: BIOCHEMISTRY, ANATOMY AND PHYSIOLOGY. A.C. Howlett, Saint Louis Univ. (Chairperson); M. Herkenham, NIMH; S. Ward, Sterling Research Group; L. Matsuda*, NIMH; S. Deadwyler, Bowman Gray Med. Sch.

The cannabinoid receptor transduces the response to Δ^9-tetrahydrocannabinol (THC), the primary active constituent in marihuana extracts. This receptor couples to G_i to inhibit adenylate cyclase. The *in vitro* structure-activity profiles for a series of synthetic cannabinoid analogs parallel a number of biological responses in humans and animals. Novel aminoalkylindole analgesics also bind to this receptor and elicit cannabinoid biological activities. Certain compounds from this series behave as antagonists *in vitro*. Autoradiographic localization studies reveal a unique cytoarchitectural distribution of cannabinoid receptors which is similar across all species examined including human. Receptors are concentrated in motor systems (basal ganglia, cerebellum) and cerebral cortex (notably the hippocampus), and are sparse in the brainstem and spinal cord. Cannabinoid receptor mRNA is prominent in the hippocampus and other CNS structures. Physiological and behavioral effects of cannabimimetic drugs are being assessed in freely moving animals, particularly with respect to impairments in cognitive processing. In addition, analyses of cannabinoid actions on single cells in culture are being studied with regard to whole cell conductances and the nature of receptor-channel interactions.

508

SYMPOSIUM. SPECIFICATION OF CEREBRAL CORTEX DURING DEVELOPMENT. M. Sur, M.I.T. (Chairperson); P. Rakic, Yale Univ. Sch. of Med. (Chairperson); S.K. McConnell, Stanford Univ.; J. Bolz, Friedrich-Meischer Lab., Tubingen; R. Linsker, I.B.M. T.J.Watson Res. Ctr.

The development of an area of the cerebral cortex involves the specification of several features that characterize the area, including the location and composition of its cells (cytoarchitecture), its external connections (inputs and outputs), its internal connections (microcircuitry), and its molecular make-up (chemoarchitecture). This symposium will review recent results on the extent to which these features are regulated by intrinsic developmental programs and by extrinsic factors such as input activity. P. Rakic will describe the radial unit hypothesis for generation of cortical laminae, the role of thalamic afferents in specifying cortical areas, and the matching of retinal, subcortical and cortical "protomaps" during development. S. McConnell will describe the processes by which the cortex generates neurons and parcels them into different laminae and different areas, in the context of layer-specific and area-specific long-distance connections. J. Bolz will show that highly specific afferent and efferent cortical connections can form in vitro, in slice cultures of thalamus and cortex; these results indicate that the cortex guides its innervation by thalamic afferents by regulating molecules that facilitate proper target selection. M. Sur will describe how a visual field map and neurons with receptive field properties similar to primary visual cortex arise in auditory cortex in ferrets that have retinal input diverted into their auditory pathway, indicating that cortical microcircuitry is both intrinsically specified as well as regulated by input activity. R. Linsker will describe theoretical studies of the self-organization of cortical microcircuitry, and present modeling results to demonstrate how cortical mechanisms may generate ordered maps and receptive fields whose properties depend upon an area's external connections, its internal structure, and correlated input activity during development.

POTASSIUM CHANNELS: MOLECULAR BIOLOGY II

510.1

TEMPORAL AND SPATIAL DISTRIBUTION OF RAT BRAIN K^+ CHANNELS.

S.Verma and R.H.Joho[1]. Division of Neuroscience and [1]Dept. of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, TX 77030.

Recently a number of related mammalian K^+ channel cDNA clones have been isolated. The biological significance of this diversity is not understood. To examine this, we looked at the distribution of K^+ channels in various neonatal and adult tissues and throughout the rat brain by *in situ* hybridization. The following cDNA isolates, each representing a subfamily, were used in this study : DRK1, R-NGK2, RCK2, K13, and IK8. We found brain specific expression of RCK2 and R-NGK2; in contrast the other clones were also present in non-neuronal tissues. *In situ* hybridization with adult rat brain showed distinct but overlapping distribution patterns for each of the isolates examined. All channels were present in some of the cortical layers. DRK1, RCK2, and K13 were all expressed in the hippocampal cell layers, whereas R-NGK2 and IK8 were expressed at very low levels. Interestingly, in contrast to others, R-NGK2 has non-homogenous expression in the cortical and hippocampal cell layers. Only K13 and R-NGK2 were present in the pons and medulla. All channels except IK8 were expressed in the cerebellum. In particular R-NGK2 was very high in the Purkinje and granular layers and RCK2 was present only in the Purkinje layer.

Since R-NGK2 expression is mainly in the adult cerebellum (the level of expression is five times less in the rest of the brain), we decided to look at the time course of appearance of R-NGK2 during development. We have found that its expression starts at postnatal day 11 in the cerebellum and continues up to and past day 20.

510.2

GLIAL- AND NEURONAL- SPECIFIC K+ CHANNEL GENE EXPRESSION IN THE CNS OF THE MOUSE.

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Voltage-gated potassium (K^+) channels are important modulators of membrane excitability in neurons and other electrically polarized cells. We have examined the cellular distribution of two different K^+ channel genes in the central nervous system (CNS) of the mouse. These genes, designated MK1 and MK2, each direct the expression, in injected Xenopus oocytes, of delayed rectifier channels with slightly different kinetic properties. Their respective patterns of CNS expression were analyzed using the technique of in situ hybridization, in which oligonucleotide probes directed against two different nonconserved regions of each gene were 3' end-labelled with ^{35}S-ATP and applied to 20 micron frontal sections of normal adult male mouse brains, using standardized protocols. We find that MK1 and MK2 have markedly different patterns of expression. MK1 is expressed in particular subsets of neuronal cell bodies, notably in regions of the brain which are known to produce high frequency actions potentials, i.e. the auditory pathway, vestibular pathway, and motor pathways, and the hippocampus. In contrast, MK2 is expressed in the major fiber tracts of the brain, and thus appears to represent a glial cell-specific K^+ channel gene.

510.3

MOLECULAR MECHANISMS OF IONIC SELECTIVITY IDENTIFIED BY SITE-DIRECTED MUTAGENESIS IN THE ShB K^+ CHANNEL. *Andrea Yool, Ross Vickery*, Robert Burgess* and Thomas Schwarz.* Molecular & Cellular Physiology, Stanford University, Stanford CA 94305-5426.

To identify the region of the K^+ channel that influences ionic selectivity, we used site-directed mutagenesis to change single amino acids in the ShB K^+ channel from *Drosophila*. Our data support the hypothesis that the H5 region (asp431 to thr449) lines the pore of the channel. A plausible model places H5 as a β-barrel in the center of a tetrameric complex of Shaker-encoded proteins. Properties of the mutant and wildtype channels, expressed in *Xenopus* oocytes, were assayed by single channel and macropatch electrophysiology in bi-ionic recording conditions with the test cation present externally, and K^+ internally (the reversal potential of the current-voltage relationship is a direct measurement of permeability of a test ion relative to K^+). In homomultimeric mutant channels, two amino acids (phe433 and thr441) each increased permeability to NH_4^+ when mutated to serine (Yool and Schwarz, 1991, *Nature* 349: 700-704). The increased permeation by NH_4^+ is consistent with the model that H5 lines the conductive pathway; both of these mutations reduce bulkiness, and maintain or increase the polar nature of the side chains. Mutations at residues outside of H5 had little effect on selectivity. Many of the mutations created within H5 produced nulls, yielding no detectable currents when expressed as homomultimers. Preliminary results have demonstrated that some of these nulls can be "rescued" by pairing the mutant subunits with wildtype subunits in tandem constructs that link the two subunits into a single polypeptide. These hybrid channels produce effects on selectivity that are consistent with the H5 hypothesis. The size of the pore in mutant channels can be probed with organic cations such as methyl-substituted ammonium derivatives, hydroxyamine and guanidine. Mutations that increase permeability to NH_4^+ also increase permeability to the larger organic cations. By the systematic mutation of residues within H5, we intend to identify the molecular mechanisms that generate the ionic selectivity of the K^+ channel. *(Supported by NIH 2HQZ-402 to TLS).*

510.4

REVERSIONS IN THE PORE OF A CHIMERIC POTASSIUM CHANNEL. J.A. Drewe, G.E. Kirsch, H.A. Hartmann, M. Taglialatela, M. De Biasi, A.M. Brown and R.H. Joho. Dept. Mol. Physiol. & Biophys., Baylor College of Medicine, Houston, TX 77030.

In the chimeric channel DRK-NGK (Hartmann, Science 1991), a stretch of cDNA encoding 21 amino acids was exchanged between the donor channel rat-NGK2 (Drewe, submitted) and the host channel DRK1 (Frech, Nature 1989). The exchange conferred upon the host channel the phenotype of the pore of the donor channel, rat-NGK2. Thus, DRK-NGK had the slope conductance of the donor rat-NGK2 channel which was about three times that of the host DRK1 channel and, DRK-NGK like NGK2, was blocked by the open channel blocker TEA at an external site whereas DRK1 was not blocked with external application but with internal application. It follows that the differences between DRK-NGK and DRK1 must have arisen from the nine amino acid differences exchanged between DRK1 and rat-NGK2. We reverted each of these nine amino acids using site specific mutagenesis and tested the results on conductance and TEA blockade. One reversion, L374V, restored an exaggerated version of the conductance of K^+ and Rb^+ by DRK1 although the TEA blocking profile was unaffected. Two reversions, Q382K and M387K, reduced inward current and blockade by external TEA. Supported by National Institutes of Health Grant Nos. NS08805 to J.A. Drewe, NS28407 to R.H. Joho, and HL39262, HL37044, HL36930 and NS23877 to A.M. Brown.

510.5

A CHIMERIC K^+ CHANNEL MUTANT WITH REDUCED SENSITIVITY TO TETRAPENTYLAMMONIUM BLOCKADE. M. De Biasi, M. Taglialatela, J. A. Drewe, H. A. Hartmann, A. M. Brown and G. E. Kirsch. Department of Molecular Physiology and Biophysics. Baylor College of Medicine, Houston TX 77030

Chimeric K^+ channels (DRK/NGK) formed by replacing the pore region of DRK1 with that of NGK2 were shown previously to have the TEA blocking profile of NGK2; extracellular TEA was much more potent than intracellular TEA. In the present study we tested a hydrophobic TEA derivative, tetrapentylammonium (TPeA) in DRK/NGK and mutant chimeras in which single amino acids in the pore region were restored to the corresponding DRK1 residues. Macroscopic K^+ currents were measured in Xenopus oocytes. External TPeA blocked DRK/NGK in a time- and voltage-dependent manner ($IC_{50} = 88\,\mu M$). Based on its voltage dependence, TPeA is thought to have an intracellular site of action. Consistent with this idea we found that TPeA sensitivity was unchanged in Q382K a mutant with markedly reduced sensitivity to external TEA. In contrast, L374V, a mutant with altered ion selectivity (Drewe, et al. this meeting), was found to be much less sensitive to TPeA (IC50 >5 mM) than DRK/NGK. Even though TPeA is an open channel blocker the observed reduction in sensitivity does not depend on decreased channel open time in L374V, since another mutant, V369I, was found to have decreased open time and high sensitivity to TPeA. Our results suggest that L374 interacts with a TPeA binding site near the intracellular mouth of the pore. This result is consistent with the idea that the pore lining is formed by a membrane-bound loop extending between two proline residues (P361 and P381) and includes L374.

510.6

GATING CURRENTS FROM A CHIMERIC K+ CHANNEL EXPRESSED IN *XENOPUS OOCYTES* [1]M. Taglialatela, [2]G.E. Kirsch, [1]A.M. Brown and [1]E. Stefani*. [1]Dept. Molecular Physiology and Biophysics and [2]Dept. Anesthesiology, Baylor College of Medicine, 1 Baylor Plaza, 77030 Houston, TX.

Structural rearrangements of the voltage sensor of voltage-dependent ion channels under the influence of the electric field generate gating currents. We have studied the gating currents of a chimeric delayed rectifier (DR) K^+ channel, DRK/NGK M3, in which the pore phenotype of the donor NGK2 was exchanged for that of the host DRK1. A single point mutation (L374V) caused a dramatic decrease in the single channel K^+ conductance of the chimeric channel. The experiments were performed with a novel open-oocyte vaseline gap method. Many properties of the DRK/NGK M3 gating currents were similar to those reported for the DR of the squid giant axon. A rising phase was resolved in the ON transient and it had little or no voltage-dependence. The gating currents appeared at more negative potentials than the ionic currents (15 mV shift) and saturated at positive potentials (+40). They showed no sign of charge immobilization during a 50-200 msec depolarizing pulse which did not inactivate the ionic current. The kinetics of the decay of the ON and OFF gating transient had the same time course of activation and deactivation of the ionic current. Hyperpolarizing conditioning pulses delayed the activation of the ON transient. The data are consistent with an early voltage-independent transition between the closed states followed by voltage-dependent transitions between later closed states and a final voltage-independent closed-open transition.

510.7

MUTATIONS IN THE LEUCINE HEPTAD REPEAT OF *SHAKER* 29-4 ALTER THE CHANNEL'S ACTIVATION WITHOUT ALTERING GATING CURRENTS

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Mutating either of the first two leucines in the conserved heptad repeat region of *Shaker* cDNA 29-4 to valines causes a 60-100 mV depolarizing shift and a dramatic reduction in the voltage sensitivity of channel activation (McCormack et al., PNAS 88: 2931). In search of an explanation for these changes, we recorded "macroscopic" ionic currents and gating currents in membrane patches from oocytes injected with mRNA encoding non-inactivating wild-type and mutant channels. All recordings were made in the inside-out mode with an NMG-Aspartate pipette solution. Bath solutions contained K-Asp and TEA-Asp for measuring ionic and gating currents, respectively. Gating current measurements showed that the voltage dependence and kinetics of most of the charge movement were similar in the wild-type and mutant channels. Moreover, the number of effective charges displaced per channel opening appeared to be similar for the two channels. Lower bounds for z obtained from the limiting slopes of the activation curves (at $P(o) < 0.01$) were similar : 9.9 ± 1.3 (N=4) and 8.7 (N=2) for wild-type and mutant channels. These values were close to those obtained from experiments which directly evaluated z. First, the number of channels in a patch was estimated from noise analysis on ionic currents; then, following the replacement of K with TEA, the charge movement was measured in the same patch. From these measurements, z was calculated to be 8.9 ± 1.8 (N=5) and 8.6 ± 1.9 (N=5) for the wild-type and mutant channels. The mutation appears to affect steps in the activation process that follow most of the charge displacement.

510.8

STRUCTURE AND FUNCTION OF VOLTAGE DEPENDENT K^+ CHANNELS: SUBUNITS AND SUBCONDUCTANCES. A.M.J. VanDongen and A.M. Brown. Dept. Mol. Physiol. and Biophys., Baylor College of Medicine, Houston, TX 77030.

Voltage dependent K^+ channels are thought to consist of four identical subunits, containing 6 transmembrane segments (S1-S6) that surround a central pore, which is formed by the linker regions between S5 and S6. Every subunit contributes to the lining of the pore. Two mechanisms could underlie channel opening and closing: a change in the physical dimension or the chemical properties of the pore. No matter what the mechanism is, each individual subunit must undergo a conformational change when the channel opens. This results in 3 intermediate states, where 1,2 or 3 subunits are in the "open" conformation. In this paper we want to investigate the role of the individual subunits in channel opening and closing. Experiments were done using *Xenopus* oocytes expressing a delayed rectifier K^+ channel, *drk1*, and a chimeric *drk1* that contains the S5-S6 linker region of *ngk2*. Single channel data from *drk1* showed subconductance levels of 2.5, 5.0 and 7.5 pS in addition to the full (10 pS) conductance. These sub-conductance states were very short lived and usually occurred as shoulders at opening and closing transitions. These results suggest that (i) the intermediate states are conducting, (ii) they are very unstable and (iii) each subunit makes an equal contribution to the conductance of the fully open channel. In another set of experiments, *drk1* was coexpressed together with the chimeric channel, which has a single channel conductance that is three time as great. From the intermediate single channel conductances it appeared that different hetero-multimers were formed. The data is being analyzed in terms of the subunit-subconductance hypothesis.

510.9

Mouse photoreceptors express mShak family potassium channels.

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Mouse potassium channels can be classified into at least four families by homology with the four potassium channel families of *Drosophila*. The most extensively characterized rodent potassium channel family, mShak, is expressed in the rodent brain. However, the diversity of expression of mShak channels within specific neronal populations is not yet known. To determine the diversity of expression of mShak channels within a specific neuronal type, RNA PCR was employed. Mouse retinas were enzymatically dissociated and individually identified photoreceptors were harvested with a broken patch pipette. Following cell lysis by detergent, mRNA was purified using bead-coupled oligo(dT). cDNA was then synthesized from purified photoreceptor mRNA. The polymerase chain reaction was used to amplify mShak sequences from photoreceptor cDNA employing primers specific for conserved mShak regions. The predicted amplification product was detected by gel electrophoresis. A control reaction without reverse transcriptase generated no such amplification product. Cloning and DNA sequence analysis of the putative photoreceptor mShak amplification products revealed the presence of MK2 sequences due to homology with RCK2 (Grupe *et al.*, EMBO J., 9: 1749-1756). Therefore, mouse photoreceptors express MK2 channels.

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510.10

A *C. ELEGANS* POTASSIUM CHANNEL GENE WITH HOMOLOGY TO *DROSOPHILA Shaw*.

A. Wei, T. Jegla* and L. Salkoff. Dept. of Anatomy and Neurobiology, and Dept. of Genetics, Washington Univ. Sch. of Med., St. Louis, MO 63110.

Four voltage-gated potassium channel gene subfamilies defined by the *Drosophila* potassium channel genes, *Shaker, Shal, Shab* and *Shaw* are represented in a wide variety of metazoans, including mammals. We now report the isolation of a *Shaw* potassium channel gene homologue from the nematode *C. elegans*. A screen of genomic DNA was performed by PCR utilizing degenerate synthetic oligonucleotides coding for highly conserved regions of the four *Drosophila* potassium channels. Though *C. elegans* is distantly related to *Drosophila*, having a common ancestor no later than six hundred million years ago, the deduced amino acid sequence of the nematode channel shares approximately 65% identity to the fly channel. Homology appears significantly greater to the fly sequence than to reported vertebrate *Shaw* homologues. The greater similarity of fly and nematode homologs is evident in an area presumed to be important in ion selectivity; ion selectivity differs between fly and mammalian homologs of *Shaw*. Functional studies of this channel by expression in *Xenopus* oocytes will reveal whether this property is conserved between fly and nematode. Efforts are underway to locate this gene on the physical map of *C. elegans*, and to identify potential mutants.

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510.11

MOLECULAR CLONING OF A NEW K⁺ CHANNEL IN *APLYSIA*.

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Potassium channels are the major targets for neuromodulation and thus contribute to neural plasticity. Since a large number of K⁺ channels exists, and different K⁺ channels play different roles in defining various aspects of the electrical properties of a neuron, we have tried to clone new families of K⁺ channels. Toward this end we have cloned at least one new K⁺ channel in *Aplysia* using a PCR-based strategy.

We carried out PCR reactions with degenerate oligonucleotides that code for conserved regions of known K⁺ channels. The right-sized PCR products were subcloned into plasmid vector and subjected to sequence analysis. At least one new K⁺ channel was found from the PCR products. This putative K⁺ channel is almost identical to other cloned K⁺ channels in the H5 region. However, surrounding this region it is at most 50% identical to *Aplysia* homologues of shab, shak, shaw and *Drosophila* shal. This type of sequence identity suggests that it is a putative K⁺ channel and represents a structurally new family of K⁺ channels, distinct from shab, shak, shaw, and shal. We are currently trying to obtain the full-length sequence in order to express it.

510.12

OVEREXPRESSION OF AN *APLYSIA* SHAKER POTASSIUM CHANNEL CHANGES THE ELECTRICAL PROPERTIES AND SYNAPTIC EFFICACY OF IDENTIFIED *APLYSIA* NEURONS.

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To examine the properties of a cloned *Aplysia* K⁺ channel (AK01a) in its native neuronal environment, we microinjected into different identified *Aplysia* neurons a plasmid expression vector (pNEX, Kaang et al., this meeting) containing the AK01a nucleotide sequence (pNEX-AK01a). As early as seven hours after microinjection of pNEX-AK01a, we observed a large transient K⁺ current. This expressed current in *Aplysia* had the biophysical and pharmacological properties of one of the native transient K⁺ currents (I_{Adepol}). A similar current was obtained when AK01a was expressed in *Xenopus* oocytes. Thus, it appears possible to generate a native current with a channel that is simply a homomultimer. Moreover, by expressing the current at high level, we could redesign critical electrical properties of identified neurons. In the bursting cell R15, overexpression resulted in an increase of the afterhyperpolarization and a narrowing of the action potential which altered the bursting pattern. In the cholinergic neuron L10, overexpression and consequent spike narrowing decreased the amount of transmitter release from the terminals and greatly weakened the synaptic connections. In many cells, prolonged period of incubation (10-30 hr after injection) induced the additional expression of a more slowly inactivating or non-inactivating component in the current that modified still further the electrical properties of neurons. For example, it silenced completely the bursting of cell R15. We are now investigating how this change in expression is brought about.

VISUAL CORTEX: FAR EXTRASTRIATE CORTEX

511.1

A BODY CENTRED COORDINATE SYSTEM IN POSTERIOR PARIETAL CORTEX.

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Models of gaze shifts suggest the existence in the brain of a visual map in body centred coordinates. A distributed map of visual targets in head centred coordinates has previously been proposed for areas 7a and LIP of posterior parietal cortex in the monkey (Andersen et al., J. Neuroscience 10(4), 1990) since cells in these areas have a retinal receptive field, modulated as a linear function of eye position (termed a gain field). Head position would need to be incorporated into the activity of these cells to produce a body centred map of the visual world. We recorded cells from areas 7a and LIP in a monkey with its head free to rotate horizontally. The animal was trained to orient its head and eyes towards independent locations and make visually guided saccades from different initial eye and head positions. This allowed us to determine separately the modulation of activity during the visual saccade task caused by eye position and that caused by head position.

82 cells from areas 7a and LIP were recorded. Both eye and head position signals were observed, often in the same cell so that the cell's activity was encoding gaze position (eye plus head position). These signals were found to linearly modulate the visual or saccadic activity of 55 cells to produce gain fields to eye and head position ($P<0.05$). In 22 cells modulation of the neuronal response occurred with eye position but not head position whereas modulation of neuronal activity by head position was seen in 33 cells. The gain fields for these 33 cells were best described as a function of gaze position rather than just head position as virtually all cells with gain fields for head position had similar gain fields for eye position. In the vast majority of these cells the gain fields were oriented towards the contralateral side of the body.

These results suggest that cells in areas 7a and LIP are of two types. One type of cell incorporates the retinal location of a stimulus with the orbital position and can thus participate in encoding the location of a target in head centred coordinates. The other type of cell modulates retinal responses as a function of gaze position rather than eye position and is thus capable of participating in encoding the location of a target in body centred coordinates.

511.2

Electrical microstimulation delineates 3 distinct eye-movement related areas in the posterior parietal cortex of the rhesus monkey.

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Previous work has subdivided the posterior parietal cortex (PPC) into distinct areas based on anatomical and recording results. We now demonstrate that this parcellation is also reflected in the effects of electrical microstimulation. Stimulation (500 Hz biphasic pulses, 0.1 msec, train duration 100 msec, max. current 200 μA) was delivered in 2 monkeys while the animals kept fixation of the remembered location of a visual target. The areas are designated on physiological criteria since the animals are still being used in experiments. **Area 7a:** Whereas many sites proved to be unexcitable, stimulation of other sites elicited eye blinks (threshold: 50-100μA), which at several sites were accompanied by fast and wavy eye movements. The eye movements were directed to a circumscribed zone in the visual field (goal-directed saccades). **Floor of IPS:** Goal-directed saccades into the contralateral hemifield were also elicited from the floor of the intraparietal sulcus (IPS). Unlike eye-movements elicited from area 7a, these saccades had straight trajectories. They were usually accompanied by brisk movements of the shoulders, pinnae and facial muscles (threshold: 25-50μA). **LIP:** microstimulation elicited saccades (threshold: 25-50μA) which were rarely accompanied by non-eye movements. Although varying initial eye position affected both the amplitude and direction of the saccades, these saccades did not show convergence to a goal within the oculomotor range. They were always directed into the contralateral hemifield with many more sites yielding upward component than downward component saccades. Our observations suggest that the function of area LIP is more confined to saccades than that of the other 2 areas.

511.3

NEURONES IN THE MACAQUE LATERAL INTRAPARIETAL CORTEX (LIP) APPEAR TO ENCODE THE NEXT INTENDED SACCADE.

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LIP, a subdivision of inferior parietal cortex, has been implicated in the planning of saccadic eye movements on anatomical and physiological grounds. In a delayed saccade paradigm, many LIP neurones maintain an elevated level of activity after the offset of the visual target until the saccade is made in that direction. We have interpreted this "memory" activity as reflecting the monkey's plan to make the next saccade. Here we present further evidence for this hypothesis:

1. When monkeys make delayed saccades to auditory targets, some LIP neurones show memory activity. Generally, this memory activity is in the same direction as for visual delayed saccades.

2. Memory double saccade experiments: the two targets are flashed briefly, as in a "traditional" double saccade, but the fixation point remains on for several hundred milliseconds after the targets are flashed. Only after offset of the fixation spot may the saccades be made. For most LIP neurones, only sustained activity related to the first saccade to be made is manifest during the memory period, even if the second saccade target appeared within the receptive field of the neurone.

3. Change of motor plan paradigm: in a variant of the delayed saccade task, we unpredictably presented 1, 2 or 3 targets (either visual or auditory) in succession during the delay period. The monkey had to saccade to the last one presented. In those trials in which 2 or 3 differing targets were presented, the monkey had to change his plan during the delay period. Activity in LIP reflected the direction of the next planned saccade. Alterations of this plan, even in the absence of eye movements, were reflected in neuronal activity: e.g., a preferred target would evoke memory activity which would be "nulled" by subsequent presentation of a non-preferred target.

Thus it appears that activity in many LIP neurones reflects the monkey's *plan* to make the *next* saccade, regardless of how the saccade is evoked, whether it is part of a sequence, and whether indeed it is made.

511.4

NEURONS IN THE LATERAL INTRAPARIETAL AREA OF THE MONKEY REMAP VISUAL SPACE IN CONJUNCTION WITH SACCADIC EYE MOVEMENTS I. PRESACCADIC EVENTS.

J.-R. Duhamel, C. L. Colby and M. E. Goldberg Laboratory of Sensorimotor Research, National Eye Institute, Bethesda, MD 20892.

Accurate processing of visual space requires that the brain compensate for changes in eye position. To study this compensation we recorded from LIP neurons in a paradigm in which a visual stimulus is presented outside the receptive field (RF) and the monkey was required to make a saccade that moved the stimulus into it. We found that half of the visual neurons studied in two monkeys began to discharge with a latency significantly less than that predicted if the response were solely due to the reafferent effect of the saccade bringing the stimulus into the RF. Some cells began to discharge even before the beginning of the saccade. Control experiments show that these same neurons did not respond to the stimulus outside the RF when the monkey made no saccade, nor did they respond when the monkey made the eye movement in the absence of the stimulus. They also did not discharge in response to the stimulus when the monkey attended to the saccade target without making an eye movement to it. The phenomenon requires that the monkey make a saccade that will bring the stimulus into the neuron's RF. When the monkey made a saccade that removed the stimulus from the retinal RF, the discharge of such neurons was truncated as compared to the case when the stimulus disappeared while the monkey maintained fixation.

These results suggest that LIP visual neurons can be excited from any retinal location as long as an impending saccade will bring that retinal location into the receptive field of the neuron. They provide evidence for a process of dynamic remapping that combines eye movement and retinal information to generate the neuronal response. This mechanism ensures the continuity of visuospatial processing across eye movements.

511.5

NEURONS IN THE LATERAL INTRAPARIETAL AREA (LIP) OF THE MONKEY REMAP VISUAL SPACE IN CONJUNCTION WITH SACCADIC EYE MOVEMENTS II. REMAPPING OF A VISUAL MEMORY TRACE.

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Visual neurons in LIP show evidence for dynamic remapping of visual space. They respond to stimuli outside the classically determined receptive field when the monkey makes a saccade that will bring the stimuli into it. We now show that for most LIP neurons, dynamic remapping is a phenomenon which extends to the memory representation of a visual stimulus. We have tested neurons in a new paradigm in which a visual stimulus is briefly flashed (< 50 msec) outside of the receptive field and is followed by a target which elicits a saccade. The saccade brings the eye to a position where the stimulus, if it were still present, would be in the receptive field. We have discovered that virtually all LIP neurons discharge in this task at a rate that is about half of their response to an actual visual stimulus. Control experiments show that this activity is not related to onset of the saccade target or execution of the saccade. Cells respond to stimuli that were flashed as long as a second before the saccade. The spatial organization of the response is retinotopic. Any saccade which brings the eye to a final position such that the stimulus would have been in the receptive field will activate the response. It does not depend on the performance of a particular amplitude and direction of saccade.

These results suggest a novel mechanism for spatial processing of visual images. LIP neurons are sensitive to the retinotopic location of a visual stimulus, whether physically present or existing as a memory trace. The retinotopic coordinates assigned to the trace are updated at the time of a saccadic eye movement as though the stimulus were fixed in space and the eye swept over it. This mechanism ensures that visual information in parietal cortex is spatially accurate at all times.

511.6

AREA 7 AND THE SPONTANEOUS CAPTURE OF ATTENTION BY UNEXPECTED STIMULI

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Attention includes both top-down processes, in which an object is chosen voluntarily, and bottom-up processes, in which an object calls attention to itself. Area 7 is involved in top-down attention as indicated by the enhancement of neuronal visual responsiveness that occurs when an animal is actively prepared to process information from a stimulus. I ask here whether there is a similar enhancement of visual responsiveness when a behaviorally irrelevant stimulus succeeds in spontaneously capturing an animal's attention.

Single-neuron activity was recorded at 105 sites in Area 7 of two cats whose eye movements were monitored with surgically implanted scleral search coils. The cats were reinforced with periodic food reward for remaining in a state of general alertness and for tolerating head restraint. No other aspect of their behavior was reinforced. Visual stimuli were presented briefly at an eccentric visual-field location under computer control. The interstimulus interval was variable and was always greater than 60 sec.

Behavioral responses to the stimulus were bimodal. Cats either responded with a large eye movement directed toward the target or did not emit a detectable response. Neuronal visual responses in Area 7 were more prolonged and more intense on trials when a behavioral response occurred than on trials when there was no orienting eye movement. Whereas the visual response might begin at a latency as short as 30 msec, the enhancement was not apparent until around 80 msec following target presentation. Enhancement of the visual response was not attributable to simple superimposition of a motor burst on the visual burst because it occurred even on trials when the saccade was delayed by several hundred msec. I conclude that the strength of the neuronal visual response in Area 7 is a predictor of whether a stimulus will or will not capture attention.

511.7

MODULAR CONNECTIONS BETWEEN AREA V4 AND TEMPORAL LOBE AREA PITv IN MACAQUE MONKEYS.

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Area V4 contains "modules" which receive input from either cytochrome oxidase thin- or interstripe compartments of V2 (DeYoe et al.,'88; Zeki and Shipp,'89). Tracer injections into these V4 modules produce segregated labeling patterns in posterior IT (Van Essen et al.,'91; and see DeYoe and Sisola, this meeting). In this study, we explore further the size, distribution, and specificity of the V4 inputs to posterior IT by making tracer injections into temporal lobe area PITv.

Anterograde and multiple retrograde tracer injections were made into loci separated by 2-6 mm in area PITv of 4 macaque monkeys. Single, 150-250 nl. injections of fluorescent dyes into PITv produced a variable labeling pattern in V4. In most instances, labeled cells were clustered in several distinct patches which ranged in size from 2x2 to 5x6 mm with an average width of 4 mm and separation of 4-7 mm. In 2 cases, 2 retrograde tracers were injected into separate loci in area PITv separated by 4-6 mm. These paired injections produced interdigitating patterns of retrogradely labeled cells in area V4. In other cases, the labeled zones in V4 were larger and irregularly shaped and may reflect labeling of several adjacent modules. Since a 4-6 mm separation between injections produce segregated clusters in V4, we suggest that "modules" in PITv are at least 4-6 mm wide. These data are consistent with the view that area PITv, like prestriate areas V2 and V4, contains a functional architecture which may reflect segregated processing of form and color information. Supported by a grant from the Whitehall Foundation and an Alfred P. Sloan Foundation Fellowship to DJF.

511.8

DISTINCT PATHWAYS LINK ANATOMICAL SUBDIVISIONS OF V4 WITH V2 AND TEMPORAL CORTEX IN THE MACAQUE MONKEY.

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Visual pathways originating in cytochrome oxidase thin- and inter-stripes of V2 remain segregated when terminating in V4 (DeYoe etal.'88). To determine if projections from other visual areas to V4 are also segregated, different fluorescent retrograde tracers were injected at 3 sites in V4 spaced 2-3 mm apart above the tip of the IOS. In two of three hemispheres injected, retrograde labeling by different tracers was overlapping but segregated within V2: thin stripes labeled by one tracer were interdigitated but separate from inter-stripes labeled by a different tracer. Within occipito-temporal cortex, cells labeled by each tracer were also markedly segregated. In one case, as many as 10 distinct patches of label ranging in size from 2X4mm to 4X8mm could be identified in the region extending from the lip of the STS ventrally to the parahippocampal gyrus. Area PITv, and possibly VOT and CITv appeared to contain more than one patch of label. Four additional foci were labeled on the lateral wall and floor of the STS though intermixing of different tracers was more common near the fundus and in all areas closer to the temporal pole. Alternating patches of different label were also observed on the anterior bank of the lunate sulcus. In a third hemisphere, comparably placed tracer injections produced significantly greater intermixing of label within both V2 and occipito-temporal cortex. These results (and those of Felleman & McClendon, this meeting) suggest that the functional division of visual pathways originat-ing in V2 is preserved in the connections of V4 with visual areas throughout occipito-temporal cortex. Supported by NIH grant RO1-EY08406.

511.9

NEURONS WITH RELATED STIMULUS SELECTIVITY ARE CLUSTERED IN THE MACAQUE INFEROTEMPORAL CORTEX.

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Neurons in the anterior 2/3 of the inferotemporal cortex (IT) of the macaque are selectively activated by visual stimuli with particular 'complex' features: 'complex' shape or combinations of texture or color with shape. Simultaneous recordings from multiple neurons with single electrodes have shown that adjacent cells have similar, but usually not identical, stimulus selectivity (Fujita et al., Soc Neurosci Abstr, 1990). To assess the spatial arrangement of neurons with similar stimulus preferences we made 2 kinds of penetrations in IT, one directed vertically and the other tangentially to the cortical surface.

Recordings were made in the crown of the anterior IT in anesthetized and immobilized monkeys (*Macaca fuscata*). For vertical penetrations, electrodes were advanced from the side at right angles to the midline of the head and tilted downward by 20°. For tangential penetrations, we directed electrodes from the side anteriorly at an angle of 45° to the midline and downward by 27°. We presented various paper cutouts and tens of 3-D objects to the monkey for the initial survey to find stimuli which activated a cell, and then determined the stimulus feature critical for the activation with the aid of a computer graphics system. We made a set of 8 to 18 stimuli ('test set') including optimal, suboptimal and ineffective stimuli for that cell. Other cells were sampled at 100 or 200 μm steps in that penetration and tested with this same set of stimuli.

Over a distance of 0.6-1.4 mm in vertical penetrations we obtained neurons which responded to related stimuli in the test set, i.e., the optimal and suboptimal stimuli for the first tested cell in each penetration. In tangential penetrations, cells with related stimulus selectivity were found within 0.2-0.5 mm. In some tangential penetrations, there existed 2 or 3 separate clusters of neurons showing similar selectivity. The gap between adjacent clusters was 0.4-1 mm. We suggest that the anterior IT is composed of modules, in which neurons with related selectivity cluster across cortical layers.

511.10

TRAINING AFFECTS TASK-RELATED PROPERTIES OF INFEROTEMPORAL NEURONS. R. Vogels and G.A. Orban. Lab. Neuro- en Psychofysiologie, KULeuven, GHB, 3000 Leuven, Belgium.

Last year we (Vogels and Orban, *Soc. Neurosci. Abstr.* 1990) reported on task related properties of inferotemporal (IT) units recorded in a monkey (Ronnie) that was extensively trained in a task in which it had to indicate whether or not two successive gratings (S1 and S2) differ in orientation. In order to determine whether the properties of IT units depend on the level of behavioral training in this task we recorded IT units at two stages of training in a second monkey (Adam). After determining orientation discrimination thresholds (6 months), we trained Adam in the same-different task for a restricted range of S1 orientations (4 months) and then recorded IT units (N=587) with S1 covering the full range of orientations (orientation difference S1-S2 26 degrees). After further intensive training (5 months) at a 15 degrees S1-S2 difference, we recorded again in the same area of IT cortex (N= 560). In the first recording session we found no significant difference in the average unit response level or response variance for learned versus unlearned S1 orientations. A comparison of the single cell responses to S1 between the two recording sessions yielded no significant difference in the response strength, response variance, response latency or orientation sensitivity. However, two task-related response properties changed in the course of training. First, significantly more cells responded stronger to S1 than to the physically identical S2 in the first than in the second recording session. In the second recording session the distribution of the difference in responses to S1 and S2 was similar to that of the extensively trained monkey Ronnie. Second, the number of cells that were responsive during the delay between S1 and S2 increased with the training. These results suggest that the properties of IT units are affected by behavioral training. Furthermore these results may lead to an understanding of the way the primate brain changes its stimulus- and task-related representations during learning.

511.11

A NEURAL CORRELATE OF RECENCY MEMORY IN INFERIOR TEMPORAL (IT) CORTEX. E.K. Miller, L. Li*, and R. Desimone. Lab. Neuropsychology, NIMH, Bethesda MD 20892

Several studies report that, in a matching-to-sample paradigm, IT neurons respond differently to a test stimulus depending on whether or not it matches an immediately preceding sample. Because an item can be actively held in memory even while viewing other items, in two rhesus monkeys we tested whether the memory of the sample influences the response of IT neurons to subsequent test stimuli, even when other stimuli intervene. On each trial, while the animal maintained fixation, the sample appeared first, followed by 0-6 nonmatching (NM) stimuli and then the matching (M) stimulus. For most cells, the responses to M stimuli were weaker than to the same stimuli presented as NM, and this difference was maintained even following 6 intervening NM stimuli. The time course of the response difference varied, but some cells discriminated between M and NM at very short latencies. Based on the response, a test stimulus could be correctly classified as M or NM on 60-90% of the trials and, for many cells, the specific sample stimulus used could also be identified from the NM response. The results suggest that many IT neurons retain short-term, dynamically updated memory traces and that their responses to stimuli are modulated according to their similarity to the trace.

511.12

INFERIOR TEMPORAL (IT) NEURONS DISTINGUISH NOVEL AND FAMILIAR STIMULI. L. Li*, E.K. Miller, and R. Desimone. Lab. Neuropsychology, NIMH, Bethesda MD 20892

In a companion study, we report that IT neurons respond differently to incoming stimuli that match items actively held in short-term memory. However, monkeys and people can also judge how well stimuli match items in a longer term store, i.e. judge familiarity. To examine this, two rhesus monkeys were trained to perform a matching-to-sample task while maintaining fixation. The sample and matching test stimuli were completely unfamiliar to the animal; a new set of 20 was used on each cell. The 4 nonmatching stimuli were familiar and held constant across cells. The responses of about a third of the cells in anterior IT cortex declined steadily with increasing familiarity, reaching a plateau by about 8 presentations of a given sample. The remainder showed either no change in response or (rarely) an increase. The response decrement was stimulus specific. For a given stimulus, the magnitude of cumulative response decrement on a given trial was related to the recency of presentation of that stimulus in the sequence of trials. A few cells were retested with a new set of stimuli, and they showed a recovery of response to the new set. The results suggest that as stimuli become familiar, connection weights in IT cortex adjust adaptively, resulting in a narrowing of activitation across the population.

PEPTIDES: PHYSIOLOGICAL EFFECTS IV

512.1

PITUITARY ADENYLATE CYCLASE-ACTIVATING POLYPEPTIDE (PACAP) INCREASES CYTOSOLIC FREE CALCIUM IN CULTURED RAT HIPPOCAMPAL NEURONS. T.Yada*, J.Tatsuno*, B.Shivers and A.Arimura. US-Japan Biomedical Research Laboratories, Tulane University Hebert Center, Belle Chasse, LA 70037; Departments of Medicine, Physiology and Anatomy, Tulane University School of Medicine, New Orleans, LA 70112.

PACAP, a 38-residue peptide, is a new member of the vasoactive intestinal polypeptide (VIP) / glucagon / secretin family of proteins and stimulates adenylate cyclase in primary cultures of rat pituitary cells, neurons and astrocytes. PACAP, derived from a 176-amino acid precursor, is expressed in primate, sheep and rat brains. Immunoreactive PACAP nerve fibers and PACAP binding sites have been detected in hypothalamic and extrahypothalamic areas suggesting that PACAP has a neurotransmitter/neuromodulator role in the brain. In the present study we investigated the effect of PACAP on cytosolic free calcium concentration ($[Ca^{2+}]i$) in hippocampal neurons. Rat hippocampal neurons were prepared from day 18 embryos and cultured on poly-L-lysine-coated coverslips for 1-10 days. Fura-2-loaded neurons were superfused at 37°C, and the effect of PACAP or glutamate on $[Ca^{2+}]i$ was determined by dual-wavelength microfluorometry with image analysis. Following a lag time of ~ 30 seconds, PACAP at 10^{-10} to 10^{-7} M elicited, in a concentration-dependent manner, an increase in $[Ca^{2+}]i$ from an 80 nM basal level to peak levels of 200-600 nM. Approximately half of the neurons responded to 10^{-8} M PACAP. The $[Ca^{2+}]i$ increase often appeared first near the plasma membrane and then spread inward. A VIP antagonist at 10^{-6} M did not alter the PACAP-induced increase in $[Ca^{2+}]i$. Glutamate at 100 μM also induced an increase in $[Ca^{2+}]i$; however, the PACAP-responsive neurons did not always respond to glutamate, and *vice versa*. These findings indicate that PACAP probably acts through a selective receptor to increase $[Ca^{2+}]i$ in hippocampal neurons and are consistent with a neurotransmitter/neuromodulator role for PACAP in the brain.

512.2

CORTICOTROPIN RELEASING HORMONE (CRH) AND ß ADRENORECEPTORS REGULATE GENE TRANSCRIPTION IN CEREBELLAR NEURONS J.P. Loeffler*, F. Barthel*, A.L. Boutillier* and P. Feltz. Institut de Physiologie et de Chimie Biologique URA 1446 du CNRS,21 rue René Descartes 67 084 STRASBOURG CEDEX FRANCE.

We demonstrate that granular neurons from rat cerebellum maintained in primary culture express functional ß adrenergic and CRH receptors. Activation of these receptors by Isoprenalin ($5 \cdot 10^{-6}$M) and CRH (10^{-8}M) increase cAMP levels and stimulate *c-fos* mRNA accumulation. The role of protein kinase A (PKA) was analyzed by gene transfer studies and expression of a mutated subunit of PKA lacking cAMP binding sites. Introduction of this dominant inhibitory mutation which inactivates PKA inhibits *c-fos* transcription from chimera genes bearing human *c-fos* promoter sequences. These results show an involvement of cAMP-dependent responses to CRH in the cerebellum and underline their importance in gene regulation in differentiated neurons.

Behr JP, Demeneix BA, Loeffler JP and Perez-Mutul J 1989 Efficient gene transfer into mammalian primary endocrine cells with lipopolyamine-coated DNA. Proc Natl Acad Sci USA 86:6982-6986
Loeffler JP, Barthel F, Sassone-Corsi and Feltz A 1990 Lipopolyamine-mediated transfection allows gene transfer studies in primary neuronal cells. J Neurochem 54:1812-1815

512.3

PHE^6-TYR^7 IS NECESSARY AND SUFFICIENT FOR ALPHA-BAG CELL PEPTIDE INHIBITORY ACTION ON ABDOMINAL GANGLION LUQ NEURONS IN *APLYSIA*. D.F. Owens, J.G. Menon, and B.S. Rothman. Department of Biology, San Francisco State University, S.F., CA 94132.

Alpha-bag cell peptide (α-BCP[1-9], APRLRFYSL) mediates the bag cell-induced inhibition of left-upper quadrant (LUQ) neurons L2, L3, L4, and L6. The purpose of this study is to determine which residues of α-BCP are necessary for its inhibitory activity, so that cleavages of α-BCP produced by *Aplysia* peptidases that we have recently characterized can be assessed for their ability to inactivate this peptide. Up to three LUQ neurons were monitored concurrently by intracellular glass microelectrodes in individual isolated abdominal ganglia that were perfused at 10 μl/min and superfused at 670 μl/min at room temperature with artificial sea water (ASW). Spike rate in 1 min intervals and interburst intervals were quantified throughout the experiment, and their changes measured when α-BCP[1-9] or one of its fragments, dissolved at 10^{-7} to 10^{-3} M in ASW, was added to the perfusion stream for 4 min. Our results indicate that the Phe^6-Tyr^7 residues are both necessary and sufficient for α-BCP-mediated inhibitory activity. This conclusion is based on the following: 1) Of all fragments tested α-BCP[6-7] had the lowest threshold (10^{-7} M) for producing inhibition. 2) When perfused individually, seven α-BCP peptides containing residues 6-7 ([1-9], [1-8], [1-7], [3-9], [3-8], [6-9], [6-8]) produced inhibition with a threshold of 10^{-6} to 10^{-5} M. 3) In contrast, when perfused individually, six α-BCP fragments lacking an intact 6-7 bond ([1-5], [1-6], [7-9], [8-9], Phe, Tyr) did not produce inhibition. Based on biochemical studies conducted in our lab, these data strongly suggest that α-BCP is inactivated after release into the extracellular spaces of the CNS by the combined actions of three ganglionic ectopeptidases and one soluble hemal peptidase. *Supported by NIH grants RO1-NS-24046 (BSR) & K04-NS-01177 (BSR).*

512.4

INTRACEREBROVENTRICULAR NEUROPEPTIDE Y INCREASES BASAL GASTRIC ACID BY DECREASING CENTRAL ADRENERGIC INHIBITION OF ACID. J.G.Geoghegan*, C.A.Cheng*, S.S.Schiffman, T.N.Pappas*. Duke Univ. Medical Center, Durham, N.C.27710.

Intracerebroventricular (ICV) NPY increases basal acid output in dogs. ICV yohimbine also increases acid in dogs, suggesting there is tonic α_2-adrenergic inhibition of acid. In this study interaction between ICV NPY and central adrenergic control of acid was examined. Six dogs with chronic cerebroventricular guides and gastric fistulas received [Leu^{31}, Pro^{34}]-NPY (250,500 pmol/kg), a Y_1 post-synaptic receptor agonist, or control into the lateral ventricle. In separate studies NPY was given either before or after ICV desmethylimipramine (DES, 50μg/kg) or vehicle (VEH) as control. Acid output was measured for two hours (shown below in mmol/hr ± SEM, *p<0.05).

Results: [Leu^{31}, Pro^{34}]-NPY had no effect on acid output (control: 1.3 ± 1.0; [Leu^{31}, Pro^{34}]-NPY: 250 pmol/kg: 1.2 ± 0.8; 500 pmol/kg: 0.9 ± 0.6).

NPY+DES	NPY+VEH	DES+NPY	VEH+NPY
9.1 ± 2.3	7.9 ± 3.5	2.0 ± 1.3*	7.3 ± 2.1

Conclusions: The effect of ICV NPY on acid is not mediated at a post-synaptic site. DES, which slows uptake of norepinephrine (NE) from the synaptic cleft, inhibits NPY-induced acid secretion when given before but not after NPY. These studies suggest that ICV NPY may increase acid output by pre-synaptic inhibition of tonic release of NE in dogs.

512.5

THE SELECTIVE POTENTIATION OF NMDA-INDUCED NEURONAL ACTIVATION BY NEUROPEPTIDE Y INVOLVES AN ATYPICAL NPY RECEPTOR. G. Debonnel, F.P. Monnet, A. Fournier and C. de Montigny. Neurobiological Psychiatry Unit, McGill University, I.N.R.S.-Santé, Montréal Québec, Canada and Institut de Recherche Jouveinal, Fresne, France.

We have previously shown that neuropeptide Y (NPY) selectively potentiates NMDA-induced activation of CA_3 hippocampal pyramidal neurons *in vivo*, and that this effect is reversed by the high affinity σ ligand haloperidol *(Eur. J. Pharmacol. 182: 207, 1990)*. To characterize the type of receptor involved in this potentiation of NMDA by NPY, we have studied the effect of peptides related to NPY: [Leu^{31}, Pro^{34}]pNPY which possesses high selectivity for the Y_1 receptor *(P.N.A.S., 87: 182, 1990)*, $pNPY_{13-36}$, a selective ligand for the Y_2 receptor *(J. Biol. Chem., 264: 6648, 1989)*, and the pancreatic peptide (PP) and peptide YY (PYY) which both activate Y_1 and Y_2 receptors.

Five-barrelled micropipettes were used for extracellular recording of CA_3 hippocampal pyramidal neurons of anesthetized male Sprague-Dawley rats and microiontophoresis of [Leu^{31}, Pro^{34}]pNPY, $pNPY_{13-36}$, PP and PYY (0.1 mM in 150 mM NaCl and 0.1% BSA, pH: 8), NMDA (10 mM in 200 mM NaCl, pH: 8) and QUIS (1.5 mM in 400 mM NaCl, pH: 8).

[Leu^{31}, Pro^{34}]pNPY and $pNPY_{13-36}$ induced a marked and selective potentiation of NMDA-induced activation which was reversed by haloperidol (20 μg/kg, i.v.). In contrast, PP and PYY did not potentiate NMDA-induced activation.

Since [Leu^{31}, Pro^{34}]pNPY and $pNPY_{13-36}$ are selective for the Y_1 and Y_2 receptors respectively, and PP and PYY activate both receptors, the present results suggest that the selective potentiation of the NMDA response by NPY is mediated by a NPY receptor distinct from the Y_1 and Y_2 receptors. This NPY receptor might represente a subtype of σ receptors.

512.6

SPINAL AND PERIPHERAL MODULATION OF GASTROINTESTINAL (GI) MOTILITY BY THE PANCREATIC POLYPEPTIDE-FOLD PEPTIDES (NPY, PYY AND PP): ROLE OF THE ALPHA (α) ADRENERGIC SYSTEM. Wager-Pagé, S., B. Ghazali*, J. Davison* and W. Veale. Univ. of Calgary, Dept. of Medical Physiology, Calgary, Alberta.

NPY, PYY and PP are distributed in the spinal cord and GI tract. Work from this laboratory has shown that NPY, PYY and PP modulate GI tone and alter intraluminal pressure at peripheral and thoracic spinal sites in the rat. Others have shown that some of the physiological effects of NPY are via modulation of the sympathetic nervous system (SNS). The role of the SNS in the PP-fold peptide's modulation of GI pressure in the rat was studied. NPY, PYY, PP (200 pMole) were injected intra-venously (IV) or intrathecally (IT) following α-1(prazosin(0.1 mg/kg) IM) (PRZ) or α -2 (yohimbine (0.5 mg/kg)IM)(YOH) blockade.

Increase In Duodenal Pressure Change from Baseline (mmHg)				Increase In Colonic Pressure Change from Baseline (mmHg)			
	Alone	+YOH	+PRZ		Alone	+YOH	+PRZ
PP(IV)	3.7*	2.3*	3.5*	PP(IV)	6.2*	2.5*	8.2*
PP(IT)	3.0*	0.9†	0.8†	PP(IT)	3.7*	0.5†	2.0*
PYY(IV)	3.2*	4.8*	2.2	PYY(IV)	3.3*	2.9	2.4
PYY(IT)	2.1*	1.3	1.1	PYY(IT)	3.3*	0.9†	2.3
NPY(IV)	1.8*	0.9	1.7*	NPY(IV)	2.0*	4.2*	2.6
NPY(IT)	1.8	0.2	1.4*	NPY(IT)	2.2*	2.0*	0.4†

(*p<0.05, from BL)(†p<0.05 from peptide alone)

As previously shown, NPY, PYY and PP increase duodenal and colonic intraluminal pressure. Both α -1 and α -2 adrenergic mechanisms may be involved in the modulation of GI tone by the PP-fold peptides.

512.7

ENDOTHELIN ACTIONS AT THE SUBFORNICAL ORGAN. K.M. Wall, M. Nasr* and A.V. Ferguson. Dept. of Physiology, Queen's University, Kingston, ON K7L 3N6, CANADA.

Endothelin (ET) is a 21-amino acid peptide with potent vasoconstrictor properties. ET administered intravenously to dogs has been shown to cause increases in plasma levels of adrenaline, noradrenaline, renin, angiotensin, aldosterone and vasopressin (VP). Evidence suggests that ET acts as a neuropeptide, but central sites of action and regulatory functions of ET in the central nervous system remain to be determined. Our initial electrophysiological studies, using extracellular single unit recordings, demonstrated excitatory responses in 44% of magnocellular neurons in the paraventricular and supraoptic nuclei of the hypothalamus in response to circulating ET. Electrolytic lesion of the subfornical organ (SFO) reduced this value to 14%. In the present study, electrophysiological and micro-injection studies were performed to further assess the role of the SFO in mechanisms whereby circulating ET alters the activity of neurohypophysial VP and oxytocin (OXY) neurons.

Male Sprague-Dawley rats anaesthetized with 1.4 g/kg urethane and fitted with femoral arterial and venous catheters were placed in a stereotaxic frame for 1) micro-injection of 0.5 pmol (0.5 μl volume) ET-1 into SFO or 2) examination of single unit responses of SFO neurons, antidromically identified as projecting to PVN, to intravenous ET (50 pmol). Micro-injection of ET into SFO caused an increase in mean arterial pressure of 10.2 ± 2.0 mmHg, while similar administration of saline had no significant effect. Single-unit extracellular recordings from SFO neurons demonstrated an excitatory response to ET for 60% of the cells. These results support the suggestion that circulating ET causes release of VP and OXY through central nervous system actions at the SFO. *Supported by a grant from the MRC of Canada. KMW is a recipient of a HSF Fellowship.*

512.8

INHIBITION OF Ca^{++} CURRENTS BY A MU OPIOID IN AN ANATOMICALLY-DEFINED SUBSET OF RAT SENSORY NEURONS. J.E. Schroeder and E.W. McCleskey. Washington University, St. Louis, MO 63110.

Selective modulation of particular somatosensory sensations occurs in humans and animals but the basis of the selectivity is unclear. We asked if there is selective modulation at the level of the primary sensory neuron in rats by comparing electrophysiological properties of randomly selected dorsal root ganglia (DRG) neurons to those of identified subsets of DRG neurons in short-term cell culture. Subsets were identified in two ways: labeling with antibodies (provided by Drs. Jane Dodd and Tom Jessell) against heterogeneously expressed cell-surface antigens and retrograde transport of dye from the peripheral target to the DRG prior to cell preparation. The antibodies labeled cells that preferentially project to specific laminae in the dorsal horn of the spinal cord, implying that they label cells of particular sensory modality. Using whole-cell voltage clamp, the following properties were examined: cell capacitance, low-threshold T-type and high-threshold Ca^{++} current amplitudes, total to sustained high-threshold Ca^{++} current ratios, Na^+ current sensitivity to tetrodotoxin, and high-threshold Ca^{++} current sensitivity to mu opioids. Only the response to opioids was different in random and labeled cells. Of 73 randomly selected cells, 12% were insensitive to a saturating concentration of the mu opioid agonist DAGO and inhibition of Ca^{++} current in the remaining cells varied from 5% to 95%. In an identified population of 44 cells enriched in pain and temperature-sensing neurons, fewer cells lacked opioid sensitivity (4%) and Ca^{++} current was never inhibited by greater than 55%. The results show that an identified DRG subset responds in a predictable manner to mu opioids, whereas randomly selected cells show wide variation in their responses. Thus, selective opioid modulation can occur at the level of DRG sensory neurons.

512.9

SELECTIVE LOSS OF ß-ENDORPHIN NEURONS AND COMPENSATORY INCREASE IN μ OPIOID RECEPTORS IN THE HYPOTHALAMUS OF ESTRADIOL-VALERATE TREATED RATS. G.C.Desjardins,J.R.Brawer and A.Beaudet. Montreal Neurol. Inst.,Dept. of Anatomy,McGill Univ.,Montreal,Que.,Canada H3A 2B4.

A single injection of estradiol valerate (EV) in female rats induces a series of hypothalamic changes which ultimately result in an aberrant pattern of LHRH release and the development of chronic polycystic ovaries. We have previously demonstrated that EV treatment is associated with a selective increase in μ opioid binding in the medial preoptic nucleus (MPO), an area of high LHRH neuron density, and with a concomitant decrease in hypothalamic ß-endorphin content. In this study, we report using quantitative immunocytochemistry that EV treatment causes an average 60% decrease in the total number of ß-endorphin immunoreactive neurons across the arcuate nucleus ($p < 0.001$;$n=5$). Adjacent sections labeled with antibodies to somatostatin, neurotensin and tyrosine hydroxylase exhibited no differences in average immunopositive neuron numbers between control and EV-treated animals, suggesting that neuronal loss was relatively specific for ß-endorphin neurons. To determine wether this effect was responsible for increases in μ opioid binding detected in the MPO, μ opioid binding densities were quantitated by in vitro radioautography in the hypothalamus of rats deprived of arcuate neurons by neonatal monosodium glutamate treatment and found to be inversely correlated with hypothalamic ß-endorphin content ($r=0.8$). These results suggest that estradiol treatment results in either destruction of ß-endorphin neurons in the arcuate nucleus or decreased synthesis of ß-endorphin in these neurons and that this reduction in endogenous peptide causes a chronic up-regulation of μ opioid receptors in the MPO.

GENE STRUCTURE AND FUNCTION III

513.1

ALTERNATIVE SPLICING OF THE PARANEOPLASTIC OPSOCLONUS GENE WITHIN SPECIFIC REGIONS OF THE HUMAN BRAIN. R.B. Darnell Cotzias Lab of Neuro-Oncology, Memorial Sloan Kettering Cancer Center & Cornell Univ Med Ctr, NY, NY 10021

Paraneoplastic opsoclonus (PO) is a neurologic disorder in which brainstem and cerebellar dysfunction are associated with malignancy. In 8 PO patients an antibody (anti-Ri) directed against their own tumors and a nuclear neuronal antigen (Ann Neurol 1991;29:241-251) has been reported. We have now identified a cDNA encoding a fusion protein recognized by anti-Ri antisera, and additional clones encoding what is most likely the full length of the Ri protein.

Because the neurologic manifestations of PO are restricted, while Ri antisera recognizes an antigen expressed throughout the brain, we have characterized Ri cDNA's from different areas of the brain. A number of Ri cDNA's appear to result from splicing of novel exons into a common transcript. One such exon ("C1"), occurs within the coding region of the Ri fusion protein, encoding a peptide rich in serine and proline. This exon is absent from all subtentorial (cerebellar and brainstem) and present in all cortical (frontal and hippocampal) cDNA clones characterized. Thus the Ri gene transcript appears to be alternatively spliced in a regionally specific manner in the nervous system. If exon C1 alters the recognition of the Ri fusion protein by PO antiserum, it would suggest that regional variations in the Ri antigen might contribute to the specificity of the neurologic symptoms.

513.2

MOLECULAR CLONING OF IMMORTALIZED MOTOR NEURON HYBRID CELL-SPECIFIC cDNAs. S.H. Pasternak, N.R. Cashman, K.E.M. Hastings.* Dept of Neurology and Neurosurgery, McGill University, Montreal PQ H3A 2B4

We have pursued a strategy which could lead to the identification of motor neuron-specific genes based on immortal hybrid cell lines produced by the fusion of N18TG2 neuroblastoma X dissociated spinal cord cell fusions (Soc Neurosci Abs 1989). One of these hybrid cell lines, NSC34, expresses many motor neuron-like characteristics not found in the parental neuroblastoma, including the ability to extend processes, to contact cultured myotubes and induce aggregation of acetylcholine receptors, and to express choline acetyltransferase, neurofilament proteins and NCAM. A cDNA library was made from NSC34, and 10 clones were isolated from it using differential and subtractive screening strategies designed to isolate cDNA copies of RNAs expressed at higher levels in the hybrid cells than in the N18 parent. Sequence analysis indicates that three transcriptional components contribute to the NSC34-enriched RNAs. These are 1. mRNAs transcribed from the mitochondrial genome, 2. small RNAs, presumably transcribed by RNA polymerase III, which correspond to the B1 highly repetitive DNA sequence element, and 3. typical cytosolic mRNAs presumably transcribed by RNA polymerase II. Among the latter group (5 clones) two have been identified as mRNAs encoding chromogranin B (a component of neurosecretory vesicles and GAP-43 (important in axonal growth cone function), while the remaining three represent previously unreported sequences. We are currently investigating the tissue expression patterns of these isolated cDNAs in order to assess their expression in motor neurons.

513.3

EXPRESSION OF A FUNCTIONAL FOREIGN GENE IN ADULT MAMMALIAN BRAIN FOLLOWING IN VIVO TRANSFER VIA A HERPES SIMPLEX VIRUS DEFECTIVE VIRAL VECTOR. M.G. Kaplitt[1], J.G. Pfaus[1], S.P. Kleopoulous*[,1], B.A. Hanlon*[2], S.D. Rabkin*[2], D.W. Pfaff[1]. [1]Laboratory of Neurobiology and Behavior, Rockefeller University, N.Y., N.Y., 10021;[2]Program in Molecular Biology, Memorial Sloan-Kettering Cancer Center, N.Y., N.Y., 10021.

We have used a herpes simplex virus type 1 (HSV1) defective viral vector for the transfer and expression of a foreign gene in the adult rat brain *in vivo*. The vector genome consists of multiple copies of an HSV1 amplicon, a plasmid-based cloning and amplifying construct which contains HSV1 cis-acting sequences, allowing packaging into defective HSV1 virions upon superinfection with a helper virus. Our amplicon, pHCL, utilizes a human cytomegalovirus promoter and bacterial lacZ gene as a reporter. Helper functions were provided by a temperature-sensitive mutant HSV1 strain, which does not replicate at physiological temperatures. The resulting defective viral vector (dvHCL) was stereotaxically microinjected into rat hippocampus and hypothalamus, and cells expressing enzymatically active β-galactosidase were detected in both regions. Expression was confined to regions at or near the site of injection. Control animals injected with helper virus alone were completely negative. Positive neurons were identified as soon as 18 hrs following injection, and expression was still detectable after 2 weeks. All animals survived and there was no behavioral, gross or microscopic anatomical evidence of a virulent neurologic infection. The unique advantages of the defective vector system include amplification of the reporter gene, localized expression without virulent spread due to a lack of autonomous amplicon replication and simple manipulation of the defective viral genome as a plasmid. Attempts are underway to express lacZ *in vivo* under the control of the vasopressin and enkephalin promoters.

513.4

EXPRESSION OF CHLORAMPHENICOL ACETYLTRANSFERASE FROM THE HUMAN MAO B PROMOTER. R. M. Denney and Abha Sharma*. Dept. of Human Biological Chemistry and Genetics, University of Texas Medical Branch, Galveston, TX 77550.

Monoamine oxidase (MAO) B oxidizes dopamine and β-phenylethylamine and activates the neurotoxin, MPTP. The X-linked, MAO B gene exhibits cell type-specific expression which differs from the similar and closely-linked MAO A gene. The MAO B promoter has not been functionally characterized. A 174 bp genomic fragment, which extends from an upstream Pst I site to a Sau 3a site in the 5'-untranslated region of exon 1, and which contains a TATA box-like sequence and a GC-box, was ligated in native orientation immediately 5' to a promoterless chloramphenicol acetyltransferase (CAT) gene in each of two vectors, one containing and one lacking an SV40 enhancer. Transient expression of the enhancer-containing construct led to detectable CAT activity in HeLa cells, which express endogenous MAO B activity and mRNA. No CAT activity was detected in HeLa transfected with an enhancer-containing CAT plasmid lacking the MAO B promoter insert, or an enhancerless plasmid containing the promoter insert. CAT vectors containing DNA extending further 5' from MAO B exon 1 are being tested to further define the promoter and to search for possible linked enhancer activity in the DNA region 5' to the MAO B gene. [Supported by NS 19543 and funds from the John Sealy Memorial Endowment, UTMB.]

513.5

TRANS-ACTING ELEMENTS AND OLFACTORY NEURON GENE TRANSCRIPTION. C. Stein-Izsak, M. Grillo, C. Behn*, M. Sakai*, J. Corbin*, F.L. Margolis, Dept. of Neurosciences, Roche Inst. of Molec. Biol., Nutley, NJ 07110

Olfactory marker protein (OMP) is an abundant, phylogenetically conserved gene product of mature olfactory receptor neurons. The OMP-gene lacks introns and CAAT or TATA box motifs characteristic of genes transcribed by RNA pol II. The pattern of OMP expression argues that it may represent a class of neuron-specific genes with novel transcriptional regulation. Previously, we described a 5' segment of the OMP gene that interacts only with extracts of olfactory epithelium (OE) (C. Stein-Izsak, M. Grillo, F. L. Margolis: Soc. for Neurosci., Abstr. 16, 158, 1990). DNase footprinting has identified a novel 17 bp motif as the binding site of this OMP gene binding protein (OMP-GBP). OMP-GBP activity is developmentally regulated and responds to olfactory nerve lesion. OMP-GBP elutes from heparin-agarose chromatography at 0.4 M KCl. A putative SP-1 site is located just 3' of the OMP-GBP sequence, but nuclear protein enriched extracts from rat OE lack an SP-1 shift in gel mobility shift assays. These extracts do contain activities that bind to consensus sequences for Oct-1, NF_KB, NF-1, and AP-3. In transgenic mice 3 Kb but not 0.3 Kb of 5' upstream OMP sequence gives appropriate, cell-specific reporter gene expression. Since the 0.3 Kb upstream sequence includes the novel footprint motif additional upstream regulatory elements are being sought.

513.6

FUNCTIONAL REGULATION OF PROGESTERONE RECEPTOR TRANSCRIPTIONAL ACTIVITY BY PHOSPHORYLATION. L.A.Denner, N.L.Weigel*, O.M.Conneely*, W.T.Schrader* and B.W.O'Malley* Dept. of Cell Biology, Baylor College of Medicine, Houston, TX 77030.

Phosphorylation regulates transcriptional activity of the progesterone receptor (PR) by ligand-dependent and ligand-independent mechanisms. We have used single amino acid substitution mutagenesis of serine 530 (S530), the progesterone-induced phosphorylation site, to elucidate the precise role of phosphorylation in functional regulation of the PR. S530 was mutated to glycine (S530G) to prevent phosphorylation and to aspartic acid (S530D) to constitutively provide negative charge, thus partially mimicking the charge provided by phosphorylation. All receptor forms had equal hormone binding and expression levels in transient transfections of CV1 cells. S530 and S530D had similar maximal transcriptional activation of the PRE-tk-CAT reporter gene while S530G was reduced 25-50%. Gel retardation studies using the PRE showed that S530D had substantially higher DNA binding than S530 and S530G, which were similar to each other. Indirect immunofluorescence indicated that S530 and S530G were localized exclusively to the nucleus after treatment with either progesterone or okadaic acid, a phosphatase inhibitor that activates PR-mediated transcription in the absence of progesterone by the ligand-independent pathway. S530D was perinuclear under all conditions. These observations suggest that the ligand-dependent pathway for transcriptional activation of the PR occurs by phosphorylation of S530, resulting in an increase in DNA binding activity. The ligand-independent pathway occurs by phosphorylation of a site distinct from S530.

513.7

POSITIVE AND NEGATIVE REGULATORY CIS-ACTING ELEMENTS IN GLUTAMINE SYNTHETASE EXPRESSION. H.J. Purohit*, R.G. King, H. Haleem-Smith*, V.P. Kedar*, and J.F. Mill. Lab. of Molecular Biology, NINDS, NIH, Bethesda, MD 20892.

Glutamine synthetase (GS) gene expression is confined to astrocytes in the CNS. The gene for GS was cloned and the 5' flanking region isolated. A series of deletion and site-directed mutants of the promoter region were generated by PCR, cloned into pSV_0CAT, and expressed in various cell lines.

We identified three regions of importance for the regulation of GS expression, an AP2 site located at -222, an enhancer region at -315, and a silencer region at -797.

The deletion mutants show that the AP2 and enhancer sites are sufficient for cell type specific expression. Inclusion of only the AP2 site created a small increase in expression while a large increase was seen when the enhancer region was included. This elevation was suppressed by the inclusion of the silencer region.

Site directed mutation studies demonstrate that while the AP2 site has only a small effect by itself, the enhancer region requires the AP2 site for its induction. Mutation of the silencer site leads to an increase in expression, though dual mutation of the AP2 and silencer sites further increase the promoter activity, suggesting that all three sites are interacting in controlling GS expression.

513.8

IDENTIFICATION OF A NOVEL REPRESSOR ELEMENT RESPONSIBLE FOR NEURAL-SPECIFIC EXPRESSION OF THE RAT BRAIN TYPE II SODIUM CHANNEL GENE. S.D.Kraner, K.M.Dains*, J.A.A.Chong* and G. Mandel*, Dept. of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, N.Y. 11794. The mammalian voltage-gated Na channnel is encoded by a multi-gene family, the members of which are expressed in a tissue-specific manner. The brain type II Na channel is expressed in a neural-specific manner, in a restricted pattern throughout the CNS. We wished to determine the molecular basis of this transcriptional specificity of the type II gene. Neural-specific expression is faithfully reproduced in cell lines transfected with fusion genes containing 5'regulatory sequences of the type II gene fused to a reporter gene (minigenes). These minigenes direct high level reporter activity in PC12 cells, a neuronal-like cell line; only minimal reporter activity of the minigene is detected in the skeletal muscle cell line, L6. By deletional analysis, we have identified a 68 base pair (bp) element within the type II Na channel gene's 5' flanking region which is responsible for the repression of minigene activity in L6 cells. The element does not repress the high level expression of the same minigene in PC12 cells. Proteins which bind specifically to DNA probes containing the 68 bp element were found in nuclear extracts prepared from L6 cells but not in those prepared from PC12 cells. Furthermore, these proteins protect 28 out of the 68 bp in DNase footprinting assays using L6 nuclear extracts. The 28 bp sequence appears to represent a binding site for novel transcription factors. These factors achieve neural-specific expression by repressing gene activity in non-neural cells. The generality of this mechanism for neural-specific gene expression remains to be determined. This work was supported by an NRSA award to SDK and an NIH grant to GM.

513.9

IDENTIFICATION OF A RETINOIC ACID RESPONSE ELEMENT IN THE HUMAN OXYTOCIN GENE PROMOTER WHICH OVERLAPS WITH AN ESTROGEN RESPONSE ELEMENT. Stéphane Richard* and Hans H. Zingg. *Laboratory of Molecular Endocrinology, Royal Victoria Hospital, McGill University, Montreal, Quebec, Canada H3A 1A1*

We previously identified an estrogen (E_2) response element (ERE) at position -164 in the human oxytocin (OT) gene promoter (JBC 265:6098). By transient expression of OT 5'-flanking sequences linked to the CAT gene, we now show that retinoic acid (RA) elicits a 7-fold stimulation of the transcriptional activity of the OT promoter in neuroblastoma cells co-transfected with either the human RA receptor (RAR) α,β or γ. We further demonstrate that the presence of 4 TGACC repeats, located at -162, -156, -103 and -83, is an absolute requirement for maximal RA induction. Since the TGACC repeats at -162 and -156 are contained within the ERE (GGTGACCTTGACC), we next determined the level of synergism and/or interference that occurs when both hormones are added together. In constructs containing an intact ERE and all 4 TGACC repeats, RA and E_2 had an additive effect. By contrast, in constructs in which the TGACC repeats at -103 and -83 were deleted, RA *decreased* E_2-induced stimulation by ~50%. A likely interpretation of these data is that, in the absence of the two downstream TGACC repeats, the RAR is forming a transcriptionally inactive complex with the ERE and is thereby preventing the ER from binding to the ERE. The overlapping elements in the human OT promoter create a natural molecular model system for the investigation of interactions between nuclear factors.

513.10

A SILENCER-LIKE ELEMENT DETERMINES CELL TYPE SPECIFICITY IN THE PROMOTER OF THE RAT CHOLINE ACETYLTRANSFERASE GENE. Håkan Persson and Carlos F. Ibáñez, Department of Medical Chemistry, Laboratory of Molecular Neurobiology, Karolinska Institute, S-10401 Stockholm, Sweden.

The cDNA coding for the rat choline acetyltransferase (ChAT) was isolated and used as a probe to investigate the developmental and regional expression of ChAT mRNA in the rat CNS. Expression of ChAT mRNA was also studied in rat peripheral tissues where high levels of two ChAT transcripts were found in the testis.

A genomic clone containing 7kb of 5' flanking sequences from the rat choline acetyltransferase (ChAT) gene was isolated and shown to contain a TATA box-like sequence and several consensus binding sites for the transcription factor AP1. Two constructs containing 450 and 1450bp, respectively of 5' flanking sequences promoted expression of a fused chloramphenicol acetyltransferase (CAT) gene when transfected into fibroblast FR3T3, Sertoli TM4, pheochromocytoma PC12 and cholinergic neuronal SN6 cell lines. In contrast, a longer construct containing 3850bp of 5' flanking sequence allowed CAT activity only in the cholinergic cell line SN6 (although at 2 to 3-fold lower levels than those seen with the shorter constructs), while CAT activity with this construct was suppressed in the three other cell lines, including PC12 cells. This result suggests that the the distal region of the ChAT promoter contains a cell type-specific silencer-like element that restricts ChAT gene expression to cholinergic cells. NGF treatment of PC12 cells increased the promoter activity of the -450 and -1450 constructs approximately 4-fold and allowed promoter activity from the -3850 construct, indicating that elements involved in NGF responsiveness of the ChAT promoter are contained in the first 450bp of upstream sequence. Taken together, our results support a model in which gene transcription controlled by cell type-specific regulatory elements contribute to the establishment, maintenance and plasticity of neurotransmitter phenotypes in the nervous system.

513.11

REGULATION OF TISSUE- AND TREATMENT-SPECIFIC ppENK RNA INITIATION. G. Weisinger, J.D. DeCristofaro and Edmund F. La Gamma. Department of Pediatrics and Neurobiology, SUNY at Stony Brook, Stony Brook, NY

We previously demonstrated that rat preproenkephalin (ppENK) RNA is initiated from at least 4 start sites by primer extension and S1 analysis of striatal RNA (JBC 265:17389, 1990). To further compare specificity of usage, we surveyed 12 tissues in the basal state using primer extension analysis. E3 + E4 start sites accounted for 80% of the 1.4 kb striatal RNA species compared to a preferential usage of the E2 site in all other tissues; including the 1.7 kb RNA species seen in testes and epididymis. To evaluate effects of induction on usage, rats were given cholinergic drug treatments (nicotine 5 mg/kg + oxotremorine 1 mg/kg; sc q 12h up to 4d). Adrenomedullary E2 transcripts peaked at 2d increasing 5-fold while newly induced E4 transcripts increased 80 fold. In contrast, after hypoglycemia (insulin 10/kg sc, rescue at 2h with 40% sucrose PO), only the E4 transcript was increased (16-fold). Interestingly, in both treatment paradigms, steady state levels of adrenal ppENK mRNA increased 50 to 100 fold. In the striatum of the cholinergic treated groups, RNA initiation increased 10-15 fold but not after insulin treatment (hypoglycemia). These studies demonstrate tissue and treatment specific differences in ppENK RNA start site usage and steady state levels of mRNA. (NSF #BNS8719872.)

513.12

EXPRESSION OF BOVINE VASOPRESSIN IN THE HYPOTHALAMUS OF THE TRANSGENIC MOUSE AND ITS REGULATION DURING OSMOTIC CHALLENGE. H.L. Ang*, J. Funkhouser*, D.A. Carter, M.Y. Ho* and D. Murphy. Neuropeptide Laboratory, Institute of Molecular and Cell Biology, National University of Singapore, Kent Ridge Crescent, Singapore 0511.

A 13.4 kilobase genomic fragment of the bovine vasopressin (VP) gene was micro-injected into mouse oocytes. Four independent transgenic mouse lines were derived and three lines had been assayed for tissue distribution of bovine VP RNA. All lines showed bovine specific ovarian VP expression. In addition, two lines showed bovine VP RNA in the hypothalamus. In-situ hybridisation of transgenic mouse hypothalamic sections identified bovine VP RNA expressing cells in neurons of the supraoptic nuclei. When the transgenic animals were subjected to an osmotic stimulus (2% NaCl in their drinking water for 7 days), the bovine message level increased 1.6 fold, similar to that of the endogenous murine message.

These studies show that the 13.4 kilobase bovine genomic vasopressin fragment contains at least some of the regulatory elements necessary to direct tissue-specific expression and physiological regulation in the transgenic mouse hypothalamus.

FORMATION AND SPECIFICITY OF SYNAPSES V

514.1

ROLE OF SYNAPSIN I IN THE MATURATION OF FUNCTIONAL NEUROMUSCULAR SYNAPSES. B. Lu, P. Greengard and M.-m. Poo, Lab. of Molecular and Cellular Neurosci., Rockefeller Univ., New York, N. Y. 10021 and +Dept. of Biol. Sci., Columbia Univ., New York, N. Y. 10027

The synapsins are a family of neuronal phosphoproteins associated with synaptic vesicles, and believed to be involved in the regulation of neurotransmitter secretion. In the developing nervous system, the expression of synapsin I correlates with neuropile development and synapse formation. Recent morphological evidence suggests that the synapsins may play a causal role in synaptogenesis. Thus, transfection of cultured neuroblastoma cells with synapsin IIb cDNA results in marked increases in the number of nerve terminals per cell, in the number of synaptic vesicles per nerve terminal, and in the number of synapse-like contacts. We have now found that synapsin I, loaded into Xenopus embryos, promotes the functional maturation of developing neuromuscular synapses in culture. Spontaneous synaptic currents occurred with higher frequency and their amplitudes showed an earlier appearance of bell-shaped distribution indicative of more mature quantal secretion. Impulse-evoked synaptic currents also showed a significant increase in amplitude. These results provide physiological evidence for the involvement of synapsin I in the maturation of neuromuscular synapses.

514.2

TARGET CONTACT REGULATES CALCIUM TRANSIENTS IN NEURITES DURING SYNAPTOGENESIS. L. R. Funte, and P. G. Haydon, Dept. of Zoology and Genetics, Iowa State University, Ames, IA 50011.

It is known that calcium channels aggregate at presynaptic terminals (Charlton, 1990), however, the mechanisms that give rise to this channel distribution are unknown. Using cultured *Helisoma trivolvis* neurons, our goal is to determine the regulatory mechanisms controlling calcium channel distribution during synaptogenesis.

Individual neurons were plated into culture, and, after 1-3 days, action potential- evoked calcium transients were imaged using Fura-2. Trains of action potentials reliably evoked homogeneous calcium transients throughout the neuritic arbor. Single, dissociated muscle fibers from the supralateral radula tensor (SLT) muscle, the normal effector muscle of neuron B19, were plated onto neurites and allowed to contact overnight. Action potentials in synaptically connected neuromuscular preparations evoked a heterogeneous pattern of calcium transients, with the largest calcium transient being localized to the site of muscle contact.

We have begun to address the mechanism(s) underlying the focal calcium transients by first determining the time course of the transition to the heterogeneous state. Neurons were plated alone, in culture, and the calcium transients shown to be homogeneously distributed. Single muscle fibers were then plated on to the neurites, and by imaging sequential calcium transients, a transition to focal heterogeneity was determined to occur within 30 minutes of muscle contact.

514.3

MOTOR NERVE TERMINALS ARE SELECTIVELY ELIMINATED FROM REGIONS OF NEUROMUSCULAR JUNCTIONS FOCALLY BLOCKED WITH α-BUNGAROTOXIN. R.J. Balice-Gordon and J.W. Lichtman, Dept. Anatomy & Neurobiology, Washington Univ. School of Medicine, St. Louis, MO 63110

Following multiply innervated neuromuscular junctions over time in living mice has shown that the postsynaptic ACh receptor regions beneath one motor axon are selectively eliminated while those beneath the other motor axon are maintained. After postsynaptic ACh receptor loss, the overlying motor nerve terminal is eliminated and that motor axon withdraws from the junction. The precocious loss of ACh receptors from beneath one input but not the other may lead to a decrease in the efficacy of the terminals of one axon and alter the competitive balance between the two inputs.

We tested this by saturating the ACh receptors with α-bungarotoxin in a portion of a singly innervated junction in adult mice using a focal labeling technique. In this way we decreased the synaptic strength of a few branches of a motor nerve terminal arbor while the other branches of that axon within the same junction were unaffected. We then followed the fate of the synaptic sites in blocked and unblocked regions over time. Several days after focal blockade was begun we found that the density of ACh receptors in the blocked region decreased, because existing receptors were eliminated from that region and no new receptors were inserted there. Furthermore, motor nerve terminals were eliminated from blocked receptor regions but not from unblocked regions. These results suggest that nerve terminals rendered inactive by postsynaptic blockade are at a disadvantage when forced to compete with nearby terminals on the same muscle fiber which have normal activity. A number of controls ruled out alternative explanations for this experimental outcome. We conclude that blocking the ACh receptors in a small region of a singly innervated adult junction simulates developmental synaptic competition between two different axons with intrinsically different activity patterns. This predictable way of inducing synaptic competition will help us learn how activity mediates the cascade of events that underlie synapse elimination.

514.4

HETEROSYNAPTIC SUPPRESSION OF DEVELOPING NEUROMUSCULAR SYNAPSES: HEBBIAN MODULATION *IN VITRO*. Y. Lo and M-m. Poo, Dept. of Biological Sciences, Columbia Univ., N.Y., N.Y. 10027

We have developed a nerve-muscle culture in which a single muscle cell was co-innervated by two embryonic spinal neurones, and the effect of electrical activity on the synaptic efficacy was examined after electrical stimulation was applied to one or both neurons. We found that brief tetanic stimulation of one neuron (100 pulses at 2-5 Hz) results in immediate functional suppression of the synapse made by the other un-stimulated neuron co-innervating the same muscle cells, as shown by a reduction in the amplitude of impulse-evoked synaptic currents recorded in the myocyte. Heterosynaptic suppression was observed regardless whether the tetanized synapse showed stronger or weaker synaptic efficacy prior to the tetanus, although stronger synapses were found to be more effective in inducing suppression. Synchronous application of tetanic stimulation to both neurons resulted in little or no suppression of both synapses, while the same tetani applied asynchronously (with 100 ms delay) to the two neurons led to marked suppression of one or both synapses. Finally, the heterosynaptic suppression was found to be absent when the distance between the sites of innervation made by two neurons was larger than 50 um, suggesting that the competitive interaction underlying the heterosynaptic suppression occurs only among adjacent synapses. These results are consistent with Hebb's postulate of synaptic modulation and provides an *in vitro* demonstration of activity-dependent synaptic competition at single-cell level.

514.5

SUPPRESSION OF DEVELOPING NEUROMUSCULAR SYNAPSE BY FOCAL IONTOPHORETIC APPLICATION OF ACETYLCHOLINE. Y. Dan and M-m. Poo, Dept. of Biological Sciences, Columbia Univ, N.Y., N.Y. 10021

Brief tetanic stimulation of a presynaptic neuron leads to rapid functional suppression of synapses made by other co-innervating neurons on the same postsynaptic muscle cell (see preceding abstract). In the present study, we tested the hypothesis that such heterosynaptic suppression was due to repetitive postsynaptic activation of myocyte acetylcholine (ACh) receptors by the stimulated neuron. We have examined the effect of repetitive focal iontophoretic application of ACh on the synaptic efficacy at a singly-innervated myocyte in 1-d old *Xenopus* cultures, using whole-cell voltage-clamp recording. The amplitude, duration and frequency of iontophoretic currents were adjusted to produce ACh-induced membrane currents of similar characteristics as those generated by ACh release from a tetanized neuron. We found that application of 100 pulses of ACh (at 2 Hz), each induced an inward current of peak amplitude of 1 to 3 nA and a duration of about 50-70 ms, consistently led to suppression of the synapse if the site of the ACh application was within 10-15 uM from the synapse. The suppression was not due to the desensitization of the ACh receptors by the ACh released during iontophoresis. Both the proximity requirement as well as the persistence of suppression following the repetitive stimulation are similar to the heterosynaptic suppression induced by nerve stimulation. The cellular events underlying the ACh-induced synaptic suppression, the involvement of Ca influx through the activated ACh channels in particular, remain to be investigated.

514.6

NMDA RECEPTOR ANTAGONIST BLOCKS SYNAPSE ELIMINATION DURING CEREBELLAR DEVELOPMENT. Y. Bailly, S. A. Rabacchi*, N. Delhaye-Bouchaud and J. Mariani*. Inst. Neurosc., CNRS & Univ. P. & M. Curie, Paris, France.

The glutamate receptor of the NMDA type has been reported to be involved in axonal remodelling in the immature visual system. During the postnatal development of the rodent cerebellum, the granule cells and the Purkinje cells exhibit a transient sensitivity to NMDA. This occurs precisely when the redundant synapses made by several climbing fibers on each Purkinje cell are eliminated, sculpting the adult one-to-one relationship between these two partners. Therefore, the cerebellum offers a unique model to test the hypothesis that the NMDA receptor participates in synapse elimination. In the present study, a selective antagonist of this receptor, the D,L-APV(1-10mM) was released chronically from a slice of Elvax polymer implanted on rat cerebellar surface during the whole period of synapse elimination (PN4-PN17). The degree of innervation of Purkinje cells by climbing fibers was then analysed by in vivo intracellular recording.

On 183 Purkinje cells recorded from 31 rats treated with D,L-APV, 47.5% remained innervated by 2 or 3 climbing fibers. By contrast, only 17.3% of 75 Purkinje cells recorded in 10 rats treated with the inactive isomer L-APV remained similarly polyinnervated; the polyinnervation persisted also in only 11.1% of the 57 Purkinje cells recorded from 8 rats implanted with Elvax alone.

These data suggest that the NMDA receptor plays an important role in the synapse elimination process, probably related to the modulation of Purkinje cell electrical activity by their afferences.

514.7

A ROLE FOR POST-SYNAPTIC NEURONS IN DETERMINING PRE-SYNAPTIC TRANSMITTER RELEASE PROPERTIES. G.W. Davis*, and R.K. Murphey. Program in Neuroscience and Behavior, University of Massachusetts, Amherst, MA 01002.

Interneurons MGI and 10-3 of the Cricket terminal abdominal ganglion have different response properties. MGI responds phasically to a 100 Hz sound stimulus while 10-3 responds tonically. This phasic/tonic difference is believed to reside in the properties of the sensory to interneuron synapses since neither accommodation of the sensory receptor nor adaptation of the sensory neuron can account for the phasic response of MGI. EPSPs recorded in the dendrite of MGI initially facilitate dramatically and then show pronounced response decrement during high frequency, 100-150 Hz, stimulation of single sensory neurons. We believe this high frequency plasticity is the basis for the phasic response of MGI and that synapses on 10-3 lack this plasticity.

To determine how synapses with different release properties are specified from a single population of sensory neurons on two different interneurons we have examined an identified sensory neuron (SN 3c) which simultaneously contacts both MGI and 10-3. 100Hz stimulation of SN 3c evokes EPSPs in MGI which facilitate dramatically followed by pronounced response decrement, while EPSPs simultaneously evoked in 10-3 are more stable, neither facilitating nor decrementing dramatically. We hypothesize that the post-synaptic interneurons have a role in determining the release properties of their presynaptic terminals. We are currently attempting a quantal analysis of these synapses and investigating the role of post synaptic activity in determining pre-synaptic release properties. Supported by NSF grant # BNS 90-96180.

PARKINSON'S DISEASE

515.1

FERRITIN mRNA EXPRESSION IN RAT BRAIN: EFFECTS OF 6-HYDROXDOPAMINE LESIONS AND LEVODOPA ADMINISTRATION. OJF Foster, A Kingsbury*, DT Dexter*, S Blunt*, SL Lightman, CD Marsden* and P Jenner*. Neuroendocrinology Unit, Charing Cross Hospital, Department of Clinical Neurology, Institute of Neurology, Queen Square, and Department of Pharmacology, King's College, London, UK.

Post-mortem studies of patients with Parkinson's disease (PD) reveal decreased levels of brain ferritin, both in the substantia nigra pars compacta (SNc) which is known to be damaged by the disease process, but also in apparently unaffected areas such as cerebral cortex. The pathogenetic significance of these changes is unclear.

In situ hybridisation histochemistry was used to study the distribution of heavy ferritin mRNA in rat brain and the effects of SNc lesioning and levodopa administration on its regional expression. Heavy ferritin mRNA was expressed chiefly in neurons. Neurons of the dorsolateral septal nucleus, hippocampus and several other brain regions were strongly labelled, moderate labelling was found in the SNc and cerebral cortex, while labelling of striatal cells was low. Following unilateral 6-hydroxydopamine lesioning of the nigrostriatal projection, ipsilateral SNc ferritin mRNA expression was reduced. Oral L-dopa administration for one month had no effect on expression of ferritin mRNA in cerebral cortex, hippocampus or SNc.

These results suggest that loss of nigral dopamine cells in PD could contribute to decreased ferritin levels in the SNc, but that the widespread reduction in brain ferritin seen in PD cases is not due to L-dopa therapy and may reflect an underlying defect of brain iron metabolism in such patients.

515.2

VULNERABILITY OF STRIATAL DOPAMINERGIC NEURONS TO FREE RADICALS: THE PROTECTIVE EFFECTS OF ZINC AND METALLOTHIONEIN. R.F. Pfeiffer* and M. Ebadi. Depts. of Pharmacol. and Neurol. Univ. Nebr. Coll. Med, 600 S. 42nd St., Omaha, NE 68198-6260.

The hallmark of Parkinson's disease is a major loss of dopaminergic neurons, causing a marked reduction in the concentration of dopamine in the corpus striatum. The surviving neurons are able to compensate by enhancing the rate of dopamine synthesis. Furthermore, it has been postulated that this may result in accumulation of peroxide-generating cell toxins causing oxidative stress, which may predispose to an accelerated rate of destruction of the remaining neurons. The altered levels of iron and transferrin in the substantia nigra of Parkinson's patients, along with the beneficial effects of α-tocopherol and/or *L*-deprenyl are additional suggestive evidence. Our laboratory has discovered and characterized metallothionein (MT) in several regions of mammalian brain, including striatum. Zinc, which is able to remove superoxide and displace iron as redox catalyst on macromolecules, is selectively reduced in 6-hydroxydopamine (8 μg in 4 μl of 0.02% ascorbic acid)-lesioned rat striatum. Similarly, MT, with its nucleophilic sulfhydryl group able to interact with intectrophilic toxins in regulating the intracellular redox potential, is also depleted in conditions causing destruction of striatal neurons. Moreover, neuroblastoma IMR-32 cells, unable to synthesize large amounts of MT in culture, exhibit far greater vulnerability to metal toxicity when compared to Chang liver cells, which synthesize copious amount of MT. The results of these studies are interpreted to indicate that zinc and MT may be involved in oxidative stress in Parkinson's disease. (Supported in part by a grant from USPHS ES-0349).

515.3

28kd CALCIUM BINDING PROTEIN IN MIDBRAIN CATECHOLAMINERGIC NEURONS FROM CONTROL SUBJECTS AND PATIENTS WITH PARKINSON'S DISEASE. E.C. Hirsch[1], A. Mouatt[1*], A.M. Graybiel[2], M. Thomasset[3], F. Javoy-Agid[1] and Y. Agid[1]. (1) INSERM U289,Hosp. Salpêtrière, 75013 Paris, France; 2) Dept of Brain and Cognitive Sciences, MIT, Cambridge, MA 02139, USA; (3) INSERM U120, Hosp. Debré, 75019 Paris, France.

Neurons expressing 28kd Calcium Binding protein (CaBP) and tyrosine hydroxylase (TH) were analyzed immunohistochemically in midbrains from 3 controls and 3 patients with Parkinson's disease (PD). Double staining experiments showed that all TH-positive neurons were CaBP-positive in the dorsal mesencephalon (central grey substance and catecholaminergic region A8) and that only 10 to 20% of the CaBP-positive neurons were TH-negative. In the ventral mesencephalon (substantia nigra and ventral tegmental area) only rare CaBP-positive TH-positive neurons were detected, whereas most TH-positive neurons were CaBP-negative.

In PD, the loss of TH-positive neurons was moderate in the dorsal part and severe in the ventral part of the mesencephalon. The number of CaBP-positive neurons was not different in the midbrain of controls and parkinsonian patients. The results suggest that CaBP may contribute to the protection of catecholaminergic neurons that are not affected by pathological process in PD. Supported by INSERM and NIH# (NIH NS 25529).

515.4

EARLY STAGES IN NERVE CELL DEGENERATION IN MPTP-INDUCED PARKINSONISM IN THE SQUIRREL MONKEY. L.S. Forno, L.E. DeLanney, I. Irwin*, P. Bolt* and J.W. Langston. From the VA Medical Center, Palo Alto, CA 94304, and the California Institute for Medical Research, San Jose, CA 95128.

Three 10-12 year old squirrel monkeys were anesthetized and perfused intracardially with paraformaldehyde-glutaraldehyde and examined by electron microscopy (EM) 2, 3 and 4 days after a single s.c. injection of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) 2.5 mg/kg. In all three animals the substantia nigra (SN) displayed distended dendrites and axons containing membranous structures. Mitochondria were generally better preserved than other organelles. Axonal swellings in myelinated fibers were observed in the 2 day animal. Some nerve cells appeared normal; others showed loss of or disorganization of Nissl substance. In the striatum focal vacuolization was noted, and some nerve cell processes contained membranous material. Immunocytochemistry with antibody to tyrosine hydroxylase (TH) revealed striking preservation of TH positive fibers in the striatum in the 3 day animal, with some decrease in the 4 day monkey. In the SN nerve cell loss was not observed, but proximal portions of the nigro-striatal pathway showed swollen axons in the TH-reacted tissue. These findings correlate well with the biochemical changes recently reported in squirrel monkeys that received a similar regimen of MPTP (Brain Res 1990;531:242-252).

515.5

COMPARISON OF NEUROTOXIC ACTIONS OF MPP^+ AND NH_2-MPP^+ ON THE SURVIVAL OF PERIPHERAL ADRENERGIC NEURONS OF THE CHICK EMBRYO. S.V. Bhave, J. Johannssen# and A.R. Wakade. Dept. of Pharmacology, School of Medicine, WSU, Detroit, MI 48201 and # FDA, Washington, D.C.

Central dopaminergic neurons are the potential targets for MPP^+ and its analogues. The neurotoxic effect depends on the uptake of MPP^+ through the dopamine carrier system. Since sympathetic neurons (SN) demonstrate the high affinity and desipramine (DMI) sensitive norepinephrine uptake we examined the effects of MPP^+ and NH_2-MPP^+ on their survival in culture. When MPP^+ or NH_2-MPP^+ was added to the medium along with NGF almost all the neurons died after 2 days in culture. Toxic effect of MPP^+ was evident at 3 nM and was maximum at 1 μM. ED_{50} for MPP^+ and NH_2-MPP^+ was about 20 nM and 200 nM, respectively. Pretreatment of SN with DMI prevented the toxic effects of MPP^+ and NH_2-MPP^+. Neurotoxic effect of MPP^+ was not evident when it was added to the medium 24 hours after the neurons were plated. NH_2-MPP^+ was toxic even when added 24 or 48 hours after the start of culture. Sensory neurons were unaffected by as high as 10 μM MPP^+ or NH_2-MPP^+. Our studies show that primary cultures of chick SN could serve as an effective model to explore the mechanism of neurotoxic action of MPP^+ and related compounds.

515.6

CHRONIC NICOTINE ENHANCES THE DOPAMINERGIC NEUROTOXICITY OF 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPRYDINE IN BLACK MICE. R.A. Behmand* and S.I. Harik. Dept. of Neurology, Case Western Reserve Univ. Sch. of Med., Cleveland, OH 44106.

There is low prevalence of Parkinson disease among cigarette smokers. Nicotine is a major component of cigarette smoke. Thus, we examined the effect of chronic nicotine on 1-methyl-4-phenyl-1,2,3,6-tetrahydroprydine (MPTP) neurotoxicity. Adult male C57 black mice (25 g) were implanted sc under general anesthesia with Alzet minipumps loaded with 15-70 mg nicotine, that discharge their loads over 14 days. Control mice were not given nicotine. Seven days later, MPTP was injected sc, twice daily (6 hr apart) for 2 days. The total dose of MPTP ranged from 20-80 mg/kg. Seven days after MPTP, mice were killed and their striata dissected and analyzed for dopamine and its metabolites by high-performance liquid chromato-graphy with electrochemical detection. We found that nicotine at all doses significantly enhanced MPTP-induced reduction in striatal dopamine and its metabolites. Also, at the higher MPTP doses, several of the nicotine-treated mice died while none of the control mice died. The results strongly suggest that nicotine accentuates rather than reduces MPTP neurotoxicity.

515.7

CHANGES IN BRAIN CATECHOLAMINES AND DOPAMINE UPTAKE SITES AT DIFFERENT STAGES OF MPTP PARKINSONISM IN MONKEYS. G.M.Alexander, R.J.Schwartzman, J.R.Grothusen*, S.Gordon* and D.L.Brainard*. Department of Neurology, Jefferson Medical College, Philadelphia, PA 19107.

1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP) has been shown to produce parkinsonism in primates. We have studied the changes in brain catecholamines and the distibution of desipramine insensitive Mazindol binding sites in MPTP parkinsonian primates. Thirty five monkeys (Macaca fascicularis) were utilized in this study. Twelve naive animals received no treatment and served as controls. Twenty three animals were rendered parkinsonian with serial injections of MPTP. All animals were given scored neurologic examinations throughout the study. Their movement was quantitated in an activity box. The animals were sacrificed 30-180 days after their last MPTP injection. The clinical exam in the MPTP parkinsonian monkeys ranged from mildly to severely affected, with dopamine depletions of 67.6-99.7% in the caudate nucleus, 48.7-99.8% in the putamen, and 0-40% in the nucleus accumbens. Frontal cortex norepinephrine was depleted 0-48.8% in these animals. Mazindol binding was decreased 30-98% in the caudate nucleus, 20-97% in the putamen, 0-26% in the nucleus accumbens, 80-96% in the substantia nigra pars compacta and 49-94% in the ventral tegmental area. In the striatum, the decreased mazindol binding was more promounced lateraly and posterior.

515.8

GENETIC EFFECTS ON DOPAMINE UPTAKE SITES IN THE STRIATUM. S. Roffler-Tarlov and A.M. Graybiel, Neuroscience Program, Tufts Univ. Sch. Med., Boston, MA 02111 and Dept. of Brain and Cognitive Sciences, MIT, Cambridge, MA 02139

Weaver is classified as an autosomal recessive mutation because its behavioral phenotype is visible only in homozygous weaver mice. However, many cellular and biochemical effects of weaver appear in less severe form in the heterozygote in both neuronal systems known to be affected.

This report describes a deficit in ^{3}H-dopamine uptake (an indicator of function for dopamine-containing terminals) in the caudoputamen of the heterozygous weaver in which all dopamine-containing cell bodies survive in the substantia nigra and striatal targets display little loss of dopamine unlike in mice homozygous for weaver. For these experiments, ^{3}H-dopamine uptake and dopamine content were measured in synaptosomal preparations of the dorsal and ventral caudoputamen of adult homozygous normal, heterozygous weaver and homozygous weaver mice. The measurements of dopamine and ^{3}H-dopamine uptake in the homozygous weaver confirmed and extended previous findings of massive depletion of dopamine and even greater reduction of uptake capacity especially in the dorsal striatum. In the heterozygote, little loss of dopamine occurred and that was confined to the dorsal striatum. In contrast, uptake capacity for ^{3}H-dopamine was severely reduced in both the dorsal and ventral caudoputamen. Loss of uptake capacity amounted to 41% in the dorsal and 53% in the ventral caudoputamen of the heterozygote compared to normal mice.

The results add to the evidence showing that weaver exerts effects on neuronal processes that are not accompanied by cell death in the midbrain of the heterozygote. We suggest that these defects may lead to cell death in the homozygote where they are present in two doses.

NS20181; MH00655 and the Parkinson's Disease Foundation.

515.9

ROTATIONAL BEHAVIOR ALTERATIONS FOLLOWING INFUSION OF GABAERGIC AGENTS INTO THE SUBTHALAMIC NUCLEUS (STN). P.M. Carvey, J.S. Kroin, D.H. Lin*, H.L Klawans*, and R.D. Penn. Depts. Neurology and Neurosurgery. Rush U, Chicago, IL.

GABAergic fibers from the basal ganglia regulate activity in the STN which in turn regulates the internal segment of the globus pallidum (GPi) via glutaminergic fibers. Lesions of the STN have been shown to reduce MPTP-induced parkinsonism in primates. We infused GABAergic agents into the STN and monitored apomorphine-induced contralateral rotation (APO-CR) in five, 6-hydroxydopamine (6-OHDA) lesioned rats to test whether modulating output affects rotation in this model. Using 0.5 µl/2.5 min injections through implanted guide cannulae the STN was infused with normal saline (NS), the GABA-A agonist muscimol (200 ng), or the GABA-A antagonist bicuculine (200 ng) at 2 day intervals. Bicuculine decreased APO-CR 79%, NS did not alter APO-CR, while muscimol increased it 157%. It appears that GABA outflow neurons from the striatum regulate activity in the STN which, in turn, influences the outflow from the GPi. Reducing the activity of the STN in the parkinsonian brain using drugs may therefore be beneficial in the treatment of Parkinson's disease.

515.10

PATHOLOGICAL CORRELATES OF DEMENTIA IN PARKINSON'S DISEASE. R.M. Zweig, J.C. Hedreen, M. Cohen*, S. Giere*, and D.L. Price. University of Nevada, Johns Hopkins, and Case Western Reserve Schools of Medicine.

Dementia in idiopathic, Lewy body positive, Parkinson's disease (PD) has been reported to be correlated with neuronal loss in the nucleus basalis (NB), substantia nigra para compacta (SN) (medial part), and locus coeruleus (LC), and with the presence of Lewy body-like (LB) inclusions in neocortex. We counted neurons at selected anatomical levels of the LC and SN, and rated NB neuronal loss and LB numbers in the anterior cingulate (ubiquitin immunocytochemistry), in 13 patients with pathologically confirmed PD without concurrent Alzheimer's disease. Individuals with dementia (7 patients, mean age 76.0) had significantly fewer neurons at all levels of the LC, but not the SN, greater NB neuronal loss, and more anterior cingulate LBs than those without dementia (6 patients, mean age 71.5). In conclusion, dementia in PD reflects patterns of extra-nigral neuronal involvement.

515.11

CELLULAR EXPRESSION OF CATECHOL-O-METHYLTRANSFERASE (COMT) IN RAT CNS AND PERIPHERAL ORGANS REVEALED BY IN SITU HYBRIDIZATION HISTOCHEMISTRY. J.G. Richards, B. Bertocci,* M. Da Prada* and P. Malherbe.* Pharma Division, Preclinical Research, F.Hoffmann-La Roche Ltd, CH-4002 Basel, Switzerland

COMT (EC 2.1.1.6), a membrane-associated enzyme, catalyzes the metabolism of dopamine, noradrenaline, adrenaline and other catechol derivatives. In the brain, COMT is responsible for the extraneuronal inactivation of catecholamines released into the extraneuronal space. cDNA clones for human and rat COMT have been recently isolated and sequenced (Bertocci et al., Proc. Natl. Acad. Sci. USA (1991) 88, 1416-1420; Salminen et al., Gene (1990) 93, 241-247). In order to identify the cells expressing COMT in rat CNS and peripheral organs, we used ^{35}S-labelled oligonucleotide probes (nucleotide sequences 92-152 and 587-647) for in situ hybridization histochemistry. Transcripts were found in discrete regions of rat brain (ependyma, choroid plexus, circumventricular organs, dentate gyrus granule cells > hippocampal CA1-4 pyramidal cells, cerebellar granule cells, Bergmann glia, brain stem nuclei - facial n., vestibular n., olive n., pontine n., > cerebral coretex, olf. bulb), leptomeninges, intracranial blood vessels, pituitary, kidney and placenta. The results suggest that non-neuronal elements provide an enzymatic barrier to the transport of catecholamines between the CSF and the brain (see also Kaplan et al., Brain Research (1981) 204, 353-360). The identity of neurones in the hippocampal formation and brainstem nuclei expressing COMT mRNA is being further investigated.

515.12

NEOSTRIATAL DOPAMINE DENERVATION FOLLOWING INTRASTRIATAL 6-HYDROXYDOPAMINE RESEMBLES WEAVER MOUSE STRIATUM. C. A. Altar, L. B. Jakeman, I. N. Acworth[1], R. H. Soriano, and M. Dugich-Djordjevic. Endocrine Research Dept., Genentech, Inc., South San Francisco, CA, 94080 and [1]ESA, Inc., Bedford, MA, 01730.

Injury to dopamine (DA) neurons was characterized following delivery of 6-hydroxydopamine (6-OHDA) to the rat striatum. Two wks after injecting 8, 25, or 100 µg of 6-OHDA, the density of striatal DA terminals, defined by [^{3}H]mazindol autoradiography, was lowered by 22, 48, and 52% respectively. Losses predominated in dorsal striatum. Caudal-rostral (1.4-fold) and medial-lateral (1.7-fold) gradients of increasing [^{3}H]mazindol binding in the intact striatum were abolished by 8 or 25 µg of 6-OHDA. Striatal contents of DA, 3-MT, DOPAC, and HVA and [^{3}H]mazindol binding were each decreased by 57-86% at 1 and 4 wks after 25 µg 6-OHDA, with a partial recovery in all measures by 4 weeks. Little or no alteration in 5-HT, NE, and related compounds were observed from 1-4 weeks post-injury. 6-OHDA or vehicle increased the density of the glial marker [^{3}H]PK 11195 but only adjacent to the cannula tract. Immunocytochemical staining at 2 wks after 25 µg 6-OHDA showed a 31-50% decrease in the number of tyrosine hydroxylase-positive neurons at 3 levels of the central and medial pars compacta of the substantia nigra but not in the VTA. Intrastriatal 6-OHDA treatment produces a reproducible, DA neuron-selective, and anatomically limited depletion of DA nerve terminals and cell bodies that closely resembles these patterns in weaver mouse striatum. The sparing of DA cell bodies relative to terminals provides a model for neurotrophic factor rescue or sprouting of DA neurons.

515.13

PERSISTENT CONSEQUENCE OF LIMITED TREATMENT WITH L-DOPA IN RATS WITH UNILATERAL NIGRAL LESIONS. P.B. Silverman. Dept. of Psychiatry and Behavioral Sciences, Univ. of Texas Health Sciences Center, Houston, TX 77030.

Rats with a unilateral 6-hydroxydopamine lesion of substantia nigra not only rotate (circle) contralaterally immediately after administration of the direct acting dopamine agonist, apomorphine, but also weeks later when they are reintroduced to the environment in which apomorphine had been administered. Here, the ability of limited L-DOPA treatment to result in similar conditioned rotation was tested. Lesioned, but otherwise drug-naive rats were administered 5 mg/kg carbidopa (ip) 30 min prior to 10 to 50 mg/kg L-DOPA (ip) and placed in hemispherical plastic bowls. Rotations in each direction were counted via a video camera and image analyzer. Two weeks after a single drug exposure, most rats briefly made rapid contralateral rotations when placed in the rotation bowls. As previously found with apomorphine, lower doses of L-DOPA were more effective than higher doses in conditioning this behavior in a single trial. Rats administered carbidopa + L-DOPA on three consecutive days generally showed an increased response to successive administrations (sensitization). Some thrice-treated animals also exhibited explosively rapid rotation when returned, undrugged, to the environment associated with L-DOPA. This striking rotation developed progressively over several months after the last drug treatment and may prove to be a useful model for study of abnormal involuntary movements associated with parkinsonism. (Supported by NIDA grant DA06269).

516.1

NMDA RECEPTOR-MEDIATED MOBILIZATION OF INTRACELLULAR Ca^{2+} STORES IN PRIMARY CENTRAL NEURONS. Sizheng Z. Lei, Dongxian Zhang, and Stuart A. Lipton. Dept. of Neurology, Children's Hospital & Program in Neuroscience, Harvard Medical School, Boston, MA 02115.

NMDA receptor-mediated increases in $[Ca^{2+}]_i$ were examined using digital imaging techniques with the dye fura-2 in primary cultures of rat neonatal retinal ganglion cell or cortical neurons. Bath application of MK-801 (12 μM), APV (200 μM), magnesium (1 mM) or EGTA (2 mM) completely blocked NMDA-evoked increases in $[Ca^{2+}]_i$. Also, a 2 min preincubation with 30-100 μM dantrolene, a drug that blocks Ca^{2+} release from sarcoplasmic reticulum and possibly from neuronal intracellular stores (Hernandez-Cruz et al., *Soc. Neurosci. Abstr.* 1989;15:17), produced a dose-dependent inhibition of the NMDA-evoked $[Ca^{2+}]_i$ increase; maximal block was 60% of the Ca^{2+} response. In whole cell patch-clamp recordings, dantrolene did not inhibit NMDA-activated currents. This result is in agreement with receptor binding studies showing no direct interaction of dantrolene with glutamate receptors (Frandsen & Schousboe, *J. Neurochem.* 1991;56:1075). With 2 mM EGTA and no added calcium in the bath, 2 μM ionomycin produced a transient increase in $[Ca^{2+}]_i$, apparently depleting intracellular stores. This effect of ionomycin could be prevented by preincubation with dantrolene, consistent with an intracellular mode of action of dantrolene. Taken together, these results suggest that NMDA-stimulated Ca^{2+} influx through ion channels leads to the mobilization of intracellular Ca^{2+} stores. Hence, both extracellular and intracellular calcium pools may be important for NMDA receptor-mediated physiologic and neurotoxic events.

516.2

EFFECTS OF THE 1S,3R- AND 1R,3S- ISOMERS OF 1-AMINOCYCLOPENTANE-1,3-DICARBOXYLIC ACID (ACPD) ON METABOTROPIC EXCITATORY AMINO ACID (EAA) RECEPTOR COUPLING IN RAT BRAIN SLICES. D.D. Schoepp, B.G. Johnson, and J.A. Monn. Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46285

The selective metabotropic EAA receptor agonist (±)*trans*-ACPD is a racemic mixture of 1S,3R- and 1R,3S- stereoisomers. These ACPD isomers were characterized for efficacy and affinity at metabotropic EAA receptors using cross-chopped slices from regions of the adult rat brain. 1S,3R-ACPD was substantially more efficacious than 1R,3S-ACPD as a stimulator of ^{3}H-phosphoinositide hydrolysis (indexed by ^{3}H-IP formation) in rat hippocampus, cerebral cortex, and striatum (see table below, N.D. = not possible to determine).

	EC50 (μM)	MAXIMAL EFFECT (% OF BASAL HYDROLYSIS)	N
Hippocampus			
1S,3R-ACPD	40.1 ± 5.7	507 ± 26 @ 300μM	5
1R,3S-ACPD	N.D.	161 ± 13 @ 1000μM	6
Cerebral Cortex			
1S,3R-ACPD	80.9 ± 9.1	462 ± 13 @ 300μM	6
1R,3S-ACPD	N.D.	171 ± 10 @ 1000μM	6
Striatum			
1S,3R-ACPD	22.9	159 ± 9 @ 300μM	8
1R,3S-ACPD	N.D.	119 ± 5 @ 1000μM	8

Further studies in adult rat hippocampus showed that1R,3S-ACPD inhibited 1S,3R-ACPD (100μM) stimulation of ^{3}H-IP formation with an IC50 = 475 ± 24 μM and a maximal effect of 65 ± 2% inhibition @1000μM (n=3). Thus, in adult rat brain tissue 1S,3R-ACPD is a full metabotropic agonist. However,1R,3S-ACPD is a partial agonist with relatively low intrinsic activity, and therefore it acts as an antagonist at fully activated metabotropic EAA receptors.

516.3

ACTIVATION OF THE METABOTROPIC EXCITATORY AMINO ACID (EAA) RECEPTORS BY INTRASTRIATAL INJECTION OF 1S,3R-1-AMINOCYCLOPENTANE-1,3-DICARBOXYLIC ACID (1S,3R-ACPD) INDUCES CONTRALATERAL TURNING. A.I. Sacaan, J.A. Monn and D.D. Schoepp. Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46285

1S,3R-ACPD is a selective agonist at the metabotropic EAA receptor (see Schoepp et al., this issue). In this study we have investigated the consequence(s) of activating the metabotropic EAA receptor *in vivo*. Unilateral intrastriatal injection of 1S,3R-ACPD produced dose-related turning behavior as indexed by contralateral rotations (ED_{50} value = 0.59 μmole / 2 μl, 32 ± 4 rotations/5min at 1 μmole / 2 μl). This effect of 1S,3R-ACPD was not mimicked by saline vehicle (2 μl) or by the less active ACPD isomer at this receptor, 1R,3S-ACPD (2 μmole / 2 μl). Administration of the NMDA receptor antagonist LY274614 (2.5 mg/kg i.p.) did not significantly alter 1S,3R-ACPD (1 μmole)-induced rotations. However, coinjection of the metabotropic receptor antagonist L-AP3 (1 μmole) significantly reduced rotations by 1S,3R-ACPD (1 μmole). Maximally effective behavioral doses of 1S,3R-ACPD did not lead to loss of striatal GABA neurons, as indexed by glutamic acid decarboxylase (GAD) activity in the injected striatum. NMDA (0.2 μmole/ 1 μl) weakly induced contralateral rotations (3.5 ± 2.4 / 5 min), but unlike 1S,3R-ACPD, this was associated with severe clonic convulsions, and a 50 ± 9% loss of GAD activity. This suggests that 1S,3R-ACPD-induced rotations are a behavioral consequence of activating striatal metabotropic EAA receptors. Furthermore, unlike ionotropic EAA receptor activation, direct *in vivo* metabotropic EAA receptor activation is not associated with convulsions and / or excitotoxicity.

516.4

EVIDENCE THAT NMDA RECEPTORS STIMULATE DEPHOSPHORYLATION OF DARPP-32 BY CALCINEURIN IN STRIATAL NEURONS. S. Halpain, G. Snyder, and P. Greengard. Laboratory of Molecular & Cellular Neuroscience, The Rockefeller University, New York, NY 10021.

DARPP-32 is enriched in the medium-sized spiny neurons of mammalian neostriatum. In most cases, dopamine and glutamate have opposite effects on the physiological activity of these cells. One mechanism for antagonistic interactions of glutamate and dopamine on these cells may involve DARPP-32. The phosphorylation state of DARPP-32 on a threonine residue (Thr-34) is increased by dopamine via activation of cAMP-dependent protein kinase. We recently showed that glutamate, via NMDA receptors, is able to antagonize this effect. We suggested that the calcium/calmodulin-dependent protein phosphatase (PP2B, also called calcineurin) might mediate this effect. NMDA receptor activation is known to trigger an increase in intracellular calcium. Both calcineurin and PP2A can dephosphorylate Thr-34 of DARPP-32 in vitro. Okadaic acid is a compound which selectively inhibits PP2A, but not PP2B. We have treated slices of rat brain striatum with okadaic acid in order to examine whether the NMDA effect is mediated by PP2A or by PP2B.

Okadaic acid increased the basal phosphorylation of DARPP-32 Thr-34 in a dose-dependent and time-dependent manner, suggesting that an okadaic acid-sensitive phosphatase does regulate DARPP-32 phosphorylation in vivo. However, okadaic acid did not inhibit the ability of NMDA to induce dephosphorylation of Thr-34. These results suggest that PP2B, but not PP2A, mediates the action of NMDA receptors on DARPP-32 dephosphorylation, and support our hypothesis that NMDA receptors are coupled, via calcium, to the activation of calcineurin.

516.5

NEW NON-NMDA ANTAGONIST DISCRIMINATES BETWEEN AMPA AND KAINATE RESPONSES ON CORTICAL NEURONES IN VITRO BUT NOT ON SPINAL NEURONES IN VIVO. M.G. Jones, A. Palmer and D. Lodge, Department of Veterinary Basic Sciences, Royal Veterinary College, NW1 OTU, U.K.

We have used bath application to cortical wedges and electrophoretic application to spinal neurones in halothane-anaesthetised rats, to characterise non-NMDA receptors in the mammalian central nervous system with a novel benzodiazepine compound, GYKI (Tarnawa et al, 1989). On cortical slices, GYKI had an IC50 vs AMPA (40uM) of 12 ± 1.5uM, whereas responses to KAIN (5uM) and NMDA (40uM) were reduced by 50 and 20% respectively. The AMPA dose-response curve was shifted in a more or less parallel manner. On spinal neurones in vivo electrophoretic GYKI reversibly reduced responses to AMPA, KAIN and NMDA by 58 ± 27, 43 ± 28 and 17 ± 18% respectively. Similar benzodiazepine analogues may prove to be useful non-NMDA antagonists with pharmacological and therapeutic benefits.

Tarnawa, I., Farkas, S., Berzsenyi, P., Pataki, A. & Andrasi, F. (1989) Eur. J. Pharmacol. 167, 193-199.

516.6

MULTIPLE QUISQUALATE UPTAKE SITES IN RAT HIPPOCAMPAL SLICES Ian C. Kilpatrick* and Peter S. Harrison* (SPON: Brain Research Association). *Department of Pharmacology, University of Bristol, Bristol, BS8 1TD, UK.*

Potentiation of the depolarising action of the excitatory amino acid (EAA) agonist, quisqualate (QUIS), by uptake blockers suggests the compound may be subject to uptake mechanisms. However, we have already shown an absence of active uptake of 16μM QUIS into rat hippocampal slices [Harrison & Kilpatrick (1991) *J. Physiol. (Lond).* **435**, 43P]. The aim of this study was to directly investigate the accumulation of higher QUIS concentrations. In addition, since active EAA transport appears to occur via 2 distinct sites: a high-affinity, Na^+-dependent carrier and a lower-affinity, Cl^--dependent/Ca^{2+}-potentiated site, the relative effects of replacement of Na^+ and Cl^- with impermeant ions has been examined.

Following incubation of 400μm slices with either 200μM or 2mM QUIS for 4 min at 25°C, homogenate levels of QUIS as measured by HPLC were 502 ± 53 and 2871 ± 128 pmol/slice (mean ± S.E.M; n=7 and 8, respectively). Both values were reduced to at least 60% of control after incubation at 2°C or by inclusion of 100μM ouabain. On complete replacement of the Na^+ content of the Krebs'-bicarbonate buffer by choline, QUIS accumulation at 200μM was greatly reduced (-83.8%, n=3; $P<0.01$, Student's *t*-test). Conversely, substitution of the medium Cl^- content by either ethanoate (CH_3COO^-) or methanesulphonate ($CH_3SO_3^-$) did not influence QUIS transport at this concentration (+0.4%, n=3 and +2.1%, n=3, respectively). At 2mM QUIS, however, Cl^- substitution with $CH_3SO_3^-$ but not with CH_3COO^- (+7.5%; n=3), induced a significant reduction in uptake of -22.7% (n=3, $P<0.05$), whereas replacement of Na^+ offset uptake by -72.2% (n=3, $P<0.01$).

It would appear that accumulation of QUIS at 200μM is predominantly via a Na^+-dependent transport site but at higher concentrations (*e.g.* 2mM), a lower-affinity, Cl^--dependent component augments uptake. The influence of medium HCO_3^- and Ca^{2+} content on QUIS uptake is presently under investigation.

We thank the Medical Research Council.

516.7

OPEN-CHANNEL BLOCK OF NMDA RESPONSES BY THE ANTIPARKINSONIAN DRUG MEMANTINE.

H.-S. Vincent Chen and Stuart A. Lipton. Depts. of Biol. Chem. & Molec. Pharm. and Neurology, Harvard Medical School, Boston, MA 02115.

Excessive activation of *N*-methyl-D-aspartate (NMDA) receptors is thought to mediate neurotoxicity during cerebral anoxia, stroke, and several neurodegenerative diseases. NMDA antagonists therefore have therapeutic potential for these diseases. One type of antagonist (e.g., MK-801) is a use-dependent NMDA open-channel blocker. Recently, memantine, an antiviral and antiparkinsonian drug, was also reported to be a use-dependent antagonist of NMDA-evoked current and to inhibit [^{3}H]MK-801 binding (Bormann, *Eur. J. Pharm.* 1989;166:591; Kornhuber & Bormann, *ibid.* 589). We used the whole-cell patch clamp technique on rat retinal ganglion cells to characterize further the mechanism of memantine block of NMDA-activated current. At a holding potential (V_H) of -60 mV, 12 μM memantine selectively and nearly completely inhibited the current elicited by 200 μM NMDA (n = 11) without affecting kainate (50 μM) and quisqualate (5 μM) responses. The memantine blockade was voltage dependent (little inhibition at V_H = +50 mV) and could be abolished by pre-exposure to 3 mM extracellular magnesium. The rate of recovery from memantine blockade was also voltage- and agonist-dependent. Taken together, the evidence suggests a mechanism of open channel block. Compared to MK-801, however, memantine has much faster macroscopic blocking and unblocking rates ($\tau \approx$ 1.1 s and 6.5 s, respectively; V_H = -60 mV, $K_d \approx$ 2 μM). With these fast kinetics at concentrations previously shown to be clinically tolerated, memantine-like drugs may eventually prove useful as NMDA antagonists for future therapeutic intervention.

516.8

EFFECTS OF POLYAMINES AND THEIR ANTAGONISTS ON NMDA-STIMULATED INCREASES OF INTRACELLULAR CALCIUM.

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The endogenous polyamines (PA) spermine and spermidine specifically modulate NMDA-receptor mediated events (e.g. [^{3}H]MK801 binding and ionic currents). Antagonists and inverse agonists suspected of interacting with this polyamine site have also been described. We have characterized the activity of various agonists, antagonists and inverse agonists at the NMDA-receptor in a system utilizing NMDA-stimulated changes in intracellular calcium (Cai) of cultured chick cortical neurons. Spermine and spermidine potentiated an increase in intracellular calcium.in the presence of maximally effective concentrations of NMDA (500 μM) and glycine (100 μM) (EC_{50}s: 346 μM and 734 μM, respectively). Both compounds demonstrated an inversion of the dose response curve for concentrations above 1.5 mM in the presence of NMDA and glycine. Both polyamines were moderately effective at elevating Cai in the absence of added NMDA and glycine (11 % of basal) likely due to endogenous glutamate and glycine. Contrary to previous reports, in the present system the purported antagonist diethyltriamine (DET) and the endogenous PA putrescine were virtually ineffective at the PA recognition site showing only moderate (12 %) reversal when administered at 2.5 mM. Arcaine and diaminodecane (DA10) were potent antagonists (250 μM, approx. 40 % reduction) of PA-stimulated increases in Cai. Arcaine and DA10 also displayed inverse agonist properties in the presence of NMDA and glycine (53 and 38 % decrease of maximal NMDA/glycine response, respectively. It is possible that the activity of Arcaine and DA10 in the absence of added PA agonists is due to interaction with endogenous polyamines.

516.9

DO PUTATIVE POLYAMINE ANTAGONISTS ACTUALLY ANTAGONIZE A FUNCTIONAL RESPONSE TO POLYAMINES: AN ANALYSIS IN THE RAT CORTICAL WEDGE MODEL

P. A. Boxer and L.J. Robichaud, Parke-Davis Pharm. Res. Div., Warner-Lambert Co., Ann Arbor, MI 48105.

Recent evidence has suggested that polyamines such as spermine and spermidine bind to a site associated with the NMDA receptor-ion channel complex and act to enhance responses to excitatory amino acids. Previously, we have demonstrated that the rate of spontaneous epileptiform discharges (SED) in the rat cortical wedge model (in Mg^{++}-free buffer) is increased in the presence of spermine (30 μM to 1 mM). This increase in rate is both time and dose dependent; however, treatment with 1 mM for >90 min or exposure to 3 mM spermine blocks the discharges. Several compounds have been described as polyamine antagonists: arcaine (1,4-diguanidinobutane), putrescine (1,4-diaminobutane), the antihypertensive, ifenprodil, and DET (diethylenetriamine); as well as the inverse agonist 1,10-diaminodecane (DA10). Arcaine (30-100 μM) and putrescine (3 mM) increased SED rate, similar to spermine. These diamines both left shifted the dose-response curve to spermine, suggesting that they are agonists at the polyamine site. Ifenprodil produced a slight increase in SED rate at 10 μM and inhibition at higher concentrations. The inhibition could not be reversed by spermine nor was the enhancement at low doses additive with spermine. Based upon this data it would appear that ifenprodil does not interact in a competitive manner with spermine at the polyamine site. On the other hand DA10 at a concentration which had no effect on its own (100 μM) blocked the increase in SED rate produced by spermine . No evidence of inverse agonism was detected. In conclusion, in a model where polyamines clearly affect NMDA mediated responses only DA10 functioned as a polyamine antagonist.

516.10

COMPETITION BETWEEN Mg^{2+} AND PCP FOR BLOCK OF NMDA ACTIVATED CHANNELS IS REDUCED AT DEPOLARIZED POTENTIALS.

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We recently showed that physiological concentrations of Mg^{2+} greatly impede the ability of phencyclidine (PCP) to block NMDA activated channels. Receptors were expressed in *Xenopus* oocytes injected with rat brain mRNA and voltage clamped at -60 mV (Lerma et al., Neurosci. Lett. 123: 187-191, 1991). Block was competitive in that Mg^{2+} decreased the potency of PCP in blocking open NMDA channels without changing its maximum effect, i.e., complete block. Channel block by Mg^{2+} or PCP alone is reduced by depolarization, and since neurons are often depolarized, it was of interest to determine the effect of depolarization on the interaction of PCP and Mg^{2+}. PCP and Mg^{2+} applied together reduce NMDA-induced currents more than do either blocker alone. However, the degree of PCP block can be estimated by removal of NMDA and blockers, thereby trapping PCP in the channel, and reapplication of NMDA alone, which provides a test of the extent of PCP trapping and of the original degree of block. By this method we found that at -20mV Mg^{2+} (0.5mM) had almost no effect on PCP block of NMDA-activated channels. This result is not unexpected, given the greater charge on Mg^{2+} and the likelihood that a substantial component of PCP binding is due to non-electrostatic interactions. Our results suggest that neuronal activity will affect Mg^{2+}/PCP interaction *in vivo* and alter the pharmacodynamics of PCP. This factor may account for differences in behavioral effects of PCP receptor ligands and competitive antagonists at NMDA receptors.

516.11

NON-COMPETITIVE NMDA-CHANNEL BLOCKERS INCREASE MEMBRANE CONDUCTANCE AND GLUTAMATE RESPONSES IN CULTURED ASTROCYTES.

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Astrocytes have been shown to express multiple transmitter-gated ion channels, including glutamate-gated kation conductances. We used the whole cell patch clamp technique to study the influence of the non-competitive N-methyl-D-aspartate (NMDA) channel blockers ketamine and MK-801 onto glutamate activated membrane currents of cultured rat cortical astrocytes. With caesium present in the recording pipette, glutamate (10^{-3}-10^{-4} M) activated an inward current ranging from 50 to 600pA. Concomitant application of MK-801 or ketamine (10^{-3}-10^{-4} M) resulted in a profound increase (up to 300%) in current amplitude. Application of the NMDA antagonists alone, also elicited a small inward current. However, the overall enhancement of the glutamate current could not be accounted for by a simple additive effect. The fact that responses elicited by glutamate, NMDA-antagonists, or the combination of both, reversed near 0 mV excludes the possibility that the currents reflect an uptake mechanism. In addition, we also observed a comparable potentiation of kainate currents by NMDA channel blockers. The data show that astroglial cells are targets of non-competitive NMDA antagonists and call for special care in the interpretation of experiments obtained with systemic application of these compounds. Supported by BMFT 316902A5.

516.12

WITHDRAWN

517.1

ALZHEIMER'S AND CONTROL BRAIN CONTAIN SOLUBLE ß AMYLOID PROTEIN PRECURSOR DERIVATIVES ENDING AT POSITION 15 OF THE ß AMYLOID PROTEIN. J. Pasternack[1], R. Cotter*[2], B. Greenberg[3], R. Heinrikson*[3], D. Lowry*[3], M. Usiak*[1], R. Wang*[2], H. Zurcher-Neely*[3], and S. Younkin[1]. [1]Case Western Reserve Univ., Cleveland, OH 44106; [2]Middle Atlantic Mass Spectrometry Facility, Johns Hopkins Univ., Baltimore, MD 21205; [3]Upjohn Laboratories, Kalamazoo, MI 49001.

To identify the carboxyl terminus of the ~125 and ~105 kDa soluble ß amyloid protein precursor (ßAPP) derivatives produced from full-length membrane-associated ßAPP, we purified these derivatives, digested with CNBr, separated the CNBr peptides by RPLC using a C18 column, identified putative C-terminal peptides by ELISA with anti-βAP_{1-40} (provided by D. Selkoe), and characterized these peptides. To implement this approach, we first analyzed the abundant soluble ßAPP derivatives secreted by Sf9 insect cells infected with baculovirus expressing full-length βAPP_{751} (Bac_{751}) or βAPP_{695} (Bac_{695}). Two ßAP-bearing peptides were readily separated and identified in the CNBr digest of these derivatives, and analysis by direct sequencing, amino acid composition, and mass spectrometry showed these peptides to be βAP_{1-16} and βAP_{1-15}. This indicates that Sf9 insect cells, like human embryonic kidney (293) cells (Esch et al., Science 248: 1122, 1990), produce soluble derivatives by cleaving full length ßAPP at βAP_{15-16}. Soluble ~125 and ~105 kDa ßAPP derivatives isolated from AD or control brain were analyzed in a similar fashion. In both the AD and control brain, C-terminal βAP_{1-15} was identified by RPLC in conjunction with direct sequencing and mass spectrometry. These data indicate that, in human brain, as in other cells, soluble ßAPP derivatives are produced by cleavage at βAP_{15-16}. It is not clear, however, that this is the only or even the major cleavage producing soluble derivatives in human brain. Additional analysis is needed to determine if cleavage producing soluble derivatives occurs at multiple sites producing the multiplicity of carboxyl-terminal ßAPP fragments that we have observed in human brain and cultured cells (see Estus et al., Golde et al., these meetings). It will be important, in particular, to determine if even a small fraction of the soluble derivatives in AD or control brain are potentially amyloidogenic forms containing the entire ßAP.

517.2

C-TERMINAL FRAGMENTS CONTAINING FULL LENGTH ß AMYLOID PROTEIN ARE PRODUCED DURING NORMAL PROCESSING OF THE ß AMYLOID PROTEIN PRECURSOR IN CULTURED CELLS. T. E. Golde, S. Estus, L. Younkin, M. Usiak, and S. G. Younkin. Institute of Pathology, Case Western Reserve University, Cleveland, OH 44106

Processing of the ß amyloid protein precursor (ßAPP) along a secretory pathway generates a soluble C-terminal truncated form and a membrane associated C-terminal fragment. Using a pulse chase paradigm followed by immunoprecipitation of cellular lysates with an anti-C-terminal ßAPP antibody (provided by D. Selkoe), we have studied the processing of the ßAPP in human embryonic kidney (293) cells overexpressing full length βAPP_{695}. Using Tris/tricine SDS PAGE, we have identified a set of at least five 8-12 kDa C-terminal fragments in these cells. These fragments appear to be derived from processing of the full length ßAPP because they appear after the synthesis of full length ßAPP. Two findings indicate that the two largest C-terminal fragments contain full length ß amyloid protein (ßAP): 1) labeling of these fragments with antibodies raised against the first 17 aa of the ßAP (provided by B. Greenberg) and 2) comigration of the second largest fragment with a band augmented in 293 cells transfected with constructs expressing the full length ßAP through the C-terminus of the ßAPP. Essentially the same set of at least 5 C-terminal fragments is present in control 293 cells (although at a much reduced level); in a variety of human cell lines including fibroblasts, neuroblastomas, and a teratocarcinoma; and in human brain (see Estus et al., these meetings) indicating that ßAPP processing in human brain is similar to that in cultured cells. However, marked quantititive differences in the proportions of these fragments are observed in various cell lines and tissues, and several cell lines produce additional fragments. We are currently investigating whether the multiple C-terminal fragments that we have identified are generated through the secretory pathway that produces soluble ßAPP derivatives or via an alternative metabolic route.

517.3

POTENTIALLY AMYLOIDOGENIC C-TERMINAL FRAGMENTS OF THE ß-AMYLOID PROTEIN PRECURSOR IN HUMAN BRAIN. S. Estus[1], T. Golde[1], T. Kunishita*[2], D. Blades[1], M. Eisen*[1], D. Lowery*[3], M. Usiak*[1], B. Greenberg[3], and S. Younkin[1]. [1]Case Western Reserve Univ., Cleveland, OH 44106; [2]Natl. Inst. of Neuroscience, Kodaira, Tokyo 187, Japan; [3]Upjohn Labs, Kalamazoo, MI 49001.

The 39-42 residue ß-amyloid protein (ßAP) deposited as amyloid in Alzheimer's disease (AD) is an internal peptide that begins 99 residues from the C-terminus of alternatively spliced, 695-770 residue, membrane-associated ßAP precursors (ßAPP). Previous studies in this and other laboratories have established that full-length ßAPPs are normally cleaved near their C-termini to produce large secreted derivatives and small C-terminal fragments. To isolate ßAPP C-terminal fragments from human brain, detergent extracts of a human cerebral cortical membrane fraction were passed over an immunoaffinity column containing a polyclonal antibody (anti-$\beta APP_{672-695}$) raised against the last 24 amino acids of full length ßAPP. Proteins eluted after extensive washing were separated using Tris/tricine SDS/PAGE, and immunoblotted. The superior resolution of small proteins afforded by Tris/tricine SDS/PAGE revealed at least 5 distinct proteins (~11.8, ~11.4, ~10.9, ~9.6, and ~8.7 kDa) migrating between the 6 and 12 kDa markers. Each of these proteins was specifically labeled with anti-$\beta APP_{672-695}$ as well as three other anti-C-terminal antibodies (provided by D. Selkoe). The ~11.4 kDa polypeptide comigrated with a 100 residue C-terminal fragment (which begins with $M\beta AP_{1-42}$) synthesized in a rabbit reticulocyte lysate system. Anti-βAP_{1-40} (provided by D. Selkoe) labeled the ~11.8 and ~11.4 kDa proteins, and this labeling was abolished by absorption with βAP_{1-15} and βAP_{1-17} as well as βAP_{1-40}. Anti-βAP_{1-17} also labeled the two largest C-terminal fragments. Using pulse-chase methodology (see Golde et al., these meetings), we identified an essentially identical set of at least 5 C-terminal ßAPP fragments in cultured 293 cells that are produced by metabolism of full-length ßAPP. Taken together these data indicate that multiple C-terminal ßAPP fragments are produced in human brain some of which contain the entire ßAP and hence are potentially amyloidogenic. Significantly, our preliminary data indicate that tissues showing marked amyloid deposition (e.g. temporal cortex) have high levels of potentially amyloidogenic C-terminal derivatives whereas relatively non-amyloidogenic tissues (cerebellum, liver, kidney) have predominantly the smaller C-terminal fragments.

517.4

CHARACTERIZATION OF β-AMYLOID PRECURSOR PROTEIN ISOFORMS.

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Several secreted or membrane-bound isoforms of amyloid precursor protein (APP) exist which may contain or lack a Kunitz-type protease inhibitor or an extradomain. Using a sensitive protease inhibitor assay as well as antibodies against different domains we have purified isoforms of APP from PC12 cells after induction with bFGF. Biochemical characterization demonstrated differences in the glycosylation pattern of membrane and secreted APP forms. Furthermore, the major secreted form contained the Kunitz-type protease inhibitor but lacked the extradomain. The extradomain, however, was present in membrane-bound APP isoforms. As compared to secreted APP, Kunitz-type protease inhibitor containing isoforms were reduced in membrane-bound APP. These biochemical differences may contribute to different as yet unidentified functions of membrane-bound and secreted APP. Currently we are testing APP isoforms for their effects on growth and survival of distinct CNS neuron types.

517.5

Heterogeneity of Amyloid Precursor Expression in Olfactory Neuroblasts. B.L.Wolozin, D. Chung*, B. Leibovics*, B.B.Zheng*, I. Lieberburg and T. Sunderland. Lab. of Clinical Science, NIMH, Bethesda, MD 20892 and Athena Neurosciences, S.F., CA 94080.

Amyloid Precursor Protein (APP) expression was examined in cell lines of olfactory neuroblasts (ON) from human donors. In basal conditions of cell culture, ON protein and gene expression was found to consist of primarily APP_{770} and APP_{751}. PCR analysis indicates the ratio of APP_{770}:APP_{751}:APP_{695} is 65:100:7.50. The amount of APP detected by immunoblotting with antibody targetting the ß/A4 region of the molecule appeared to vary among individuals. Antibodies targetting the N and C termini also detected variation in APP immunoreactivity, but to a lesser extent. We have looked at cell lines from 10 donors less than 50 years old who died of non-neurologic causes and 3 donors with AD. APP was abundantly expressed in 7 of the young donors. However, 3 of the cell lines from young donors and all 3 AD cell lines expressed APP at much lower levels. Calcium stimulation of the cells increased APP immunoreactivity and resulted in approximate equalization of APP levels for all the cell lines. Because immunoprecipitation experiments show a calcium induced decrease (rather than increase) in APP levels, we suspect that the apparent calcium induced increase in APP levels seen by immunoblot is due to a post-translational modification of APP. Analysis of APP expression in a non-neuronal cell model (lymphoblasts) did not indicate any variation in baseline or calcium-stimulated APP levels between AD and controls.

517.6

ß-AMYLOID PEPTIDES DESTABILIZE CALCIUM HOMEOSTASIS IN HUMAN CORTICAL NEURONS AND INCREASE THEIR VULNERABILITY TO NEUROFIBRILLARY DEGENERATION. M. P. Mattson, R. Rydel, B. Cheng and I. Lieberburg, Center on Aging and Anatomy & Neurobiology, Univ. of Kentucky, Lexington KY 40536; Athena Neurosciences, South San Francisco, CA 94080.

Human cerebral cortical cell cultures were employed to test the hypothesis that ß-amyloid peptides (ßAPs) can render neurons more vulnerable to calcium-mediated excitatory amino acid (EAA) neurotoxicity. Synthetic ßAPs corresponding to amino acids 1-38 or 25-35 of the ß protein enhanced glutamate neurotoxicity, while a peptide with a scrambled sequence was without effect. ßAPs 1-38 and 25-35 alone had no effect on neuronal survival. ßAPs enhanced both kainate and N-methyl-D-aspartate (NMDA) neurotoxicity. The effects of ßAPs on EAA-induced neuronal degeneration were concentration-dependent (10nM - 40 µM) and required prolonged exposures. Measurements of intracellular calcium levels indicated that ßAPs made neurons vulnerable to EAA-induced calcium elevation. The ßAPs also rendered neurons more vulnerable to calcium ionophore neurotoxicity indicating that the ßAPs destabilize calcium homeostasis. Finally, the ßAPs made neurons more vulnerable to neurofibrillary tangle-like antigenic changes induced by EAAs or calcium ionophore (i.e., increased staining with tau and ubiquitin antibodies). Neuronal calcium destabilization leading to neurofibrillary degeneration was also seen with a peptide corresponding to amino acids 25-28 of the ß protein. We propose that in Alzheimer's disease, ß-amyloid destabilizes neuronal calcium homeostasis and so renders neurons more vulnerable to environmental insults. (supported by the Alzheimer's Association).

517.7

PLASMA AMYLOID PRECURSOR PROTEIN IS A MARKER FOR ALZHEIMER'S DISEASE. A.I.Bush*, R.D.Moir*, Q.-X.Li*, J.Currie*, D. Small, B. Rumble*, U. Mönning#*, K.Beyreuther#* and C.L.Masters. Dept. of Pathology, Univ. of Melbourne, Parkville, VIC, Australia and # Center for Molecular Biology, Univ. of Heidelberg, Heidelberg, Germany.

βA4 amyloid deposition in brain, characteristic of Alzheimer's disease (AD), may result from the failure of the amyloid precursor protein (APP) to be correctly processed. A blood marker reflecting this abnormal metabolism would be of predictive and diagnostic value, as well as provide a means of monitoring the efficacy of therapeutic interventions. We have previously sequenced APP from the human platelet where it is detected by Western blotting as 130 and 110 kDa proteins and may be released with the βA4 domain intact[1]. Plasma contained these and additional forms of 65 and 42 kDa. We analysed immunoblots of plasma APP from AD cases (n=26) and controls (n=34) and found a 60% increase of the 130 kDa species of APP in AD ($p< 0.001$), no differences in 110 and 65 kDa forms and a 35% decrease in the 42 kDa form ($p< 0.001$). Studies of protease action on APP indicated that plasma APP forms may be proteolytic cleavage products. The APP abnormalities in the circulation in AD may reflect a failure of constitutive proteolysis due to modifications of APP, or anomalies involving degradative pathways, co-factors or inhibitors. Abnormalities of constitutive APP proteolysis may allow APP to be processed through an alternative, amyloidogenic, pathway. Our studies show that the source of plasma APP and the mechanism responsible for the APP differences detected may lie outside the vascular compartment.

1. A.I. Bush *et al.*, (1990) *J. Biol. Chem.* **265**, 15977.

517.8

FORMS OF β-AMYLOID PRECURSOR PROTEIN (β-APP) IN HUMAN PLATELETS AND LYMPHOCYTES. M. Schlossmacher*, B. Ostaszewski*, I. Lieberburg+, K. Kosik, D. Selkoe. Brigham & Women's Hosp., Harvard Medical School, Boston,MA 02115;+Athena Neurosciences, San. Francisco 94080.

Amyloid-β-protein (AβP) is progressively deposited in cerebral plaques and meningocerebral vessels in Alzheimer's disease (AD). AD shares numerous features with amyloidoses of circulating origin. The soluble, C-truncated ~125 kDa form of βAPP (i.e., PN-II), which we have identified in plasma, has also been localized to platelet α-granules. Full-length (FL) βAPP has been reported to be released from agonist stimulated platelets as membranous microparticles. We have examined peripheral blood cells with regard to βAPP isoforms. After purification of platelets from contaminating leukocytes, we detected the mature, N- plus O-glycosylated ~140 kDa FL-βAPP in platelet membranes but only trace amounts of immature, N-glycosylated βAPP. In the cell-free releasate of activated platelets, we found abundant PN-II but failed to detect FL-βAPP associated with microparticles. Membrane extracts of resting and stimulated platelets also revealed comparable amounts of a ~10 kDa fragment, which probably represents the C-terminal portion of βAPP after cleavage of PN-II. B and T lymphocytes isolated from peripheral blood and cultured EBV-transformed B cells contained small amounts of internal membrane-associated mature and immature FL-βAPP. The presence of amyloidogenic FL-βAPP molecules in peripheral blood cells has potential relevance for the pathogenesis of AβP deposition in AD.

517.9

PROCESSING OF ß-AMYLOID PRECURSOR PROTEIN IN ASTROCYTES AND MICROGLIA FAVORS A LOCALIZATION IN INTERNAL VESICLES C. Haass*, A. Hung* E.H. Koo, and D.J. Selkoe. Center for Neurologic Diseases, Harvard Medical School, Brigham and Women's Hospital, Boston MA.

We report the expression of ßAPP in microglial cells and astrocytes in primary cultures of cerebral cortex of newborn rats. Both cell-types express substantial amounts of ßAPP 695, 751 and 770 as determined by metabolic labeling followed by immunoprecipitation and by PCR-mediated amplification of the corresponding mRNAs. Both cell types show a reduced secretion of soluble fragments of ßAPP into media compared to other cell types transfected with cDNAs coding for ßAPP. This can be monitored by using the remaining membrane-bound 10 kDa fragment following constitutive proteolytic cleavage as a marker. The amount of the 10 kDa fragment in both cell types is low, correlating with the relatively low amounts of the soluble ßAPP fragments in media. Immunocytochemistry reveals that ßAPP is located intracellularly in Golgi-like structures, with very little cell surface staining. From these data, we propose that at least in astrocytes and microglia cells, ßAPP may primarily occur in an intracellular, membrane-associated form rather than being cleaved and secreted or inserted at the cell surface. It may thus perform a principally intracellular function. We also present preliminary data showing that microglial cells secrete C-terminal-containing βAPP fragments into the media. These fragments are potentially amyloidogenic since they contain the C-terminus and ß the peptide epitopes.

517.10

EFFECTS OF BETA AMYLOID I: INDUCTION OF ALZ-50. J. Busciglio and B.A. Yankner. Dept. of Neurology, Children's Hospital and Harvard Medical School, Boston, MA 02115.

The β amyloid protein causes neuronal degeneration in primary rat hippocampal neurons after 3 days in culture (Yankner et al. Science 250:279, 1990). We have assayed for neuronal expression of the neurofibrillary tangle-associated Alz-50 immunoreactive proteins (Alz-50 i.p.s.) by quantitative immunoblot analysis and immunocytochemistry. The Alz-50 i.p.s. are expressed at low levels during neuronal development in primary hippocampal cultures as two isoforms with molecular weights 55 and 68 kD. Treatment with β amyloid induced a 4-5-fold increase in Alz-50 i.p.s. which was not observed after addition of control peptides or a reverse sequence β amyloid peptide. The levels of several other cytoskeletal proteins were unchanged. The β amyloid dose response for induction of Alz-50 i.p.s. was quite similar to the dose response for the neurotoxic effect. Treatment with the excitotoxin glutamate did not induce the Alz-50 i.p.s. In addition, coincubation of β amyloid with the excitatory amino acid antagonists kynurenate and APV did not affect the induction of Alz-50 i.p.s. Preincubation of neurons with cycloheximide prevented subsequent induction of Alz-50 i.p.s. by β amyloid. Thus β amyloid can specifically induce the altered cytoskeletal proteins associated with Alzheimer's disease by a mechanism which requires protein synthesis and which differs from that of excitatory amino acids.

517.11

EFFECTS OF BETA AMYLOID II: PROTECTION BY SUBSTANCE P. M. Flint Beal, Neil W. Kowall, Jorge Busciglio, Lawrence K. Duffy and Bruce A. Yankner. Neurology Service, Mass. General Hospital, and Children's Hospital, Boston, MA 02114.

The role of β amyloid protein in the pathogenesis of neuronal degeneration in Alzheimer's disease (AD) is a central issue in the illness. We found that focal deposition of β amyloid in adult rat cerebral cortex causes profound neurodegenerative changes including neuronal loss, degenerating neurites and induction of Alz-50-immunoreactive proteins associated with AD. In the present study we examined the effects of local or systemic administration of substance P on the neurodegenerative effects. β-amyloid (3 nmol) or control peptides were stereotactically injected into parietal cortex in a volume of 1 µl. Animals were sacrificed at 1 week and perfused for histologic evaluation. Neuronal counts were made in a 0.64 mm^2 square in the area of maximal neuronal degeneration. Coinjection of substance P (2-200 pmol) with β amyloid dose-dependently prevented neuronal loss. Induction of Alz-50 immunoreactivity by β amyloid as detected by either immunocytochemistry or immunoblot analysis, was also blocked by substance P. Peripheral administration of substance P in a dose of 2-200 nmol/kg i.p. 30 min before intracortical injection of β amyloid dose-dependently blocked the neurotoxicity. Administration of substance P for up to 24 hr after the β amyloid injection was effective in preventing neurotoxicity, but the effect dissipated by 72 h. These results show that β amyloid neurotoxicity in vivo is prevented by either local or systemic administration of substance P.

517.12

EFFECTS OF BETA AMYLOID III: THE PRIMATE BRAIN A.C. McKee, N.W. Kowall, M.F. Beal, J. Schumacher and B.A. Yankner Depts. of Neuropathology, Neurology and Neurosurgery, Massachusetts General Hospital, Children's Hospital and Harvard Medical School, Boston, MA 02114

Deposition of ß amyloid protein in the brain is a universal feature of Alzheimer's disease (AD). To determine the effects of ß amyloid deposition, we performed intracerebral microinjections of a ß amyloid peptide (ß1-40) in adult Cynomolgus monkeys. Bifrontal craniotomies were performed with the resection of a dural flap. Microinjections of ß1-40 and control peptides were performed under stereotactic guidance. After short survival intervals, the brains were perfusion-fixed, cryoprotected and cut at 50 µ on a freezing microtome. Sets of serial sections were processed for histopathology and immunohistochemistry with the monoclonal antibody Alz 50, and antibodies to tau, ß amyloid and ubiquitin. The ß1-40 peptide injections produced a defined ß amyloid-immunoreactive deposit surrounded by neuronal degeneration and abnormal ubiquitin and Alz 50 positive neurites. The extent of degeneration was dose-dependent. Control peptides produced cortical disruption at the injection site without a significant lesion. A substituted ß1-40 peptide produced a smaller lesion than that caused by native ß1-40. These findings suggest that ß amyloid protein is neurotoxic and may, therefore, be responsible for neuronal degeneration in AD.

518.1

GANGLIOSIDE GM1 MAY COUNTERACT THE MPTP-INDUCED UNILATERAL DECREASE IN NUMBER AND MEAN VOLUME OF PIGMENTED NEURONS IN THE SUBSTANTIA NIGRA OF THE PIG-TAILED MACAQUE. [1]A.M. Ianson,[2]A. Møller,[3]H. Nakashima,[4]G. Toffano*,[3]M. Goldstein and [1]K. Fuxe. [1]Dept of Histology and Neurobiology, Karolinska Institutet, S-104 01 Stockholm, Sweden; [2]NeuroSearch, Copenhagen, Denmark, [3]Dept of Psychiatry, New York, New York Univ. Medical Center, New York, USA, [4]Fidia Research Laboratory, Abano Terme, Italy.

Two female pig-tailed macaques (b.wt. 5.9 kg) were infused with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, 2 mg/kg) in the left internal carotid artery. Starting 30 min before the MPTP injection one macaque was treated with the ganglioside GM1 (20 mg/kg) daily, i.m. for 18 days. The other macaque received saline injections. The day after the last GM1 injection the animals were sacrificed, perfused with 4 % paraformaldehyde and the brains were processed for immunohistochemistry after cryotome sectioning of the entire substantia nigra (35µm coronal sections). After Nissl staining the total number of pigmented neurons in the substantia nigra were estimated unbiased using the optical disector (Olympus) and the total volume of nigral pigmented neurons were estimated using Cavalieri's principle. The coefficient of error of the estimates was 4-7 %.

The degree of rigidity was milder in the GM1-treated animal, which sometimes used the right hand when eating, which was never the case in the saline treated macaque. The unbiased estimates of the total number of pigmented neurons in the substantia nigra were: saline treated animal: 89,000 (unlesioned side), 42,000 (lesioned side); GM1 treated animal: 101,000 (unlesioned side), 58,000 (lesioned side).

The estimates of the mean volume were: saline treated animal: 9,200 μm^3 (unlesioned side), 7,400 μm^3 (lesioned side); GM1 treated animal: 10,800 μm^3 (unlesioned side), 13,200 μm^3 (lesioned side).

This pilot study gives support for the previously observed effect of GM1 to counteract the MPTP-induced shrinkage of nigral tyrosine-hydroxylase immunoreactive neurons in the male C57 BL/6 mouse. Thus, ganglioside GM1 may exert anti-parkinsonian actions.

518.2

THE NEURONOTROPHIC ACTION OF N-METHYL-D-ASPARTATE (NMDA) IS ASSOCIATED WITH AN INCREASE OF IMMEDIATE EARLY GENE MESSENGER RNA IN CEREBELLAR GRANULE NEURONS. M. Favaron*, J. Rimland, S. Romanello*, N. Gabellini*, A. Leon and R. Dal Toso. Fidia Research Labs, 35031 Abano Terme, Italy.

In primary cultures of cerebellar granule neurons the stimulation of Excitatory Amino Acid (EAA) receptors leads to a rapid increase of immediate early gene messenger RNA (mRNA). The induction elicited by NMDA was dose-related and dependent on the concentration of extracellular KCl. At day 2 in vitro the NMDA EC50 for c-fos mRNA induction was 10uM at 10mM KCl, and 30uM at 25mM KCl. Higher levels of NMDA-mediated c-fos mRNA induction were observed at 10mM KCl compared to 25mM KCl. The differential activation related to KCl concentration was maintained up to 5 days in vitro, at which time the levels of c-fos induction reached a plateau independently of the extracellular concentration of KCl and NMDA. A similar trend was observed monitoring neurofilament mRNA, suggesting that the increased activation of c-fos mRNA elicited by NMDA results in a time-related pattern of activation of neurofilament mRNA. This system could provide a useful approach for examining the ability of compounds to effect neurotrophism.

518.3

EYE EXTRACT COMPONENTS PROMOTE ACETYLCHOLINE SENSITIVITY IN CHICK DORSAL ROOT GANGLION NEURONS. J. F. Margiotta. Dept. of Physiol. & Biophysics., Mount Sinai School of Medicine, NY, NY 10029.

Eye tissue extracts contain at least two activities that support the survival and development of chick ciliary ganglion neurons in culture: a neurotrophic activity and a 50-kDa fraction that increases acetylcholine (ACh) sensitivity. Recent studies indicate that the neurotrophic (growth promoting) activity can substitute for nerve growth factor (NGF) in supporting short-term survival of chick dorsal root ganglion (DRG) neurons (Eckenstein et al., Neuron 4:623-631, 1990), and that DRG neurons express both ACh receptor (AChR) mRNA and functional nicotinic AChRs (Boyd et al., J. Neurobiol. 22: 1-14, 1991). In order to examine factors influencing the appearance of functional AChRs on sensory neurons, embryonic day 13 (E13) DRG neurons were grown in basal culture medium supplemented with either NGF (10 ng/ml) or eye extract (3% v/v) for up to 7d, and examined for responses to 500 uM ACh using whole-cell recording methods. Close to 100% of the cells identified as neurons after 1d in NGF and eye extract-supplemented culture media survived for 6d, while <50% of neurons in basal medium survived. After 5-6 d, 95% of the neurons grown with eye extract displayed ACh responses well-above a criterion level of 2 pA/pF (geometric mean response=12±1 pA/pF, n=35) while only about 50% of the neurons grown in basal media alone or with NGF did so, and the responses were much smaller [2±1 pA/pF (n=20) and 3±1 pA/pF (n=25), respectively]. At an equivalent developmental age (E18,19) 95% of freshly dissociated DRG neurons were sensitive to ACh, identical to the proportion seen after 6d in culture medium containing eye extract, and representing a >2-fold increase from the proportion seen for neurons obtained at E13,14 (n=18). These results indicate that eye extract components, different from NGF, promote the ACh sensitivity of DRG neurons in culture, and suggest that similar components may influence AChR appearance *in vivo*. Supported by NIH NS24417 & March of Dimes 5-742.

518.4

DEVELOPMENTALLY REGULATED EXPRESSION OF THE CALCITONIN GENE-RELATED PEPTIDE (CGRP) IN THE MOUSE OLFACTORY SYSTEM S.Denis-Donini, B.Chini+ and M.Vitadello. Biology Department, University of Milan, 20133 Milan, Italy.

We have shown previously, using an in vitro system, that in the embryonic mouse olfactory bulb the expression of dopaminergic phenotypes is induced by the neuropeptide CGRP which is synthesized and released in vitro by the chemosensory neurons from the olfactory epithelium (Nature 339:701,1989). It was therefore interesting to study the expression of the peptide during development. Cells involved in the synthesis of CGRPα in the olfactory epithelium have been studied by using in situ hybridization and immunohistochemistry. Sections processed for in situ hybridization with the type αCGRP probe demonstrated specific mRNAs at the level of the growing olfactory axons at stages between E13-15 with a gradual decrease at E17-18 to reach the background level at birth. Immunostaining demonstrated specific CGRP also at the level of the olfactory axons with a kinetics closely matching that of its mRNA.

These results show that the expression of CGRP is developmentally regulated and confirm its role as a differentiation factor during olfactory bulb ontogenesis.

518.5

IBOTERNIC LEISON IN STRIATUM TRIGGERS EXPRESSION OF ONE NEW AND INCREASES FIVE EXISTING PROTEINS. F.C. Zhou., Y. Kim,* C.F. Pu, and B.S. Kwon,* Departments of Anatomy and microbiology, Indiana Univ. Sch. Med. Indianapolis, IN, 46202, USA.

Neuronal toxin, ibotenic acid (IB), injection caused neuronal degeneration and glial activation and proliferation in the adult rat brain. The region of non-neuronal and reactive glial environment was subsequently heavily invaded by serotonergic fibers beginning three days and prevailed after 20 days. Such an environment also fostered a high survival rate of transplanted fetal neurons as compared to normal striatal environment. A trophic signal was suggested in the injured area where the growth of 5-HT fibers and transplanted neurons was facilitated. A gel analysis was performed to detect protein expression in the injured brain region. Three and eight days after IB lesion in the striatum, a 10ul of 1137ci/mmole ^{35}S-methionine was injected in the same area to label the newly synthesized protein / peptide; injection into pair-caged normal animals were used as controls (n=5 each). Two hours (n=3 each) later the striatum was homogenized in the 0.5% Triton-added isotonic lysis buffer, and centrifuged. The protein in supernatant was precipitated and analyzed by 2D-polyacrymide gel electrophoresis. The expression of 5 peptides (IBNF1,2,6=49KD, IBNF3=35KD, IBNF4=30KD) significantly increased in 3- and 8-day-post-IB-lesion animals. A protein/peptide of 45KD newly appeared in the lesioned striatum. This study provides evidence that a group of proteins other than prominent glial proteins, GFAP (40KD) and S100 (32KD), increased their expression level in the brain where neurons were injured and 5-HT fibers grew responsively (supported by NIH grant NS23027 to FCZ).

518.6

BIOLOGICAL ACTIVITY OF THE SECRETED FORM OF AMYLOID β/A4 PROTEIN PRECURSOR I: SEARCH FOR THE APP RECEPTOR. J.-M. Roch[a], I.P. Shapiro[a], L.M. Refolo[b*], N.K. Robakis[b], and T. Saitoh[a]. [a] Dept. of Neurosciences, 0624, Univ. of California, San Diego, La Jolla, CA 92093 and [b]Dept. of Psychiatry, Mt. Sinai School of Medicine, NY, NY 10029

Amyloid β/A4 protein precursor (APP) is a transmembrane glycoprotein, the extracellular domain of which is secreted by different cell types. This process includes the cleavage of the β/A4 domain preventing formation of amyloid β/A4 protein. The secreted form of APP, with and without the Kunitz type protease inhibitor domain, is involved in growth regulation of fibroblasts (Cell 58:615-622, 1989). This biological activity is present in a bacteria-made fusion protein corresponding to the N-terminal two-thirds of APP-695 and the first 13 amino acids of CII protein of phage λ . This APP fragment has high affinity binding sites on the surface of fibroblasts and it presumably triggers the signal transduction mechanisms, resulting in alteration of PKC distribution. This supports the idea that the secreted form of APP-695 has a cell surface receptor. To further analyze APP and APP-triggered cellular signaling mechanisms, large quantities of pure APP are needed. Therefore, we used a prokaryotic expression vector allowing the production of large amounts of APP in bacteria. Specifically, we inserted into this plasmid the 1.7 kb KpnI-BglII fragment of APP-695 cDNA which encodes almost the entire secreted form of APP-695. Bacteria carrying this plasmid expressed high levels of APP which was detected by anti-APP antibodies and biologically active in terms of growth stimulation of fibroblasts. This bacteria-made APP was purified, labeled with ^{125}I and used as a radioligand in binding experiments. This is a first step toward the identification and isolation of APP receptor.

518.7

A FACTOR IN CONDITIONED MEDIUM FROM RETINAL PIGMENT EPITHELIUM (RPE) MEDIATES PHOTORECEPTOR SURVIVAL IN VITRO. V.P. Gaur*, A.J. Sweatt and J.E. Turner. Departments of Neurobiology and Anatomy and Ophthalmology, Bowman Gray School of Medicine, Winston-Salem, NC. 27157

Maintenance of visual gene expression in photoreceptor cells (PRC) of dystrophic RCS rat retinas by RPE transplants has been reported (Exp. Eye Res., 1991). These studies indicated that a RPE secreted factor mediated PRC rescue. We have used an in vitro PRC culture system to characterize this factor. We report PRC survival and neurite promotion by medium conditioned by cultured normal RPE (RPE-CM). Up to 87% of cells expressed immunocytochemically detectable opsin in RPE-CM. In RPE-CM from RCS retinas only 14% cells expressed opsin. Analysis of basic fibroblast growth factor (bFGF) levels (shown effective in achieving PRC rescue in vivo) in RPE-CM from normal and dystrophic rats indicated no significant differences by ELISA. Analysis of metabolically labeled proteins in normal and dystrophic RPE-CM indicated specific differences. Efforts are currently underway to test various components of the RPE-CM for PRC rescuing abilities.

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518.8

INSULIN-LIKE GROWTH FACTOR I (IGF I) STIMULATES THE DIFFERENTIATION OF DOPAMINERGIC (DA) NEURONS *IN VITRO*

S.L. Bradshaw, A. Smith*, I. Reisert and V.K.M. Han*. MRC Group in Fetal and Neonatal Health and Development, Lawson Res. Inst., London, Ont, Canada N6A 4V2.

Insulin-like growth factors (IGFs) are potent mitogenic and differentiating promoters of many developing tissues including brain. To determine the role of IGF I on the differentiation of DA neurons in the developing mesencephalon , we have studied the effects of IGF I on primary DA neurons cultured in serum free medium. Mesencephalon of 14 d rat embryos were dissociated and cultured in 10% FCS for 48 hours, then maintained in SFM with or without IGF I. At 7 days *in vitro*, ^{3}H-dopamine total uptake, K^+ stimulated release and Ca^{2+} stimulated release were measured. Total uptake of ^{3}H-dopamine was increased from 861 dpm/min (SFM) to 1334 dpm/min (IGF I 100ng/ml), ($p<0.001$) indicating an increased capacity for uptake and storage of neurotransmitter with IGF I treatment. K^+ stimulated release of stored ^{3}H-dopamine was increased significantly ($p<0.05$) from 419 dpm/min to 715 dpm/min (IGF I 50ng/ml). Ca^{2+} stimulated release of ^{3}H-dopamine was not significantly altered upon treatment with IGF I. Immunocytochemistry with antiserum against tyrosine hydroxylase and morphometric measurements of the identified neurons demonstrated a significant increase in the soma size, and in the number and length of neurite processes, following treatment with IGF I. We conclude that IGF I promotes uptake, storage and K^+ mediated release of ^{3}H-dopamine, as well as neuronal morphology of DA neurons *in vitro*.

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518.9

INSULIN-LIKE GROWTH FACTOR-I ENHANCED SECRETION IS BLOCKED IN PROTEIN KINASE C DEFICIENT CHROMAFFIN CELLS. M.K. Dahmer, C. Hardaway* and K.L. Leach. Dept. of Biochemistry, Univ. of Tennessee College of Medicine, Memphis, TN 38163 and Dept. of Cell Biology, The Upjohn Co., Kalamazoo, MI 49001.

We have previously shown that bovine chromaffin cells cultured in medium with 10 nM insulin-like growth factor-I (IGF-I) secrete ~2 fold more catecholamine when exposed to secretory stimuli than cells cultured without IGF-I. The purpose of these studies was to determine whether the effect of IGF-I on secretion from chromaffin cells might involve protein kinase C. High K^+-stimulated release of [^{3}H]norepinephrine previously loaded into the cells was used to assay secretion. We found that high K^+-stimulated secretion from cells cultured without IGF-I was unaffected by overnight treatment with 100 nM β-phorbol didecanoate (PDD), which depleted protein kinase C in the cells as determined by immunoblots, however, secretion from cells cultured with IGF-I was reduced by β-PDD treatment to a level comparable to that in cells cultured without the peptide. α-PDD (100 nM) had no effect on secretion from untreated or IGF-I treated chromaffin cells. Overnight treatment with 100 μM H7, but not HA1004, prevented the enhanced secretion normally seen in IGF-I treated cells. High K^+-stimulated $^{45}Ca^{2+}$ uptake in IGF-I treated cells was reduced to that seen in untreated cells when the cells were incubated overnight with β-PDD (200 nM). Immunoblots using antibodies to protein kinase C isozymes suggested that the ε-isozyme of protein kinase C is more abundant in IGF-I treated cells than in untreated cells, yet α-protein kinase C levels are relatively unchanged. These observations suggest that protein kinase C is required for IGF-I enhanced secretion from chromaffin cells and that ε-protein kinase C may be the specific isozyme involved in this effect.

518.10

DELAYED DELIVERY OF A CORTICALLY DERIVED TROPHIC FACTOR RESCUES AXOTOMIZED, SLOWLY-DEGENERATING NEURONS OF THE DORSAL LATERAL GENICULATE NUCLEUS (DLGN) AND IMPROVES VISUAL DISCRIMINATION. F. Haun, T. Jaret and T.J. Cunningham, Dept. of Anatomy & Neurobiology, Medical College of Pennsylvania, Philadelphia, PA 19129

Visual cortex lesions in adult rats lead to cell death in two populations of dLGN neurons; in one population (generated late in neurogenesis) loss is maximal by 2wks. post-lesion, while for earlier generated neurons a similar maximal loss is not reached until 2mos. post-lesion. Using an implanted osmotic minipump, we delivered a 2-week supply of a neuron survival factor to the visual cortex lesion site beginning 2wks. post-lesion, and tested the animals behaviorally starting 2mos. post-lesion. This factor was was derived from an HPLC fraction of culture medium conditioned by embryonic occipital cortex explants. Animals receiving the factor learned a difficult visual discrimination more than twice as fast as animals with comparable lesions that received a control fraction of unconditioned culture medium. 3H-thymidine counts of labeled dLGN neurons showed that the factor rescued all neurons that otherwise die between 2wks. and 2mos. post-lesion. These results show that delayed delivery of a trophic factor following CNS injury can nonetheless rescue slowly degenerating neurons, with a resulting behavioral improvement.

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518.11

A NOVEL PEPTIDE ENHANCES CHOLINERGIC STIMULATION ACTIVITY OF NGF IN MEDIAL SEPTAL NUCLEI CULTURE. K.Ojika, S.Kojima*, Y.Ueki*, N.Fukushima*, M. Taiji and M.Yamamoto*. Second Dep. of Internal Medicine, Med. Sch., Nagoya City Univ., Mizuho-ku, Nagoya, Japan 467 and Res. Inst. Sumitomo Pharma. Co.Ltd., Konohana-ku, Osaka, Japan 554.

A novel peptide(HCNP) that enhances acetylcholine(AcCho) synthesis of the medial septal nuclei(SEPT) culture has been purified from rat hippocampus. Structure of HCNP is acetyl-Ala-Ala-Asp-Ile-Ser-Gln-Trp-Ala-Gly-Pro-Leu.

To study the disparity of cholinergic stimulation activity between NGF and HCNP, saturable amount(1ng/ml) of mouse 2.5S-NGF and/or 30pg/ml of synthetic de-acetylated HCNP(free-HCNP) were applied to explant cultures of anterior spinal cord(SPC), corpus striatum(STRI) and SEPT, from 14, 15 and 16 day Wistar rat embryo, respectively. Compared with control or either component alone, application of both components to the same SEPT culture exerted a striking additive or more than additive enhancement of AcCho synthesis as well as choline-acetyltransferase activity at day 6 in vitro. However, neither NGF nor free-HCNP influenced AcCho synthesis of SPC culture, and only NGF stimulated AcCho synthesis of STRI culture.

518.12

GENERATION OF ANTIBODIES AGAINST NATIVE CHOLINERGIC NEURONAL DIFFERENTIATION FACTOR (CDF) WHICH BLOCK CHOLINERGIC-INDUCING ACTIVITY. Keiko Fukada and Marie F. Towle*. Anat./Cell Biol., SUNY-HSCB, Brooklyn, NY 11203.

A glycoprotein from heart cell-conditioned medium (CM), CDF, causes a transition from noradrenergic to cholinergic phenotype in cultured rat sympathetic neurons. We developed an efficient protocol for purification of naturally-occurring CDF from CM using antipeptide antibodies raised against a synthetic peptide homologous with the N-terminal region of CDF. Using this protocol we obtained ~ 44 nmoles of pure CDF from 150 liters of CM. Both amino acid composition analysis and N-terminal sequence analysis showed that this preparation was ≥ 96 % pure (the working accuracy of procedures used). This pure CDF was used to raise antibodies in three rabbits. All three antisera specifically immunoprecipitated CDF from a preparation where CDF constituted ~0.07 % of the total protein, while preimmune sera did not. Moreover, all three antibodies completely blocked the cholinergic-inducing activity of CDF when tested on cultured sympathetic neurons, although high concentrations of antipeptide antibodies also showed partial inhibition. When concentrated CM was used instead of pure CDF, the three antibodies at 100 ug/ml blocked ~95% of the cholinergic-inducing activity of the CM, whereas no inhibition was observed by the same concentration of antipeptide antibodies. These antibodies will be useful for assessing the physiological role of CDF. (NINCDS to KF.)

519.1

AUTORADIOGRAPHIC DISTRIBUTION OF CHOLINERGIC MUSCARINIC RECEPTORS IN OLFACTORY BULBECTOMIZED (OB) RATS AFTER CHRONIC TREATMENT WITH MIANSERIN AND DESIPRAMINE.

B. Earley[1*], M. Glennon[1*], M. Lally[1*], B. E. Leonard[1*], J-L. Junien[2*], J.G. Wettstein[2*]. Pharmacology Department[1], University College, Galway, Ireland and Institut de Recherche Jouveinal[2], 94265, Fresnes, France.

Bilateral removal of the olfactory bulbs in rats produces a behavioural syndrome that is defined by a retention deficit in a passive avoidance test and hyperactivity in the open-field test. This syndrome may be related to depression since these behavioural effects can be attenuated by antidepressant drugs. Moreover, abnormalities of the cholinergic system may be involved in the pathogenesis of depression. Thus, muscarinic receptor density was measured by autoradiography with [^{3}H]QNB after the bilateral removal of the olfactory bulbs from rats. In OB rats, muscarinic receptor density was decreased in several brain regions including the amygdaloid nuclei, the basal ganglia, hippocampus, hypothalamus, cortex and olfactory regions. When OB rats were treated chronically for 24 days with mianserin (5 mg/kg i.p.) or desipramine (7.5 mg/kg i.p.) the behavioural abnormalities in the passive avoidance and open field tests were reversed and muscarinic receptor density was increased in the hippocampus and cortical regions. The results are consistent with a cholinergic involvement in depressive illness and suggest that the cholinergic modulatory properties of antidepressant drugs may contribute to their therapeutic effectiveness.

519.2

EFFECT OF THE CHOLINESTERASE INHIBITORS SOMAN AND PHYSOSTIGMINE ON BRAIN MUSCARINIC RECEPTORS.

X-H. Yang, W. Li*, L. Erwin* and J.J. Buccafusco. Dept. Pharmacology & Toxicology, Medical College of Georgia & Dept. Vet. Affairs Med. Ctr., Augusta, GA

The ability of cholinesterase inhibitors (ChEI) to enhance the effects of endogenously released brain acetylcholine has often been reported to result in muscarinic receptor down-regulation. This is not always a consistent finding for different brain regions or for different inhibitors. In this study two dissimilar ChEI were employed in two species and under different conditions of acute and chronic administration. Soman, an irreversible organophosphorous ChEI, and physostigmine, a reversible carbamate ChEI were employed for the purpose of comparing their effects on regional muscarinic receptor regulation in synaptosomal fractions. Acute, s.c., administration of soman did not significantly alter the density of receptors as measured by [^{3}H]methylscoploamine binding in cortex, midbrain or hindbrain, at doses ranging from LD_{20} to LD_{90} in rats. Acute administration of soman to guinea pigs at doses in the $LD_{80\text{-}90}$ range also did not alter binding parameters in the respective brain regions. Chronic treatment once daily (7 days) of rats with soman at doses which resulted in 95% inhibition of ChE at the time of receptor measurement did not alter binding parameters. Physostigmine administration twice daily (25µg/kg,s.c.) for 7 days produced a significant decrease in the density of cortical muscarinic receptors of 28% without affecting the Kd, although this treatment did not alter binding parameters in the midbrain and hindbrain. These results support the concept that brain ChE inhibition is not the only factor which determines whether down regulation will occur and that receptor regulation may be different in the various brain regions.

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519.3

PHARMACOLOGICAL CHARACTERIZATION OF A NOVEL MUSCARINIC PARTIAL AGONIST, (2,8-DIMETHYL-3-METHYLENE-1-OXA-8-AZASPIRO[4.5]DECANE (YM796) IN TRANSFECTED CELLS EXPRESSING THE m1 OR m2 MUSCARINIC RECEPTOR (mAChR) GENE.

H. B. Wei*, W. R. Roeske, J. Lai, F. Wanibuchi, K. Hidaka, S. Usuda and H. I. Yamamura. U. of AZ, Tucson, AZ 85724 and Yamanouchi Pharm. Co., Tsukuba, Japan.

To investigate the pharmacological effect of a novel compound YM796, we performed binding and correlative biochemical experiments using the transfected murine fibroblast B82 cells which expressed the m1 and m2 mAChR genes (defined as LK3-3 and M2LKB2-2 cell lines). [^{3}H](-)MQNB binding in these transfected cell lines was displaced by different optical isomers of YM796, atropine, pirenzepine, AF-DX 116, as well as selected agonists. (-)YM796, (+)YM796 and (±)YM796 displaced [^{3}H](-)MQNB from its binding site in LK3-3 cells with K_i values of 16.5, 30.4 and 21.9 µM and in M2LKB2-2 cells with K_i values of 43.3 , 93.2 and 79 µM, respectively. From functional assays we found the two isomers had different intrinsic activities for the M_1 and M_2 mAChRs. (-)YM796 stimulated IP_1 accumulation in LK3-3 cells with an EC_{50} value of 27.3 µM and inhibition of cAMP formation in M2LKB2-2 cells with an EC_{50} value of 91.2 µM although (-)YM796 failed to achieve a maximal response in either assay compared to carbachol (a full agonist). (+)YM796 showed no obvious efficacy for the M_1 and M_2 mAChRs. In conclusion, (-)YM796 represents a muscarinic partial agonist with selectivity for the M_1 mAChRs and may be potentially useful in Alzheimer's disease whereas (+)YM796 might be an antagonist at the M_1 and M_2 mAChRs.

519.4

PHARMACOLOGY OF THE M4 MUSCARINIC RECEPTOR MEDIATING CA CURRENT INHIBITION IN NG108-15 CELLS.

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Molecular biological techniques have shown genes coding for five muscarinic receptors in neuronal tissue. NG108-15 cells express message exclusively for m4 receptors and ACh inhibits the Ca current (I_{Ca}) in these cells (Higashida et al., Proc. Roy. Soc. Lond.(B). 242:68-74, '90); we have studied the effects of some muscarinic agonists and antagonists on I_{Ca} inhibition to define the pharmacology of the m4 gene product in a functional assay. ACh (+ neostigmine), oxotremorine-M and carbachol were "full" agonists (max. inhibition 39.5% ± 1.9); -log EC_{50} values (±se) were 6.95 (0.1), 6.85 (0.18) and 5.7, respectively. Muscarine, methylfurmethide and APE were "partial" agonists, with -log EC_{50} values of 6.27 (0.3), 6.1 and 6.71 (0.28), respectively. The effect of ACh was blocked by omega-conotoxin (100nM), but not by nifedipine (5µM). Antagonist potencies (-log K_B ± se) were atropine, 9.8±0.39; pirenzepine, 7.74±0.23; methoctramine, 7.63±0.23; himbacine, 8.83±0.13. This antagonist profile is similar to that seen in binding studies on these cells (Lazareno et al., Mol.Pharmacol., 38:805-815, '90) and shows himbacine can distinguish M_4 receptors from M_1 receptors, as both are blocked by pirenzepine with high potency.

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519.5

EVIDENCE THAT MUSCARINIC STIMULATION OF ADENYLATE CYCLASE AND GENERATION OF INOSITOL PHOSPHATES ARE MEDIATED BY DIFFERENT RECEPTOR SUBTYPES IN RAT OLFACTORY BULB.

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Often agents that stimulate phospholipid hydrolysis increase cyclic AMP formation. We have previously shown that in rat olfactory bulb (o.b.) muscarinic agonists enhanced adenylate cyclase (a.c.) activity and stimulated phosphoinositide breakdown. In the present study we have examined whether the two responses were mediated by the same pharmacological receptor subtype. In miniprisms of rat o.b., carbachol (CCh) increased the accumulation of inositol phosphates (IPs) by about 110 % of basal level with an EC50 of 96 µM. Oxotremorine M behaved as a full agonist, whereas oxotremorine and arecoline exibited only 20 % of the CCh efficacy. In contrast, oxotremorine and arecoline were 70% and 90% as effective as CCh in enhancing a.c.. The CCh stimulation of IPs generation was potently antagonized by the M1 blocker pirenzepine (pA2=8.26), but was less sensitive to the M2 antagonist AF-DX 116 (6.12). Conversely, the muscarinic stimulation of a.c. showed the opposite sensitivity. Thus, in rat o.b. different muscarinic receptor subtypes stimulate a.c. and increase phosphoinositide hydrolysis.

519.6

IN VITRO LOCALIZATION OF A RADIOLABELLED VESAMICOL ANALOG IN THE HUMAN BRAIN.

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The prominence of the cholinergic lesion in Alzheimer's disease has provided the impetus for the development of novel radiotracers which can detect this lesion. The phencyclidine isomer 2-(4-phenylpiperidino) cyclohexanol (vesamicol) is a potent inhibitor of vesicular ACh transport. This ligand binds with high affinity to a cytoplasmically-oriented site, the vesamicol receptor (VR), on the cholinergic synaptic vesicle. Thus, the vesamicol receptor may be a potentially useful target for the development of novel tracers for mapping central cholinergic innervation. This study reports the characterization of a novel vesamicol-like radiotracer, (-)-2-hydroxy-3-(4-[^{125}I]iodo-phenyl)-1-(4 phenylpiperidino) -cyclohexanol (4-HIPP) in the postmortem human brain. Saturation analysis revealed that 4-HIPP binds to two sites in the human brain (KD = 3.5 nM, BMAX =42.5 pmol/g; KD = 31.1 nM, BMAX =102.1 pmol/g). We have used a low concentration of 4-HIPP (3 nM) to selectively label the high affinity site in slide-mounted sections of the human brain. Quantitative autoradiography demonstrates that [^{125}I]-4-HIPP binding sites are heterogeneously distributed throughout the human cortical mantle. The highest levels of binding sites were observed over the basal forebrain. High binding site concentrations were seen throughout the cortex, with lesser amounts in the the caudate, putamen and nucleus accumbens. Preliminary localization studies in Alzheimer's disease cerebral cortex indicate that this binding site persists despite the marked disruption of cholinergic synapses. Studies are underway in the human brain to characterize the residual binding sites in relation to the topography of cholinergic innervation. Funded by the AHAF.

519.7

LOCALIZATION OF CHAT mRNA IN THE RAT BRAIN AND SPINAL CORD. Justin D. Oh, Nancy J. Woolf, Ali Roghani, Robert H. Edwards, and Larry L. Butcher. Dept. Psychology and Neurology, UCLA, Los Angeles, CA 90024-1563, U.S.A.

Gene expression of choline acetyltransferase (ChAT) in the central nervous system was studied by use of *in situ* hybridization histochemistry. RNA probes against ChAT DNA templates were synthesized using *in vitro* transcription in the presence of digoxigenin-11-UTP. The probe was detected by an antibody against dioxigenin coupled to alkaline phosphatase. This method resulted in a large number of cell bodies and occasional proximal processes being intensely labeled against a virtually undetectable background. Telencephalic cells expressing ChAT mRNA were found in the following striatal and basal forebrain nuclei: the caudate-putamen, nucleus accumbens, olfactory tubercule, medial septal nucleus, vertical and horizontal diagonal band nuclei, substantia innominata, and nucleus basalis. Cortical cells were not labeled with this probe. In the diencephalon, only cells of the medial habenula were labeled with the ChAT mRNA probe, other thalamic areas and the hypothalamus did not contain labeled cells. In the brainstem, only the pedunculopontine and laterodorsal tegmental nuclei and motor cranial nuclei 3, 4, 5, 7, 9, 10, and 12 contained cells labeled with the ChAT mRNA probe. In the spinal cord, ventral horn neurons exclusively exhibited ChAT mRNA label. The distribution of ChAT mRNA labeled cells in this study corresponds conservatively to previously identified cholinergic cells. [Support: USPHS grant NS 10928 to L.L.B.].

519.8

THE RAPID KINETICS OF ACETYLCHOLINE RELEASE FROM RAT BRAIN SYNAPTOSOMES: ANALYSIS BY RAPID SUPERFUSION. L. B. Pearce, and E. Adamec* Department of Pharmacology, Boston University School of Medicine, Boston, MA 02118.

Spontaneous and evoked [^{3}H]ACh efflux from rat brain synaptosomes was investigated on the second and millisecond time scale using the technique of rapid superfusion. Synaptosomes, radiolabeled by preincubation with [^{3}H]choline, were superfused with Krebs-bicarbonate buffer, pH 7.4 at 0.3 to 0.5 ml/second. The mixing half-life was 119 milliseconds (ms) and efficiency of superfusion > 85%. Superfusion for 48 seconds with isoosmotic Krebs buffer resulted in 1.57, 5.68, 7.19, 8.80, and 18.32 percent release of tritium in the presence of 10, 20, 30, 50, 75, and 100 mM potassium ion, respectively. Kinetic analysis of net potassium-stimulated release revealed a single component of release that fit a single exponential function with a $t_{1/2}$ = 12.7, 13.1, 15.4, and 12.7 seconds for 20, 50, 75, and 100 mM KCl. Analysis of the area under the tritium efflux curves observed for serial collection of 50 ms samples over 750 ms revealed that 0.111, 0.550, 0.614 % net tritium release was evoked by superfusion with isoosmotic buffer containing 20, 50, and 100 mM KCl, respectively. Potassium-evoked release observed on both the second and millisecond time scales was totally calcium-dependent whereas, only a small fraction of spontaneous release was dependent on calcium. This work was supported by a grant from The Eppley Foundation for Research.

519.9

MUSCARINIC TYPE-2 ANTAGONISTS INCREASE HIPPOCAMPAL ACETYLCHOLINE RELEASE IN UNANESTHETIZED RATS.

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Hippocampal muscarinic receptors belong to two main pharmacologic subtypes, M_1 and M_2. The majority of the latter subtype is predominantly located presynaptically, where these receptors could modulate acetylcholine (ACh) release by functioning as inhibitory autoreceptors. M_2 antagonists may inactivate this receptor, leading to increased ACh release. The purpose of this study was to assess changes in extracellular ACh via *in vivo* microdialysis following administration of various M_2 antagonists. Microdialysis guide cannulae were implanted into the CA1 hippocampal region of male Fischer rats. After a minimum of two days to recovery from surgery, each rat was tested while awake and unrestrained. Drug or placebo was perfused via the microdialysis probe. High performance liquid chromatography with electrochemical detection was used for on-line analysis of the dialysates. M_2 antagonists significantly enhanced ACh release in a dose-dependent fashion as compared to placebo. The present data corroborate studies which show increased ACh levels *in vitro* following application of M_2 antagonists.

519.10

DOPAMINERGIC REGULATION OF STRIATAL ACETYLCHOLINE RELEASE: IMPORTANCE OF D1 AND NMDA RECEPTORS G. Damsma†, G.S. Robertson§, C. Tham§* and H.C. Fibiger§, †PF/CNS 70/310, Hoffmann La Roche, CH-4002, Basel, Switzerland; §Division of Neurological Sciences, Dept. of Psychiatry, University of British Columbia, Vancouver, B.C., Canada, V6T 1Z3

The dopaminergic regulation of striatal cholinergic activity was studied using *in vivo* microdialysis to measure the interstitial concentration of acetylcholine (ACh) and choline in the striata of freely moving rats. *d*-Amphetamine (2 mg/kg, s.c.) and nomifensine (5 mg/kg, s.c.) increased the concentration of ACh in the striatal dialysate by 40-60%. The selective D1 receptor antagonist SCH 23390 (0.3 mg/kg, s.c.) decreased the concentration of ACh in the striatal dialysate by 15-20%, while the selective D2 antagonist raclopride (1 mg/kg, s.c.) increased striatal ACh release by 50-60%. Raclopride blocked the increase in locomotor activity produced by *d*-amphetamine but did not interact with the stimulant to further enhance ACh release. In contrast, SCH 23390 completely antagonized both the increase in locomotion and the enhancement of striatal ACh release produced by *d*-amphetamine. SCH 23390 also blocked the raclopride-induced increase in ACh overflow. These results indicate that *d*-amphetamine increases ACh release in the striatum via a D1 receptor mechanism. Consistent with this hypothesis, the selective D1 receptor agonist CY 208-243 (1 mg/kg, s.c.) increased striatal ACh release by approximately 60%. Inclusion of the N-methyl-D-aspartate (NMDA) receptor antagonist MK-801 in the striatal perfusion solution significantly attenuated the increase in striatal ACh release produced by CY 208-243. Consequently, increased striatal ACh release produced by enhanced D1 receptor stimulation appears to be mediated, at least in part, by activation of striatal NMDA receptors.

519.11

CHOLINERGIC MICROSTIMULATION OF THE PERIBRACHIAL NUCLEUS: PROLONGED ENHANCEMENT OF PGO WAVES AND REM SLEEP. S.Datta, J.M.Calvo, J.Quattrochi and J.A.Hobson. Lab. of Neurophysiology, Harvard Med. School, Boston, MA 02115 and Instituto Mexicano de Psiquiatría, México D.F.

During REM sleep ponto-geniculo-occipital (PGO) waves are generated by cholinergic neurons in the peribrachial pons. We report here the effect of cholinergically stimulating this brain region. Five cats were chronically prepared for recording wake-sleep parameters. After recovery, carbachol (4.0 μg/250nl) was microinjected into the peribrachial PGO bursting cell zone. Carbachol produced an immediate onset of state-independent PGO waves lasting 4 days and the duration of state-dependent PGO waves remained high for 6 days (49.3±13.8%, $p<0.0001$) compared to control values (18.9±6.3%). Beginning on day-2, increased PGO activity was associated with a threefold increase in REM%. REM% also remained elevated for 6 days (22.2±6.4% , $p<0.001$) compared to control values (13.0±3.2%). We postulate that the immediate and sustained increase in PGO activity is due to cholinergic activation by carbachol of the PGO burst cells and the delayed, sustained increase in REM is a secondary consequence of membrane excitation. Supported in part by NIH grant MH-13923.

520.1

MOLECULAR CHARACTERIZATION OF A STRIATAL ENRICHED PROTEIN TYROSINE PHOSPHATASE.

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Modulation of tyrosine phosphorylation is one mechanism for regulating cell differentiation and is controlled by opposing actions of protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPases). A number of PTPases have been cloned and their amino acid sequence determined. Two classes have been identified. The first class is intracellular and the second class has extracellular regions linked to cytoplasmic, catalytic domains through single transmembrane regions. Although Northern analyses of different tissues indicate wide variability in the regional expression of PTPases, none have been nervous system specific.

Here, we report the isolation and characterization of a cDNA clone which encodes for a PTPase found predominantly in the brain. Within the brain, it shows further tissue specificity by appearing to be a STriatal Enriched Phosphatase and has been designated STEP. This new PTPase contains regions homologous to ones found in previously characterized PTPases, and appears to be a distinct member of the cytoplasmic class of PTPases. The deduced amino acid sequence predicts a protein of approximately 369 amino acids. *In vitro* translation produces a protein with an apparent molecular weight of 46 kDa. Northern analyses of various regions of rat brain reveal a 3 kb and a 4.4 kb mRNA. *In situ* hybridization experiments support the Northern analyses by showing a high degree of localization of the 3 kb message in the striatum.

520.2

TIME-COURSE OF PEPTIDE ALTERATIONS IN STRIATAL EFFERENT NEURONS AFTER UNILATERAL CORTICAL LESION BY THERMOCOAGULATION.

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We have previously reported (Chesselet and Salin, Neurosci. Abst. '90) a bilateral increase in the levels of mRNAs encoding enkephalin (ENK) and substance P (SP), two neuropeptides present in striatal efferent neurons projecting to the globus pallidus (GP) and entopeduncular nucleus (EP) respectively, 5 and 21 days after unilateral lesions of sensory-motor cortex by thermocoagulation in adult rats. Levels of ENK and SP were measured in GP and EP after similar lesions, by quantitative immunohistochemistry with 125I- secondary antibodies and film autoradiography. After an initial increase, ENK immunoreactivity in GP markedly decreased contralaterally at 6 weeks, and bilaterally at 3 months post-surgery, without a parallel loss of ENK mRNA-positive neurons in striatum. SP levels in EP were decreased, but to a lesser extend. The results suggest a delayed alteration in peptide synthesis or transport in striatal efferent neurons after cortical lesions in rats. The greater severity of effects in the side contralateral to the lesion points to a role of the contralateral cortex in these effects. Supported by BNS 86-16841 and NS 29230.

520.3

ULTRASTRUCTURAL CHARACTERIZATION OF DOPAMINE IMMUNO-POSITIVE FIBERS AND THEIR RELATION TO POSTSYNAPTIC STRUCTURES IN THE STRIATUM AND NUCLEUS ACCUMBENS OF THE LIZARD GEKKO GECKO.

J.M.L. Henselmans* and F.G. Wouterlood. Department of Anatomy, Free University, Amsterdam, The Netherlands.

In the lizard *Gekko gecko*, efferents of mesencephalic dopaminergic neurons project to the striatum (STR) and the nucleus accumbens (ACC). We studied this innervation by means of immuno-EM, using an antibody against dopamine (DA). DA-immunoreactivity in the striatal complex is localized in boutons which mainly contain clear round vesicles and a few dense-cored vesicles. The great majority of the DA-immunopositive boutons form symmetrical synapses with dendritic shafts. In addition, symmetrical axo-somatic contacts were found. Only a few synapses were found with dendritic spines. In STR, one DA-immunopositive bouton established both an axo-somatic and an axo-axonic contact. Occasionally, convergence on dendrites was observed of a DA-immunopositive terminal and an unlabeled axon terminal. Since symmetrical synapses have been associated with inhibition, the DA-immunopositive elements in the STR and ACC may be involved in an inhibitory action of dopamine. In the rat, asymmetrical, non-dopaminergic and symmetrical, dopaminergic synapses frequently converge on a common postsynaptic element. The absence of this phenomenon in the STR of *Gekko* suggests a different organization of inhibitory and excitatory inputs than in mammals.

520.4

DISTINCT SUBSETS OF NEUROTENSIN IMMUNOREACTIVE NEURONS REVEALED IN THE RAT STRIATUM FOLLOWING ANTAGONISM OF THE DOPAMINE-MEDIATED SUPPRESSION OF NEUROTENSIN IMMUNOREACTIVITY.

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The distribution of neurotensin immunoreactive structures in the rat striatum was evaluated after blockade of dopamine neurotransmission by drugs that act presynaptically (6-OHDA, reserpine) and postsynaptically, preferentially at the D-2 (eticlopride, haloperidol) and D-1 (SCH-23390) receptor sites. Calbindin-D 28 kD immunoreactivity was used to delineate patch(striosome) and matrix in the caudate-putamen and core and shell in the nucleus accumbens. Antagonism at the D-2 dopamine receptor and 6-OHDA lesions caused dense axonal immunoreactivity and moderate numbers of neurotensin immunoreactive neurons to be distributed preferentially in the matrix of the caudate-putamen. D-1 receptor antagonism was significantly less effective at eliciting neurotensin immunoreactive neurons in the caudate-putamen. Reserpine or co-administration of the D-1 and D-2 receptor antagonists produced many neurotensin immunoreactive neurons in both striatal compartments throughout the caudate-putamen and dense axonal neurotensin immunoreactivity in the medial patch compartment. This dense axonal neurotensin immunoreactivity may therefore be a site of convergence of neurotensin immunoreactive axons from nearby patch and matrix. To varying degrees, with SCH-23390 being least effective and reserpine most effective, all of the drug treatments elicited neurotensin immunoreactivity in neurons in the olfactory tubercle, rostral nucleus accumbens, accumbal shell and ventrolateral caudate-putamen, i.e., most of ventral striatum. Support: NS-23805.

520.5

THE SYNAPTIC CHARACTERISTICS OF Leu-ENKAPHALIN IMMUNOREACTIVE TERMINALS IN THE MARGINAL DIVISION OF THE MONKEY (Macaca Mulatta) STRIATUM.

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A new subdivision, the marginal division has been found in the striatum of the monkey as well as in the rat striatum (Shu, et. al., J Chemical Neuroanatomy 1988). The marginal division is more densely filled with enkephalin(Enk) - and substance(SP) P -immunoreactive terminals than does the rest of the striatum. In the present study we examined the ultrastructure of L-Enk immunoreactive terminals in the monkey (Macaca Mulatta) striatum. A pre-embedding staining technique was used. The tissues of the darker stained marginal division were dissected out under a macroscope and processed for EM examination. The L-Enk immunoreactive product is deposited around the vesicles in the positive reacted terminals. Four types of synapses consisting of L-Enk immunoreactive terminals are observed in the marginal division of the monkey striatum. They are axo-dendritic(A-D), axo-somatic(A-So), axo-axonic(A-A) and compound synapses. Axo-spinous(A-Sp) synapses are found but not connecting with L-Enk reactive terminals. Most of the synapses with L-Enk immunoreactive terminals are compound with more than three components. The compound synapses are demonstrated in different ways: A-A-D, D-A-D, A-D-A, D-A-So, or A-A-So.

520.6

A SECOND INPUT SYSTEM FOR BODY REPRESENTATIONS IN THE PRIMATE STRIATAL MATRIX.

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Only one sensorimotor input system to the primate striatal matrix, the projection from ipsilateral sensorimotor cortex, has been mapped both anatomically and electrophysiologically. We show here the existence of a second system, from the contralateral motor cortex, which projects to a different set of interdigitating zones within the matrix. Intracortical microstimulation and multiunit recording guided deposits of distinguishable anterograde tracers in motor cortex (area 4) and SI somatosensory cortex (area 3b) in 4 squirrel monkeys. Striosomes and matrix were distinguished by enkephalin immunohistochemistry.

In one monkey we examined foot area inputs from ipsilateral motor cortex and from area 3b (which projects almost exclusively ipsilaterally). The regions innervated overlapping zones in the matrix of the putamen (cf. Fotuhi et al., *Soc.Neurosci.Abstr.* '89), zones that also receive input from corresponding parts of ipsilateral areas 3a and 1 (Flaherty and Graybiel, *J.Neurophys.*, in press). In two monkeys we deposited one tracer in nearly all of SI bilaterally, and the other tracer, on one side only, in the motor cortex leg representation. Ipsilaterally, the leg representations of motor cortex and SI projected to largely overlapping zones. In sharp contrast, contralateral motor cortex sent its strongest inputs to matrix zones that largely interdigitated with inputs from ipsilateral SI. In a fourth monkey we labeled motor cortex in each hemisphere with differentiable tracers. Ipsi- and contralateral motor cortex projected to largely interdigitating matrix zones.

These experiments suggest that inputs from cortex representing predominantly the contralateral and ipsilateral body alternate in the primate striatal matrix, an arrangement reminiscent of the alternating ocular dominance columns in visual cortex.

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520.7

OROFACIAL MOTOR CONTROL IN THE VENTROLATERAL STRIATUM OF RAT: GLUTAMATERGIC-DOPAMINERGIC INTERACTIONS. M. Pisa. Dept. Biomed. Sci., Div. Neurosci., McMaster Univ., Hamilton, Ont. Canada L8N 3Z5.

Previous lesion studies indicated a role of the ventrolateral striatum in orofacial motor control (Pisa, Neurosci., 24:453, 1988). The aims of this research were to examine the roles of corticostriatal glutamatergic (GLU) and nigrostriatal dopaminergic (DA) transmission in orofacial motor control. Rats were administered microinfusions of kynurenic acid (KYN), an antagonist of GLU transmission, into the ventrolateral striatum through chronically implanted cannula guides (12 to 36 nmoles/0.2 ul PBS, pH 7.4), with or without prior infusion of the dopamine receptor blocker haloperidol (HAL: 0.1 to 0.5 ug/0.2 ul of 1% lactic acid), and their orofacial movements were videotaped for 1h. Data were number of grimacing movements and non directed (in vacuo) chewing movements. KIN injections produced a dose-dependent, significant increase of grimacing and chewing. HAL injections dose-dependently reduced the KYN-induced dyskinesias. These results indicate that 1) an impairment of corticostriatal GLU transmission can produce oral dyskinesias; 2) a concomitant blockade of nigrostriatal DA transmission can counteract this effect. The implications of these findings for normal brain function and mechanisms of oral dyskinesias in humans will be discussed. (Supported by the Scottish Rite Schizophrenia Res. Prog. M.P. is a Research Associate of the Ontario Mental Health Foundation).

520.8

ORGANIZATION OF NIGROSTRIATAL AND STRIATONIGRAL PROJECTIONS IN THE PRIMATE. E. L. Lynd-Balta, S.J. Mitchell, and S.N. Haber. Department of Neurobiology and Anatomy, University of Rochester, Rochester, N.Y. 14642.

The substantia nigra and striatum are reciprocally connected. Lesions of the nigrostriatal pathway result in profound deficits as seen in Parkinson's Disease. The striatum receives topographical inputs from cortex which impose a cortical map on the striatum. It has been hypothesized that striatal output projections maintain this topography so that different parallel circuits travel through the basal ganglia. We propose that the organization of efferent projections of the substantia nigra pars compacta does not maintain a strict topography in its relationship with the striatum and that some integration between regions such as dorsolateral (motor) and ventromedial (limbic) striatum may occur through the inputs and outputs of the substantia nigra.

A combination of retrograde and anterograde tracers were placed in the dorsolateral motor-related region and the ventromedial limbic-related region of the striatum. Results indicate that there is overlap in the substantia nigra between the inputs and outputs of these different regions. For example, terminals labeled from an injection of PHA-L into the ventral striatum overlap with retrogradely labeled neurons in the substantia nigra after an injection of HRP-WGA into the dorsal striatum. These results suggest that the substantia nigra may be an important integrator of striatal function by allowing different areas of the striatum to communicate via pathways through the substantia nigra. Supported by NIH NS22511.

520.9

HALOPERIDOL + SCH 23390 PREVENTS THE INCREASE IN RAT STRIATAL 'PERFORATED' SYNAPSES DUE TO EITHER DRUG ALONE: POSSIBLE INVOLVEMENT OF SEPARATE D-1/D-2 PATHWAYS. C.K. Meshul, A. Janowsky, D.E. Casey*, R.K. Stallbaumer*, B. Taylor*. V.A. Medical Center and Depts. of Pathology, Medical Psychology, Psychiatry, and Pharmacology, Oregon Health Sciences Univ., Portland, OR 97201

Haloperidol (0.5 mg/kg/d/14d), a neuroleptic associated with extrapyramidal syndromes (EPS) and tardive dyskinesia (TD), blocks dopamine (DA) D-2 receptors, while SCH 23390 (1 mg/kg/d/14d) blocks DA D-1 receptors. These drugs cause an increase in striatal DA D-2 and D-1 receptors, respectively, and in the percentage of 'perforated' synapses. Co-administration of haloperidol and SCH 23390 for 14 days prevented the increase in striatal perforated synapses while the DA D-2 and D-1 receptors were increased to the same level as if administered separately. We have shown that the perforated synapse originates from motor cortex and speculate that the increase is due to activation of the corticostriatal pathway. It has been suggested that many striatal neurons possess separate DA D-1 and D-2 receptors. The D-1 pathway projects to the thalamus via the entopeduncular nucleus and the D-2 pathway also projects to the thalamus via the globus pallidus, subthalamic nucleus, and substantia nigra-zona reticulata. Simultaneous blockade of both receptor subtypes with haloperidol and SCH 23390 could up-regulate the two DA receptors, while converging input to the thalamus could eventually decrease activation of the corticostriatal pathway. Administration of SCH 23390 could be effective in reducing the incidence of EPS and TD associated with haloperidol. Supported by the Dept. of Veterans Affairs and NIMH.

520.10

SEGREGATED EFFERENT PROJECTIONS FROM NEOSTRIATAL COMPARTMENTS TO THE ENTOPEDUNCULAR NUCLEUS IN THE RAT. N. Rajakumar, K. Elisevich and B.A. Flumerfelt. Dept. of Anatomy and Clinical Neurological Sciences, Univ. of Western Ontario, London, Canada, N6A 5C1.

The compartmentalization of afferent projections and neurochemical markers into well demarcated striosomes and matrix regions is an established feature of the mammalian neostriatum. The observation of distinct types of neuroactive substances, receptors and their interactions in striosomes and matrix suggests a functional segregation between these compartments. The present study was undertaken to determine the organization of efferent projections from each of the neostriatal compartments to the entopeduncular nucleus (EP), one of the main output centers of the striatum. Fluorogold, a fluorescent retrograde tracer, was placed into the lateral habenula (LH) or the ventrolateral nucleus of the thalamus (VLT) of adult Wistar rats to identify the topographical organization of neurons projecting to these two major output centres of the EP. Subsequently, fluorogold was placed into the rostral or caudal region of the EP, identified from the above experiment as areas that project to the LH or VLT respectively. The fluorescent retrograde labelling was combined with immunocytochemistry for calbindin, a specific marker for the projection neurons of the matrix. The results reveal that the rostral part of the EP projects to the LH and receives input exclusively from the striosomes, whereas the caudal part of the EP projects to the VLT and receives input exclusively from the matrix. These observations were substantiated by the finding that calbindin immunoreactive fibres lie in relation to those EP neurons that project to the VLT. The data suggest that the striosome is involved in a pathway through EP, LH and the dorsal raphe nucleus which in turn projects to the neostriatum, while the matrix is involved in a pathway through EP and VLT which projects to the cerebral cortex. [Supported by MRC Canada and London Upjohn Neurosciences Program.]

520.11

LIMBIC INTERACTION IN THE ROSTRAL CAUDATE NUCLEUS: A NEUROANATOMICAL STUDY IN THE CAT. A. Rosell*, S. de las Heras*, B. Hontanilla*, P.Barreiro* and J.M. Giménez-Amaya. Dept. Morfología, Fac. Medicina, U.A.M., 28029-Madrid, SPAIN.

The limbic component of the basal ganglia has been mainly assigned to the so-called ventral striatum. The dorsal striatum has, however, a remarkable innervation from limbic territories located in the medial wall of the cerebral cortex (Powell et al.,1974). This study was designed to explore in detail the organization of these projections, and their hodological relationship with two other afferent striatal systems: the thalamus and the substantia nigra. Eleven cats were deeply anesthetized and stereotaxically injected with horseradish peroxidase free (HRP) and/or conjugated with wheat germ agglutinin (HRP-WGA) in different portions of the rostral caudate nucleus. Next, we focussed our anatomical evaluation of retrogradely labeled cells in the limbic areas of the medial wall of the ipsilateral hemisphere, rostral intralaminar nuclei of the thalamus (ILr) and the pars compacta of the substantia nigra (SNc). Adjacent sections processed for acetylcholinesterase and Nissl were used to complete, histochemically and cytoarchitectonically, our tracer study. The most prominent results of this study are: a) the anterior limbic area projects heterogeneously to the rostromedial striatum (RmS) following a dorsoventral topology, with rostral cortical portions projecting chiefly to more dorsal striatal aspects, and caudal territories to more ventral parts of RmS; b) the rostral and dorsal pole of the cingulate area (Cg) projects mainly to dorsal areas of RmS; c) more caudal zones of Cg project conspicuously to central portions of RmS; d) the rostral half of the retrosplenial area projects to dorsal, central and ventral fields of RmS; e) regarding the ILr, dorsal injections in RmS elicited a remarkable labeling in the central lateral and paracentral thalamic nuclei, central injections into RmS labeled noticeably the so-called central dorsal thalamic nucleus, and more ventral injections in RmS yield retrogradely labeled cells mainly in the midline thalamic nuclei; and, finally, f) the two caudal thirds of the SNc were most abundantly labeled after retrograde tracer injections in RmS. Supported by CICYT PB88-0177.

Powell, E.W., K. Akagi and J.B. Hatton, J. Hirnforsch. 15, 269-278 (1974).

520.12

PHARMACOLOGICAL ACTIVATION OF DOPAMINERGIC PATHWAYS IN THE BABOON STUDIED WITH PET. C.C. Rowe* and J.S. Perlmutter. Washington University School of Medicine, St. Louis, MO 63110.

We developed an in-vivo method to measure regional cerebral blood flow (rCBF) responses to dopamine receptor manipulation in the anesthetized baboon. A surgically implanted skull cap that locks into a head holding device equipped with 3 orthogonal N-shaped fiducial markers for PET and MRI was employed for head fixation. Comparison of PET fiducial measurements to those from an MRI obtained in the same plane in each animal permitted precise anatomical identification of rCBF responses. Quantified CBF studies were performed using the bolus O-15 water method and regional responses were identified by subtraction of studies performed before and after I.V. drug administration. We found as much as a 25% reduction in rCBF in the lentiform nucleus and thalamus after i.v. injection of the selective D2 agonist quinpirole. These reductions were dose dependent over the range of 0.002 to 0.2 mg/kg and were reproducible within and across different animals. Furthermore, different regional responses occurred after the D1 agonist SKF 38393. Functional effects of specific dopamine pathways manipulation can be demonstrated in-vivo in primates with PET.

520.13

TASK SPECIFIC MOVEMENT INITIATION IMPAIRMENTS AND EFFECTS OF L-DOPA IN PATIENTS WITH BASAL GANGLIA DISEASE. V. J. Brown, E. M. Bowman, U. Schwarz, T. A. Zeffiro, D. L. Robinson, and M. Hallett*.
Laboratory of Sensorimotor Research, N.E.I. and *Human Motor Control Section, N.I.N.D.S., Bethesda, MD 20892.

The symptoms of Parkinson's disease include a slowness to initiate movement. However, the impairment may be task specific. It has been suggested that patients may be unimpaired when the responses are cued by stimuli which are compatible with the response, but when the stimulus-response (S-R) relationship is arbitrary, there is an impairment.

To test this hypothesis, two choice reaction time tasks were compared. In both tasks, the subjects responded by lifting the right or left index finger as rapidly as possible following a cue. In one task (S-R compatible), the stimulus was to the left or the right. In the other task (arbitrary S-R) the side of the required response was symbolically cued.

Performance of patients with idiopathic Parkinson's disease was compared with that of patients with Parkinsonian-like symptoms which were not responsive to L-dopa. Both groups were tested with and without L-dopa on each type of choice reaction time task. Only the patients with Parkinson's disease were differentially impaired in the two tasks, but it was the task with high S-R compatibility which was more impaired and not the task with an arbitrary S-R relationship. The differential deficit was ameliorated with L-dopa treatment. These results demonstrate a task specific impairment in reaction time performance with differential response to dopaminergic therapy.

INVERTEBRATE LEARNING AND BEHAVIOR III

521.1

BLOCKADE OF ADENYLATE CYCLASE INCREASES THE RATE OF SYNAPTIC DEPRESSION IN SIPHON SENSORY NEURONS IN APLYSIA. B.A. Goldsmith and T.W. Abrams Dept. of Biology & Institute of Neurological Sciences, Univ. of Pennsylvania, Phila., PA 19104.

We have found that an inhibitor of adenylate cyclase, 9-(tetrahydro-2-furyl)adenine (THFA or SQ 22,536) effectively blocks cAMP-mediated effects produced by 5-HT in siphon sensory neurons (SNs) including spike broadening, reduction of accommodation, and reversal of depression at SN synapses. We observed that synaptic depression occurred more rapidly in the presence of THFA. When SNs were stimulated at an ISI of 15 s, the second EPSP was 35 ± 6% (± SEM) of initial amplitude in THFA vs. 71 ± 6% in controls ($p<.001$). After 10 SN action potentials were elicited, EPSPs still showed significantly more depression in THFA experiments (29 ± 6% initial amplitude in THFA vs. 42 ± 3% in control, $p<.001$). In addition to acting as a cyclase inhibitor, THFA produces a modest broadening of SN action potentials, possibly by blocking K^+ channels. Comparable broadening of the SN APs by the K^+ channel blocker 3,4-DAP had no effect on the rate of synaptic depression. These results suggest that cAMP plays an important role in maintenance of normal levels of synaptic transmission.

521.2

POSSIBLE MOLECULAR BASIS OF THE ORDER REQUIREMENT FOR CS-US PAIRING DURING CONDITIONING: BACKWARDS PAIRING OF Ca^{2+} WITH FACILITATORY TRANSMITTER ACCELERATES THE DEACTIVATION OF *APLYSIA* NEURAL CYCLASE. H.E. Jarrard*, Y. Yovell, T.W. Abrams. Dept. of Biology & Inst. of Neurological Sciences, Univ. of Penn., Phila., PA 19104.

During classical conditioning of the gill and siphon withdrawal reflex of *Aplysia*, Ca^{2+}/calmodulin-sensitive cyclase serves as a molecular site of convergence within siphon sensory neurons for Ca^{2+} and serotonin, the cellular representations of the conditioned and unconditioned stimuli (CS and US). Using a perfused membrane cyclase assay, we previously found that a prepulse of Ca^{2+} resulted in both a faster rate of cyclase activation and greater peak activation by a brief pulse of 5-HT than did a backwards pulse of Ca^{2+}. These results suggested that Ca^{2+}/calmodulin binding to the cyclase might enhance the rate of activation by receptor and G_s. If Ca^{2+}/calmodulin binding influences the rate of G_s-cyclase coupling, we hypothesized it might also affect the rate of G_s-cyclase uncoupling once transmitter leaves the receptor. To test this possibility, we measured the time course of cyclase deactivation, in the presence and absence of a pulse of Ca^{2+} delivered after a transient exposure to 5-HT. The Ca^{2+} pulse, after 5-HT had been washed out, accelerated the rate of decay of cyclase activity to basal. These results suggest that the CS-US sequence requirement of classical conditioning may result from effects of Ca^{2+}/calmodulin on G_s-cyclase coupling and uncoupling.

521.3

A MOLECULAR MECHANISM FOR MEMORY IN *APLYSIA* : UBIQUITIN-MEDIATED PROTEOLYSIS OF REGULATORY SUBUNITS OF THE cAMP-DEPEDNDENT PROTEIN KINASE. Ashok N. Hegde and James H. Schwartz. Howard Hughes Medical Institute, Columbia University, New York, NY 10032

In *Aplysia*, behavioral sensitization of defensive reflexes and the underlying presynaptic facilitation of sensory-to-motor neuron synapses lasts for several min (short-term) or days to weeks (long-term). Short-term facilitation has been explained by cAMP-dependent modulation of ion channel function. Long-term facilitation requires additonal molecular processes including new protein synthesis. A key molecular event is persistent activation of the cAMP-dependent protein kinase (PKA). Greenberg et al (*Nature* **329:** 65-69, 1987) showed that regulatory (R) subunits are lost in long-term sensitization and this could account for persistent activation (Sweatt & Kandel, *Nature* **339:** 51-54, 1989). Despite dependence on new protein synthesis the loss of R results from proteolysis rather than diminished synthesis (Bergold et al, *PNAS* **87:** 3788-3791, 1990). In order to understand the molecular events that produce the loss of R in long-term facilitation, we investigated how R is degraded. A prominent degradation pathway (involved in stress, cell cycle and other developmental events) is mediated by ubiquitin. When several molecules of ubiquitin are conjugated to a protein, it is degraded. We find that *Aplysia* R subunits in nervous tissue (and early embryos) are degraded through the ubiquitin pathway. First, high molecular-weight *Aplysia* R-ubiquitin conjugates (ladders) form in cell-free rabbit reticulocyte lysates, suggesting that R subunits can be multi-ubiquitinated. Second, we observe ATP- and ubiquitin- dependent degradation of R. We suggest that the ubiquitin-mediated proteolysis of R operates constitutively in *Aplysia* neurons (which contain ubiquitin) and, further, that in facilitated sensory cells a protein targeting R for multi-ubiquitination is synthesized that is limiting in naive neurons. The *induction* of this postulated targeting protein would result in enhanced degradation of R, which in turn would *maintain* PKA in an activated form.

521.4

INHIBITORS OF PROTEIN AND RNA SYNTHESIS BLOCK STRUCTURAL CHANGES ACCOMPANYING LONG-TERM PRESYNAPTIC FACILITATION AND INHIBITION IN *APLYSIA* SENSORY NEURONS. S. Schacher, P.G. Montarolo, E.R. Kandel, Mary Chen, and C.H. Bailey Ctr. Neurobiol. & Behav., Columbia P&S, NYSPI, & HHMI, N.Y., N.Y. 10032.

Long-term presynaptic facilitation by 5-HT of sensorimotor synapses in culture is accompanied by increases in both the size of the EPSP evoked in the motor cell L7 and in a number of sensory neuron varicosities. By contrast, long-term presynaptic inhibition by FMRFamide of sensorimotor synapses is accompanied by a decrease in sensory neuron varicosities. Since in both cases the changes in the EPSPs are dependent on macromolecular synthesis, we now examined whether the structural changes also require protein or RNA synthesis. As previously reported, both transmitters evoked long-term changes in the EPSP in L7 that are paralleled by changes in the number of sensory neuron varicosities. Thus, the EPSP increased by 61% ± 10 and the number of varicosities by 46% ± 7 with 5-HT ($N = 15$), and the EPSP decreased -41% ± 4 and the varicosities decreased by -26% ± 5 with FMRFamide ($N = 10$). The protein synthesis inhibitor, anisomycin, and the RNA synthesis inhibitor, actinomycin-D, blocked both the changes in EPSP and in the number of varicosities evoked by each transmitter. The inhibitors alone had no significant effect on the structure of the sensory cell or the EPSP. These results indicate that structural changes require the expression of genes and proteins not required for the short-term changes, and suggest that the long-term structural changes are tightly coupled with long-term modulation in the efficacy of synaptic transmission.

521.5

EARLY STEPS IN LEARNING-RELATED SYNAPTIC GROWTH: 5-HT INDUCES ENDOCYTIC ACTIVATION AND INTERNALIZATION OF N-CAM RELATED CELL ADHESION MOLECULES IN *APLYSIA*: C.H. Bailey, M. Chen, F. Keller and E.R. Kandel. Ctr. Neurobiol. & Behav., Columbia P&S, NYSPI, & HHMI, NY, NY 10032.

We have found the synaptic growth that accompanies 5-HT induced long-term facilitation of the sensory to motor connection in *Aplysia* dissociated cell culture requires new protein and mRNA synthesis. This growth is associated with a down-regulation of cell adhesion molecules related to N-CAM (Ap CAM, Barzilai et al., 1989; Mayford et al., in prep). By combining thin section E.M. with gold-conjugated mAb specific to Ap CAM (Keller and Schacher, 1990), we have found that 5-HT leads, within one hour of its application, to a 50% decrease in the density of gold-labeled complexes at the surface membrane of the sensory neuron (9.4/ μm ± 0.7, SEM = 7) vs. Control (19.5 ± 1.4, $p<0.001$). Down-regulation is achieved by activation of the endosomal pathway, leading to internalization and subsequent degradation of Ap CAM. The endocytic activation and resultant internalization triggered by 5-HT increases the gold found within the cell seven-fold (29.4 ± 2 vs. 4.1 ± 0.3; n = 7, $p<0.001$), requires new protein synthesis and is particularly prominent at sites of homophilic interactions. These findings suggest that an early step in learning-related synapse formation involves endocytic activation and internalization of cell adhesion molecules via a clathrin-dependent pathway. The removal of cell adhesion molecules from the neuronal surface may lead to a destabilization of cell-cell contacts that might be important for disassembly. In addition, we suggest that endocytosis may lead to a redistribution of membrane components to sites of new synapse formation.

521.6

FURTHER BEHAVIORAL AND CELLULAR STUDIES OF DISHABITUATION AND SENSITIZATION IN *APLYSIA*. T.E. Cohen*, V. Henzi, E.R. Kandel and R.D. Hawkins. Ctr. Neurobiol., Columbia Univ., HHMI, NY, NY 10032.

We have developed an isolated mantle organ preparation for simultaneous behavioral and cellular studies of plasticity of the gill-withdrawal reflex in *Aplysia*. In our initial behavioral studies, we reinvestigated the effect on dishabituation and sensitization of test time, tap strength, and shock intensity. Sensitization occurred in most conditions, with significant effects of time, tap, and shock. By contrast, for dishabituation there were no effects of tap or shock level 2.5 min after the shock, and the post-test was always slightly less than the first (pre-habituation) test. 12.5 min after the shock there were significant effects of both tap and shock level (as in sensitization), and in most conditions the post-test was greater than the first test. This pattern of results suggests that at 2.5 min dishabituation of this reflex may be due to removal of habituation, whereas at 12.5 min it may also be due to sensitization. In our initial cellular studies we reinvestigated the contribution of the LE mechanosensory neurons. We used a mechanical tapper driven by a solenoid and varied force by varying the travel distance. In 9 out of 21 preparations, a tap that did not actually touch the siphon elicited a gill-withdrawal response. By contrast, 32 out of 32 LE cells in those preparations fired action potentials if and only if the tapper touched the siphon. Moreover, the latency of firing of the LE neuron was always slightly longer than the latency of the onset of the response in a motor neuron. We have obtained similar latency results in a second series of experiments with a controlled force stimulator. These results confirm that the LE cells participate in the reflex, and suggest that a second, unidentified population of sensory cells with lower thresholds and slightly shorter latencies may also contribute.

521.7

ACTIVITY-DEPENDENT ENHANCEMENT OF ELECTROPHYSIOLOGICAL MEASURES CONSISTENT WITH ENHANCED PRODUCTION OF cAMP IN PLEURAL SENSORY NEURONS OF *APLYSIA*. R.D. Hawkins, T.E. Cohen*, L.S. Eliot and E.R. Kandel. Ctr. Neurobiol. & Behav., Columbia Univ., & HHMI, NY, NY 10032.

Activity-dependent facilitation of synaptic transmission from sensory neurons to motor neurons in *Aplysia* is thought to be due in part to enhanced production of cAMP in the sensory neurons (Kandel et al., 1983; Ocorr et al., 1985). Recently, Eliot et al. (1989) demonstrated activity-dependent facilitation in isolated cell culture, using a modified one-trial training procedure. We have begun to investigate whether this training procedure also produces electrophysiological changes consistent with enhanced production of cAMP in isolated clusters of pleural sensory neurons. In a first series of experiments, we produced tetanic firing (20 hz, 2 sec) of the neurons with extracellular shock either 0.5 sec (paired) or 1 min (unpaired) before a 30-sec application of 5-HT and measured the excitability (number of spikes produced by a constant intracellular current pulse) of one cell in the cluster. Paired training produced a significantly greater maximum ($\bar{X}$ = 425 vs. 292%) and duration ($\bar{X}$ = 2.4 vs. 1.5 min) increase in excitability than unpaired training ($N = 12$, $t = 2.26$, $p < .05$). Tetanus alone had no effect. Preliminary results from a second series of experiments suggest that paired training also produces a greater increase in frequency-broadened spike width (12.8%) than either 5-HT alone (7.7%) or tetanus alone (-1.6%). These results are consistent with enhanced production of cAMP in the sensory neurons, and lay the groundwork for future experiments relating these electrophysiological changes to biochemical measurements in the same preparation.

521.8

ACTIVATION OF THE FACILITATORY INTERNEURON L29 PRODUCES INHIBITION OF REFLEX INPUT TO SIPHON MOTOR NEURONS IN *APLYSIA*. T.M. Fischer & T.J. Carew, Yale Univ., Dept. Psychology, New Haven, CT 06520.

The identified interneuron L29 has a well characterized facilitatory role in the siphon withdrawal reflex (SWR) of *Aplysia* (Hawkins et al.,1981). We report here that L29 can also transiently inhibit this reflex via recruitment of recurrent inhibition from the identified interneuron L30.

The SWR was elicited by siphon taps (5 min ISI) in an isolated mantle/abdominal ganglion preparation. As a measure of net SWR circuit activity, we integrated the initial 500 msec of the tap-evoked complex EPSP in LFS siphon motor neurons (hyperpolarized to prevent spiking). Intracellular activation of L29 (5 sec, rate ≈ 30 Hz) 10 sec prior to a tap produced a significant decrement of the complex EPSP ($\bar{x}$ = 79% of baseline, $N = 7$, $p < .001$) which recovered by the next trial. In separate experiments, L29 activation 20 sec prior to a tap still produced significant inhibition ($\bar{x}$ = 85%, $N = 11$, $p<.01$), whereas 40 sec prior did not ($\bar{x}$ = 89% , $N = 10$, n.s.), indicating that L29-induced inhibition has a time course of approximately 30 sec.

Several preliminary observations suggest that the mechanism of L29-induced inhibition involves, at least in part, L29 removing itself from the SWR circuit through recruitment of recurrent inhibition from interneuron L30. First, voltage clamping L29 during reflex activation significantly reduces the complex EPSP in LFS neurons ($\bar{x}$ = 85%, $N = 11$, $p < .05$). Second, intracellular activation of L30 20 sec prior to tap inhibits both LFS complex EPSPs ($\bar{x}$ = 83%, $N = 9$, $p < .05$) and L29 spiking ($\bar{x}$ = 62%, N=5). Third, the monosynaptic IPSP from L30 to L29 shows substantial post-tetanic potentiation both directly (by L30 activation) and indirectly (by L29 activation). These observations suggest that L29/L30 interactions can serve as a module providing use-dependent gain control in the neural circuit for siphon withdrawal.

521.9

A NETWORK MODEL OF INHIBITORY INFORMATION PROCESSING IN THE SIPHON WITHDRAWAL REFLEX OF *APLYSIA*. D.E.J. Blazis, D. A. Berkowicz*, E. W. Kairiss, and T.J. Carew. Dept. Psych. and Prog. Neuroscience, Yale Univ., New Haven, CT, 06520.

Two forms of inhibition have recently been described in the siphon withdrawal reflex (SWR) of *Aplysia:* (1) Tail-shock (TS) induces transient inhibition of the SWR (Marcus et al, 1988). This inhibition is mediated, at least in part, by inhibitory interneuron (INT) L16. L16 responds to TS, activation of L16 inhibits reflex-induced complex EPSPs in siphon motor neurons (MNs), and voltage clamping L16 abolishes this TS-induced inhibition (Wright & Carew, 1990). (2) Activation of excitatory INT L29 inhibits reflex-induced complex EPSPs in MNs; this inhibition arises in part from L29's recruitment of inhibition onto itself from inhibitory INT L30, and is modulated both by electrical coupling between the cells and by post-tetanic potentiation (PTP) of the L30-L29 inhibitory synapse (Fischer & Carew, 1991).

We have used the network simulator GENESIS to incorporate the connectivity and synaptic interactions described above into a biologically plausible model of inhibition in the SWR. Our model includes elements representing siphon and TS input, INT processing, and MN output. This network generates both forms of inhibition observed in the SWR: (1) Activation of the simulated L16 inhibits reflex-induced EPSPs onto MNs. Following TS, which activates L16, the EPSP is reduced by ≈ 75%. TS-induced inhibition is abolished under simulated voltage clamp of L16. (2) Activation of simulated L29 recruits inhibition onto itself via activation of INT L30, which in turn reduces excitation to MNs. We are currently examining the computational significance of the L29-L30 electrical connection and inhibitory PTP at the L30-L29 synapse.

As our model more completely simulates inhibitory interactions in the SWR circuit, it will be of interest to apply it to different forms of learning exhibited by this reflex.

521.10

LATERALIZATION OF LONG-TERM HABITUATION IN TAIL-INDUCED SIPHON WITHDRAWAL IN *APLYSIA*. M. Stopfer, X.V. Chen*, and T.J. Carew. Department of Psychology, Yale University, New Haven, CT.

The tail-induced siphon withdrawal reflex (T-SWR) in *Aplysia* is mediated by bilateral clusters of sensory neurons. Each cluster conveys sensory information from one side of the body, raising the possibility that learning mediated by these sensory neurons might be lateralized. Indeed, Scholz and Byrne (1987) have demonstrated lateralization of long-term sensitization in the T-SWR. In this study we report that another form of learning, long-term habituation, also can be lateralized in the T-SWR.

Aplysia were bilaterally implanted with silver wire electrodes so that weak electrical stimuli (behaviorally equivalent to tactile stimuli) could be delivered consistently to the same tail sites. All experiments were conducted blind in freely-moving animals. Animals first received a series of bilateral pre-tests at a non-habituating (10 min) ISI; the T-SWR duration was measured following each stimulus. Next, computer-controlled unilateral habituation training ensued: four stimulus blocks were spaced 90 min apart (each block= 30 stim at a 30 sec ISI). Finally, bilateral tests were conducted 24 hr after training. Two experiments were conducted: in Exp 1 (N=13) the control side received no stimuli during the training period; in Exp 2 (N=15) the control side received an equivalent number of stimuli as the trained side, but at a non-habituating (10 min) ISI. An ANOVA revealed a significant overall effect of training in both experiments (interaction: $p<0.001$ in each). Furthermore, trained side test scores indicated significant habituation relative to their own pre-scores (Exp 1: $p<0.05$; Exp 2: $p<0.001$) whereas control sides showed no habituation.

Our results show that long-term habituation can be lateralized in the T-SWR. Since this reflex is well suited for a cellular and molecular analysis of behavior, it should now be possible to use the within-animal control provided by our findings to explore the detailed mechanisms of long-term memory for habituation.

521.11

DIFFERENTIAL EFFECTS OF THREE STIMULI PRODUCING LEARNED CHANGES OF RESPIRATORY PUMP RATE IN *APLYSIA*. M. Levy* and A.J. Susswein, Dept. of Life Sciences, Bar-Ilan University, Ramat Gan 52 900, Israel

In *Aplysia*, the gill contracts during 2 behaviors: 1) the gill withdrawal reflex; 2) respiratory pumping. Learning affecting the withdrawal reflex has been intensively studied by others. We have examined learned changes in respiratory pumping.

Three stimuli affecting respiratory pumping were identified: 1) decrease in pH 2) increase and 3) decrease in salinity. Effects of all 3 have sharp thresholds. For any of the 3, threshold can be altered by pairing subthreshold stimulation (pH 7.0, 120% or 85% seawater) with head shock. Shock alone, pre-exposure to a sub-threshold stimulus, or unpaired exposure to shock *and* a sub-threshold stimulus lead to sensitization of respiratory pumping that is shorter-lasting than is effect of pairing. Thus, pairing may amplify sensitizing effects produced by shock and by the 3 stimuli.

The head ganglia and the abdominal ganglion contain neurons capable of sensitizing the gill withdrawal reflex. These neurons also could affect respiratory pumping. When the pleural-abdominal connectives were cut, thereby separating the abdominal and head ganglia, no response was seen to decreased pH, but responses were normal to increases and decreases in salinity. These data suggest that sensitizing neurons in the head ganglia respond to decreased pH, while neurons in the abdominal ganglion mediate responses to altered salinity.

We tested this hypothesis further, by examining generalization of learning between the 3 stimuli. When animals were shocked in 85% seawater and tested in 120%, and vice versa, there was complete generalization of learning between the 2 stimuli. By contrast, there was no generalization of learning when animals were shocked in decreased pH and tested in either 85% or 120% seawater. These data support the suggestion that a common sensitizing site in the abdominal ganglion is responsive to increases and decreases in salinity, while a different site in the head ganglia responds to decreased pH.

DEVELOPMENT OF CEREBRAL CORTEX AND LIMBIC SYSTEM III

522.1

Development of amygdaloid projection of the prefrontal cortex in the rat studied with Phaseolus vulgaris-leucoagglutinin (PHA-L). R.W.H. Verwer, J.F.M. van Uum and E.H.S. van Vulpen*. Netherlands Institute for Brain Research, Meibergdreef 33, 1105 AZ Amsterdam, The Netherlands and *Neuroscience Unit, Ottawa Civic Hospital, Ottawa, Canada. (Spon: European Neuroscience Association)

The amygdaloid innervation pattern in the prefrontal cortex (PFC) was studied at 3 early postnatal ages. PHA-L was injected in the rostral part of the basolateral amygdala at postnatal days (dpp) 1, 6 and 12 and the pups survived for 2 days. At 1 dpp a few diffusely distributed very thin fibers were observed throughout the PFC. The most rostral and caudal parts were void of fibers. At 6 dpp more fibers were present and in the medial PFC (mPFC) fibers showed a slight preferrence for layers I, II, III and VI. The caudal part of the prelimbic area was almost empty. In the lateral PFC (lPFC) the fibers were mainly in layers V and VI. At 12 dpp there was a marked increase of the fiber density and a distinct segregation into two bands. In mPFC the bands comprised layers (II, III) and VI, which were less pronounced more caudally. Again the density became less in the caudal mPFC. In lPFC the bands were II and (V, VI). In adult rats (day 34) the pattern seemed to stay more or less the same, while the density further increased as compared with 12 dpp.

522.2

POSTNATAL DEVELOPMENT OF THE SEROTONERGIC INNERVATION IN THE FERRET CEREBRAL CORTEX. T.Voigt and A. D. de Lima. Max-Planck-Institut für Entwicklungsbiologie, Spemannstr.35/I, 7400 Tübingen, Germany

We have investigated the serotonergic innervation of the ferret cortex from the day of birth to adulthood with immunohistochemical techniques. Due to the premature birth of ferrets, this period spans the entire generation of cells located within the upper cortical layers and their subsequent migration to their final positions. Already at birth, serotonergic fibers innervate the developing cortex. This innervation is most dense within the marginal zone, the subplate region and the lower portion of the cortical plate. As long as cell migration continues, serotonergic fibers enter the expanding portions of the cortex. Only the region just below the marginal zone where newly arriving cells are added to the cortical plate is not innervated by the ingrowing fibers. When the bulk of cell migration ceases, during the third postnatal week, this gap disappears and the fibers gradually form a continuous innervation from the pia to the ventricle. As the cortex matures the serotonergic fibers become successively confined to the upper layers, to generate the adult pattern. In the adult ferret cortex, the highest innervation density is found within layers 1, 2 and 3, with a much sparser innervation within the lower layers. The dense innervation in the deep cortical layers is only transient, virtually disappearing towards adulthood. These results suggest that serotonergic axons innervate cortical layers as soon as newly arriving cells reach their final positions within the cortex. This early innervation lends support to the idea that serotonin may play a role during development of the cerebral cortex.

522.3

ISOFORMS OF MICROTUBULE-ASSOCIATED PROTEIN 2 (MAP2) IN DEVELOPING CAT CORTEX AND CORPUS CALLOSUM. B.M. Riederer, and G.M. Innocenti. Institute of Anatomy, University of Lausanne, Rue du Bugnon 9, 1005 Lausanne, Switzerland.

MAP2, an essential protein for dendritic microtubules and morphogenesis, was shown on immunoblots to occur in only two isoforms, MAP2b and c, during cat brain development. *In cat cortex*, MAP2b was abundant during the first postnatal month, and decreased towards adulthood, while no or little MAP2b was detectable in corpus callosum during the same time. MAP2c, present in three forms of close molecular weights, changed composition in cortex and corpus callosum during the first postnatal month, possibly due to dephosphorylation, and then disappeared toward adulthood. In adult brain tissue, MAP2a could not be detected with different MAP2 antibodies. Immunocytochemical localization revealed the presence of MAP2 mainly in differentiating neurons in all cortical layers. *In corpus callosum*, axons were stained - probably containing MAP2c forms. In additon in the same tissue, MAP2b positive cells of different size and shape, and with neuronal characters (i.e. positive to neuron specific enolase and GABA) were identified. They were numerous at early postnatal ages, and remained in the frontal part, but decreased in middle and rostral parts of the corpus callosum towards adulthood. Function and fate of these cells are unclear. *In conclusion*, MAP2 isoforms differ in distribution and structural association, and change at a time of intense dendritic and axonal growth and reorganization. This work was in parts supported by Sandoz Foundation, the University of Lausanne, and SNF grants Nr. 31.26624-89 and 3.359-0.86.

522.4

DEVELOPMENTALLY TRANSIENT ACETYLCHOLINESTERASE ACTIVITY IN CORTICAL PYRAMIDAL NEURONS OF RAT BRAIN. C. Geula and M-M. Mesulam, Harvard Medical School, Boston, MA 02215.

Using a histochemical method for visualization of cholinesterase activity, we have observed transient expression of acetylcholinesterase (AChE) in cortical pyramidal neurons during postnatal development in the rat.

Practically no AChE-positive cortical pyramidal neurons are seen at birth. At 1-4 days postnatally (P1-P4), many of these neurons emerge and can be detected in most cortical areas. The emergence of these neurons occurs earlier in paralimbic cortical areas (P1-P2) as compared with other areas of the cerebral cortex (P2-P4). Between P5 and P8, the density of these neurons increases and a leveling off is observed at P8-P11. During this period of peak density, AChE-positive pyramidal neurons can be detected in all cortical areas. Their density and staining intensity, however, appear to be higher in paralimbic cortical zones as compared with other cortical areas. Thereafter, the density of AChE-positive cortical pyramidal neurons gradually decreases. At P21 and longer, very few of these neurons can be observed and this pattern continues into adult life.

The developmental pattern of AChE-positive cortical pyramidal neurons in the rat is quite different from that observed in the human, in which such neurons are absent in childhood and become established in adult life.

522.5

AN ANTISERUM TO THE LOW AFFINITY NERVE GROWTH FACTOR RECEPTOR LABELS SUBPLATE CELLS OF DEVELOPING NEOCORTEX IN THE MOUSE AND A SUBSET OF NEURONS AND GLIA IN TISSUE CULTURE. D.B. Wayne, A.A. Zupan, and A.L. Pearlman Depts. of Cell Biology, Neurology and Pharmacology, Washington University School of Medicine, St. Louis, MO 63110

The preplate cells of embryonic mammalian neocortex are a unique population of early-generated, predominantly transient cells that make up the subplate and marginal zone after cortical plate formation. They are closely associated with specific extracellular matrix components (Stewart and Pearlman, 1987; Chun and Shatz, 1988; Sheppard et al., 1990), and express the nerve growth factor receptor (NGFR) in the cat and ferret (Allendorfer et al., 1990). Many preplate/subplate cells are neurons that form synapses and extend the first efferent axons (McConnell et al., 1989), but some may be glia (Rickmann and Wolff, 1985). To define this important population further, we immunolabeled sections and dissociated cell cultures of the developing neocortex of the mouse with a new antiserum produced in the chicken to an extracellular domain of recombinant human NGFR (Zupan et al., Soc. Neurosci. Abst., 1991). The cell bodies and processes of subplate cells are clearly labeled by the antiserum on embryonic day (E) 16 and postnatal day 1. In cell cultures dissociated from cortex on E13 and double-immunolabeled three days later, the cell bodies and processes of a subset of the neurons identified with an antibody to neurofilaments (RM108 provided by Dr. V. Lee) are labeled with the NGFR antiserum, as are a subset of the glial cells identified with the monoclonal antibody RC1 (provided by Dr. M. Yamamoto). Taken together, these findings indicate that subplate neurons express an NGFR-like molecule on their surface, and that NGFR expression also defines a subset of embryonic glial cells.

522.6

ANATOMIC AND FUNCTIONAL CORRELATES OF THE SUBPLATE IN THE SOMATOSENSORY CORTEX OF KITTENS. S.L. Juliano, R.A. Code, D.E. Eslin Department of Anatomy & Cell Biology, USUHS, Bethesda, MD 20814.

In kittens less than 2 weeks of age, little stimulus-evoked functional activity (as revealed by 2-deoxyglucose [2DG] uptake) is seen in the somatosensory cortex. Dense metabolic label is present in these animals, however, as a band of increased activity at the interface between layer VI and the white matter. This region corresponds to the subplate, which is normally observed in developing animals. We found in kittens aged 3 days to 2 weeks, that the subplate was heavily labeled with 2DG uptake, whether or not a somatic stimulus was delivered. After this age, a metabolic correlate of the subplate was not visible and stimulus-evoked activity became gradually more apparent, until about 5 weeks of age, when it was adult-like. A dense band of cytochrome oxidase activity is also found in the same layer VI-WM location, normally not observed in the adult. Histologic evaluation of this region established an increased number of Nissl-stained cells at the layer VI-WM junction, which conformed in morphology to neurons of the subplate. In addition, the cortical laminar organization of cell bodies is immature with layers V and VI being easily distinguished, while layers II-IV are relatively indistinguishable. An immature pattern of acetylcholinesterase activity is also seen in animals less than 2 weeks of age. In the young kittens, a dense network of fibers extends from the WM to into layers V and VI. Cortical layers above this site possess only sparse AChE+ fibers, in contrast to the adult distribution, which displays a dense distribution of fibers in all cortical layers. These structural correlates indicate that the subplate is present and metabolically active in the somatosensory cortex of kittens up to 2 weeks of age. Further studies may interpret the significance of the subplate in forming a mature pattern of functional activity in the somatosensory cortex. Supported by NS-24014.

522.7

MODIFICATIONS IN MYSTACIAL VIBRISSAE AND THE BARREL CORTEX IN RAGGED MUTANT MICE. R.O. Kuljis. Department of Neurology, The University of Iowa College of Medicine, Iowa City, Iowa 52242-1053.

The functional and cytoarchitectonic units called barrels in the murine somatosensory cortex are among the most distinct examples of modular ensembles in the cerebral cortex. Postnatal cues from sinus hair receptors to this region are necessary for their development. However, since early prenatal vibrissal follicle ablation has so far been unsuccessful, virtually nothing is known about their role in the specification of barrels in embryos. The barrel cortex of 6 specimens of Ragged, opposum (Ra^{OP}) mutant mice and 4 controls was analyzed in coronal or tangential sections, using the Nissl, cytochrome oxidase and succinate dehydrogenase methods. Flat-mounted muzzle skin from each animal was analyzed in Bielschowsky and Masson's stains. The vibrissal pad of mutants has rows of small elevations or mounds in a location similar to that of sinus hair follicles and in a pattern identical to them in normal animals. Only 3-4 short and thin vibrissae originate from the most posterior of those mounds. Histology confirms that each mound - with and without vibrissa - is a sinus hair follicle. Barrels corresponding to the existing vibrissae are almost twice as wide as those not supplied by vibrissae, but exhibit about the same level of staining intensity for oxidative enzymes as the rest. Only barrels immediately surrounding the enlarged ones have a reduced diameter and a polygonal appearance, suggesting that they have either shrunk or grown less than their neighbors. These observations indicate that barrels can develop in the absence of pre- and postnatal vibrissal stimulation, but do not necessarily imply that they can develop in complete absence of cues other than action potentials from vibrissal receptors. It appears also that vibrissal stimulation may be a factor influencing the amount of cortex devoted to each barrel.

522.8

SPONTANEOUS ACTION POTENTIAL ACTIVITY AND SYNAPTIC CURRENTS IN THE EMBRYONIC TURTLE CEREBRAL CORTEX. Mark G. Blanton and Arnold R. Kriegstein, Department of Neurology and Neurological Sciences, Stanford Univ. Sch. of Med., Stanford, CA 94305.

We used loose-patch and whole-cell recording techniques to study spontaneous action potential activity and excitatory and inhibitory synaptic currents in embryonic neurons in the cerebral cortex of turtles. Sporadic action potentials appeared early in development at stage 17. As the cortical plate matured in mid-embryonic stages, action potential activity became more regular and fell into one of two distinct patterns, slow waveform, tonically active neurons (pyramidal) and fast waveform, repetitively firing neurons (nonpyramidal).

Spontaneous synaptic currents with rapid rise and slow exponential decay were observed in embryonic neurons at stage 18. Spontaneous excitatory synaptic currents appeared first, and while IPSCs could be elicited by repetitive stimulation at this stage, spontaneous IPSCs appeared suddenly at stage 20 and soon surpassed EPSCs in frequency. These results, and the presence of inhibitory synapses ultrastructurally at stage 18 (Parnavelas, Blanton and Kriegstein, unpublished), indicate that the time of onset of spontaneous IPSCs is not determined by the formation of a functional synaptic apparatus but is dependent on a later developmental event. EPSCs increased in frequency, rise time, and tau, but amplitudes remained stable despite dendritic elongation. In contrast, IPSCs showed a significant change in mean amplitude, increasing from 9.0 ± 0.6 pA at stage 18 to 13.0 ± 2.2 at stage 26 at a holding potential of -20 mV, and showed no change in tau. EPSCs and IPSCs were sensitive to TTX, and were thus dependent on spontaneous action potential activity.

Spontaneous IPSCs appear just prior to the time excitatory synapses can synchronize epileptiform bursts, and after the time inhibitory activity can be evoked. Inhibitory synapses are therefore present in advance of their role in controlling excitability and are recruited as spontaneous excitatory activity increases. Spontaneous neuronal activity emerges in a characteristic sequence and provides a substrate for shaping development *in vivo*.

522.9

CHANGES IN GRAY AND WHITE MATTER VOLUME IN NORMAL DEVELOPMENT: AN MRI STUDY. A. Pfefferbaum, E. V. Sullivan, D. H. Mathalon*, B. B. Zipursky*, and K. O. Lim. Dept. of Psychiatry and Behavioral Sciences, Stanford University School of Medicine and Mental Health Clinical Research Center, Dept. of Veterans Affairs Medical Center, Palo Alto, CA .

Significant changes in brain morphology occur during childhood. The brain grows in size, white matter continues to be myelinated, and gray matter increases and then decreases with regressive processes, such as neuronal pruning. To track these changes in vivo, we quantified volumetrically MRI scans obtained from 40 children referred for a clinical scan. The group comprised 17 boys, age 2-18 yrs (mean=11.4 yrs) and 23 girls, age 1 mo-19 yrs (mean=9.7 yrs); children known to have CNS disorders were excluded. All scans were read clinically as normal. An automated image quantification procedure segmented each image into compartments of gray matter, white matter and cerebral spinal fluid (CSF). Volumes, based on 7, 5-mm thick sections, provided estimates of whole brain volumes of each compartment. Volumes, expressed as percentages of head size, measured changes in CSF and tissue proportions over age, controlling for head/brain size differences. Absolute volumes provided an index of growth patterns of each compartment, regardless of head size. Because sex differences were not significant, all data were pooled. Percentage gray matter decreased and white matter increased linearly with age ($r=-.86$, $r=.80$, $p<.0001$); percentage CSF increased curvilinearly from about age 10. Absolute volumes changed curvilinearly with age: white matter volume increased more steeply over the first than second decade; gray matter volume peaked around age 8-10 and then decreased over the second decade, complementary to an increase CSF volume. This pattern is consistent with neuropathological studies; thus, MRI may provide a valid tool for longitudinal developmental studies of the brain. *[Supported by MH30854, AA05965, Norris Mental Health Center, the Meyers Foundation]*

523.1

DIRECT MEASUREMENT OF PEPTIDE CO-TRANSMITTER RELEASE FOLLOWING INTRACELLULAR STIMULATION OF A SINGLE IDENTIFIED MOTORNEURON IN *APLYSIA*. F.S. Vilim[1], D.A. Price[3], W. Lesser[3], I. Kupfermann[1], and K.R. Weiss[2]. [1]Cntr. Neurobiol. & Behav., Columbia Univ., NYS Psych Inst., NY, NY, [2]Dept. Physiol. & Biophys., Fishberg Res. Ctr. in Neurobiol., Mt. Sinai School of Med., NY, NY, and [3]Whitney Labs St Aug. FL.

The accessory radula closer muscle (ARC) and its innervation provide a model system for studying the role of neuromodulation and co-transmission. Motorneuron B15, which innervates the ARC, synthesizes peptides that modulate ARC contractions when applied exogenously. These peptides fall into two classes (coded for by different genes), the SCPs and the buccalins. The SCPs potentiate (postsynaptically) and the buccalins depress (presynaptically) motorneuron induced ARC contractions. Previous work provided indirect evidence for SCP release in the ARC. We have developed a method for directly measuring SCP and buccalin release and provide evidence that they are co-released in response to physiological ranges of B15 stimulation. Radioimmunoassay, with femtomolar sensitivity, was used to detect buccalin and/or SCP in the perfusate of ARC muscle. B15 was intracellularly stimulated to fire at either 7.5 or 12 Hz for 3.5 sec every 7 sec for 10 min or continuously at 6 Hz for 10 min. The muscle was kept at 15°C which matched the temperature of the tanks housing the animals. Under these conditions, release of both SCP and buccalin was greater at 12 Hz than at 7.5 Hz. Continuous stimulation at 6 Hz for 10 min produced intermediate levels of release indicating that stimulus parameters other than frequency affect release. No release was observed if Mg++ was substituted for Ca++ in the perfusate. Blocking muscle contractions with 10^{-4}M hexamethonium had no effect on peptide release. The present findings, together with previous findings that SCP and buccalin are colocalized in the same dense core vesicles suggest that they are co-released. The results provide good evidence that the peptides are released in vivo when the animal feeds.

523.2

CHARACTERIZATION OF A cDNA CLONE ENCODING MULTIPLE MYOMODULIN-RELATED NEUROPEPTIDES IN *APLYSIA*. M.W. Miller, S. Stamm*, E.C. Cropper, F.S. Vilim, S. Beushausen, J. Brosius, I. Kupfermann, and K.R. Weiss. Center for Neurobiol. & Behavior, Columbia Univ., Coll. P&S and NYS Psychiat. Inst., NY, NY 10032; and Fishberg Res. Center for Neurobiol., Mount Sinai School of Medicine, 1 Gustave Levy Pl., NY, NY 10029.

The neuropeptide myomodulin A, originally purified and sequenced from the accessory radula closer (ARC) muscle of *Aplysia*, is present in one of the cholinergic motor neurons innervating this muscle, where it has been proposed to act as a modulatory cotransmitter (Cropper et al., 1987). Clones encoding myomodulin A were isolated from a buccal ganglion cDNA library using degenerate oligonucleotide probes. The sequence of the longest clone (≈1.4 kb) encodes a 370 amino acid open reading frame containing a potential myomodulin precursor. This sequence includes 10 contiguous copies of myomodulin A, each flanked by dibasic potential cleavage sites, and each ending with a C-terminus glycine amide donor. The precursor also contains 6 myomodulin-related peptides, each of which is present in a single copy. Four of these peptides, the heptapeptides myomodulin D, F, and G (Miller et al., 1990) and the octapeptide myomodulin B (Vilim et al., 1989), have been previously purified from the ARC muscle. Whereas all of the heptapeptides are flanked by dibasic (Lys Arg) residues, a single basic residue (Arg) is present at the carboxyl end of the octapeptide. The precursor sequence includes two novel related peptides, myomodulin H (GLHSMLRLa) and myomodulin I (SLSMLRLa). Synthetic myomodulin H and I were tested for bioactivity and found to modulate evoked contractions of the ARC. Probing Northern blots of *Aplysia* nervous system poly(A) RNA at high stringency revealed a single band approximately 1.5 kb in length, suggesting that this clone corresponds to a full length transcript. *In situ* hybridization experiments demonstrate that abundant expression of this messenger RNA occurs in specific neurons in each of the central ganglia. These results indicate that the myomodulins comprise a family of neuropeptides which may participate in diverse neural circuits in *Aplysia*.

523.3

RFa PEPTIDES ARE PRESENT IN THE ARC NEUROMUSCULAR SYSTEM OF *APLYSIA*. E.C. Cropper, F. Vilim, A. Vitek*, M.W. Miller, I. Kupfermann, and K.R. Weiss. Dept. Physiol. & Fishberg Ctr. for Neurobio., Mt. Sinai Med. Schl., NY, NY 10029; Ctr. Neurobio., Columbia Univ., NY, NY 10032.

A striking feature of the ARC neuromuscular system is its chemical complexity. The ARC motor neurons B15 and B16 each contain at least four neuropeptides in addition to their primary neurotransmitter ACh. All four of the B15 peptides appear to be packaged in the same dense core vesicles, and therefore are presumably coreleased. Thus, modulatory effects of the ARC neuropeptides are not likely to be exerted independently. When peptide cotransmission occurs, it is likely that multiple peptides are released, and that released peptides have interactive effects. This suggests that a comprehensive investigation of peptide cotransmission in the ARC neuromuscular system will be difficult unless all major bioactive peptides present in B15 and B16 are characterized. Towards this end, material was extracted from neuronal processes attached to 100 g of ARC muscle and was subjected to RP-HPLC. Bioactive fractions were purified and the following amino acid sequences were obtained: G-G-A-L-F-R-F, G-S-L-F-R-F, and S-T-L-F-R-F. To localize these peptides, and to verify their structures, B15 neurons were incubated in ^{3}H-Phe and chromatographic properties of radiolabeled peptides were compared to those of synthetic amidated versions of the above RFa peptides. B15 radioactivity and synthetic peptides coeluted through sequential chromatography, indicating that B15 contains the novel RFa peptides. All three RFa peptides decrease the size of muscle contractions elicited by motor neuron stimulation, presumably by acting presynaptically. They decrease the size of motor neuron-elicited EJPs, but have no effect on contractions produced by direct application of ACh to the ARC. Thus, a new family of bioactive neuropeptides has been localized to the ARC motor neuron B15.

523.4

SCP AND MYOMODULIN UTILIZE SEPARATE SIGNAL TRANSDUCTION PATHWAYS AND CONVERGE ON COMMON SUBSTRATES IN THE ARC MUSCLE OF *APLYSIA*. S.L. Hooper, W.C. Probst*, I. Kupfermann and K. Weiss. Dept. Phys. & Biophys., Mt. Sinai Sch. of Med., NY NY 10029; Cntr. Neurobiol. & Behav., Columbia Univ. and NYS Psych. Inst., NY NY 10032

The peptides SCP and myomodulin (MM) are contained in the ARC motorneurons B15 and B16 and increase ARC contraction amplitude and relaxation rate. SCP is known to increase ARC cAMP levels; the MM second messenger is unknown, but is not cAMP. Application of pcpt cAMP or forskolin, an adenylyl cyclase activator, resulted in dramatic increases in ARC contraction amplitude and relaxation rate; threshold concentrations for each were *ca.* 10^{-5} M. SCP application and B15 stimulation, but not MM application or B16 stimulation, increased the level of activated cAMP dependent protein kinase (PKA). SCP application to intact ARCs induced dose dependent increases in active PKA in the 12K supernatant of muscle homogenates. The threshold was 10^{-8} M and PKA was maximally activated (a 10 fold increase) at 10^{-5} M SCP, at which point 100% of total PKA was activated. This change was associated with an approximate two fold drop in total (activated + inactive) PKA levels in this fraction, suggesting that SCP translocates PKA to the pellet fraction. Protein phosphorylation was investigated in ARC muscle by back-phosphorylation after treatment with MM and SCP. The ability of cAMP to stimulate incorporation of ^{32}P from γ-^{32}P-ATP into proteins in homogenates of control and peptide treated (before homogenization) ARCs was assessed by SDS-PAGE and autoradiography. The incorporation of radiolabel into three protein bands (300 kD, 57 kD, and 41 kD) was dramatically reduced after treatment of intact muscles with either peptide, indicating increased phosphorylation of these proteins in intact ARC. This change in protein phosphorylation state indicates that even though SCP stimulates cAMP and MM does not, their second messengers converge on the same protein substrates.

523.5

MODULATION OF ION CURRENTS IN THE ARC MUSCLE OF *APLYSIA*: CONVERGENT ACTIONS OF SEROTONIN, SCPs AND MYOMODULINS. V. Brezina, C. G. Evans, I. Kupfermann & K. R. Weiss. Dept. of Physiology & Biophysics, Mount Sinai School of Medicine, New York, NY 10029, and Ctr. for Neurobiology & Behavior, N.Y. State Psychiatric Institute, New York, NY 10032.

Potentiation of feeding movements during food-induced arousal in *Aplysia* is implemented partly by release onto the accessory radula closer (ARC) muscles of small cardioactive peptides (SCPs) and myomodulins (MMs) from the muscles' own motorneurons and serotonin from separate modulatory neurons. Although acting via different second messengers (SCPs and serotonin via cAMP, MMs unknown), all of the modulators similarly increase both the size and the relaxation rate of ARC contractions, and phosphorylate common protein substrates in the muscle (Hooper *et al.*, these abstracts). We have characterized ion currents in voltage-clamped dissociated ARC muscle fibers to determine whether these effects might reflect modulation of a common set of ion channels. Differences in voltage dependence, ion selectivity and pharmacological sensitivity enabled us to distinguish several currents including a hyperpolarization-activated Cl, inwardly rectifying K, 'A'-like K, delayed rectifier-like K, and a depolarization-activated Ca current. Two currents were indeed modulated similarly by each of serotonin, MM and SCPs, as well as by elevation of intracellular cAMP. Modulation of the first, as yet unidentified, current (which could conceivably also represent the activity of an ion pump) appeared as a small outward current shift at potentials more positive than -80 mV. The second current modulated was the Ca current, which we usually studied as inward Ba current in TEA-containing solution; it was activated at potentials more positive than -40 mV, and eliminated by addition of 1 μM nifedipine, 1 mM Cd^{2+}, or replacement of extracellular Ba^{2+} with Co^{2+}. This current was increased by 50-100%, in a mutually occlusive manner, by 1 μM (a supramaximal dose) of each of serotonin, MM and SCP_B, and 50 μM forskolin. This enhancement of Ca current by the modulators may underlie their potentiation of the size of ARC muscle contractions.

523.6

A TYROSINE KINASE RECEPTOR FOR INSULIN IN *APLYSIA* BAG CELLS. D.H. Solomon*, A.M. Elste*, P.J. Bergold and J.H. Schwartz. Center for Neurobiology and Behavior, Howard Hughes Medical Institute, College of Physicians & Surgeons, Columbia University, New York, NY 10032.

In invertebrates, insulin-like peptides have been convincingly localized to neurons, and, in several instances, fulfill some of the criteria expected of neurotransmitters. Much less secure is the status of insulin and insulin-related peptides as neurotransmitters in vertebrate brain. In the marine mollusc *Aplysia*, Shapiro et al (*J. Neurobiol.* **22**, 55-62, 1990) provided evidence that vertebrate insulins can act on certain identified neurons to modulate ion channels through activation of a phospholipase C to release the complex sugar, inositol phosphate glycan (IPG). Using immunocytochemistry with an insulin-receptor α subunit antibody, we find strong immunoreactivity in *Aplysia* bag cell bodies and axon processes that extend into the neuropil of the abdominal ganglion. Immunoblot analysis using this insulin-receptor antibody, which was affinity purified with the peptide immunogen, reveals an M_r 130,000 protein in bag cell extracts similar in size to the *Drosophila* insulin-receptor α subunit (the α subunit is the extracellular insulin-binding component of the receptor). Using the polymerase chain reaction (PCR) with degenerative primers spanning a conserved domain in human and *Drosophila* insulin receptors, we obtained a partial cDNA sequence from bag cells with an inferred amino acid sequence similar to the tyrosine kinase domain of human and *Drosophila* insulin receptors. These two results suggest that both extracellular and intracellular domains of an insulin-type receptor are present in *Aplysia*. Our working hypothesis is that, in addition to their roles as growth factors and hormones, insulin-like peptides can function as peptide transmitters to modulate neuronal function through tyrosine specific protein phosphorylation.

523.7

FURTHER CHARACTERIZATION OF NEUROPEPTIDES THAT MIMIC PROLONGED INHIBITION OF BAG CELL TARGET NEURONS IN APLYSIA. S.M. Rajpara*, J.C. Eliassen* and E. Mayeri. Department of Physiology, University of California, San Francisco, CA 94143

The bag cell neurons of *Aplysia* are part of a neural system that utilizes four peptides as neurotransmitters. The peptides are derived from the egg-laying hormone/bag cell peptide (ELH/BCP) precursor and produce various effects in abdominal ganglion neurons following initiation of a bag cell burst discharge. In cells L3 and L6, bag cell activity produces inhibition that lasts for 2 or more hours. α-BCP mediates an early component of this prolonged inhibition.

To further characterize two peptides that are candidates for mediating the prolonged component of inhibition, we purified material from an acid extract of abdominal ganglia by molecular sizing HPLC, followed by one or more steps of reverse-phase HPLC on C4. One peptide is 4687 Da and consists of 40 amino acids, as determined by mass spectroscopy and amino acid composition analysis. A two min. application of the peptide at a concentration of 4 μM produced sustained hyperpolarization and a reduction in spontaneous spike activity that lasted 100 min. The second peptide has an apparent molecular weight of 3300 Da and was 5-fold less potent. Amino acid composition analysis suggests that neither peptide is derived from the ELH/BCP precursor. Since the 4687 Da peptide is effective at a lower concentration, it is the best candidate for the true inhibitory transmitter.

Supported by NIH grants NS 16490 and NS 16033.

523.8

MODULATION OF NEUROMUSCULAR EFFICACY BY EXCITATORY AND INHIBITORY MOTOR NEURONS IN *APLYSIA*. P. J. Church and P. E. Lloyd. Committee on Neurobiology, The University of Chicago, Chicago, Il 60637.

Buccal muscle I3a is innervated by two excitatory motor neurons, B3 and B38 and a newly identified inhibitory neuron, B47. B3, B38, and B47 synthesized FMRFamide, the SCPs, and myomodulin (Mm) respectively. Bath application of FMRFamide, SCPs or Mm (10nM) enhanced B3-evoked contractions. We monitored the modulation of B3-evoked muscle contractions in response to stimulation of B38 and B47 using paradigms designed to release conventional transmitters alone and with peptide cotransmitters. When B38 was stimulated in high frequency bursts during B3 interburst intervals, subsequent B3-evoked contractions were enhanced. We interpret these results as B38 releasing its peptide modulatory cotransmitters (SCPs) during high frequency bursts. Stimulation of B47 simultaneous with B3 inhibited the B3-evoked contractions. However, high frequency B47 stimulation during B3 interburst intervals enhanced subsequent B3-evoked contractions. It appears that B47 can have different effects on neuronally evoked muscle contractions depending on the temporal characteristics of stimulation. We interpret these results as B47 releasing its inhibitory conventional transmitter alone during low frequency bursts and along with its peptide cotransmitter (Mm) during high frequency bursts. Supported by NS 23569.

523.9

SEROTONIN AND FMRFamide INHIBIT THE RELEASE OF NEUROPEPTIDES FROM IDENTIFIED *APLYSIA* NEURONS IN CULTURE. M.D. Whim and P.E. Lloyd. Dept. Pharm. Physiol. Sci. & Comm. on Neurobiol. Univ. Chicago, Chicago, IL 60637.

To examine the modulation of the release of neuropeptide cotransmitters we have used *Aplysia* neurons B1 and B2 in primary culture which incorporate ^{35}S-met into 2 neuropeptides (SCPA and SCPB). The SCPs are transported into the regenerated neurites, and can be shown to be released in a stimulus- and Ca-dependent fashion (Lloyd et al, 1986). Initial experiments determined that stimulating individual B1 or B2s using intracellular microelectrodes at a frequency of 6 Hz for 500 msec with 500 msec interburst intervals, evoked a reproducible release of radiolabeled SCPs into the bathing solution which could be detected using a combination of HPLC and liquid scintillation counting. This release showed only a small decline wth repetitive stimulation periods. Bath application of FMRFamide and serotonin both transiently inhibited release. 1μM FMRFamide reduced the release of the SCPs to less than 5% of control. The effect of serotonin was dose-responsive, with a ~60% inhibition of release at 100μM. Although serotonin inhibited the release of the SCPs, it increased excitability as monitored by the number of spikes evoked by depolarizing current pulses. We are currently examining the possibility that the effects of serotonin are mediated via the actions of a second messenger. Supported by NS 23569.

523.10

NEURON SPECIFIC SPLICING OF FMRFamide GENE TRANSCRIPTS IN THE MOLLUSC *LYMNAEA*. J.F. Burke, S.E. Saunders*, K. Bright*, E. Kellett*, N. Santama* and P. Benjamin*. Sussex Neuroscience Research Centre, Biological Sciences, University of Sussex, Brighton, E. Sussex BN1 9QG, (U.K.)

The FMRFamide gene of *Lymnea* contains at least three exons. One encodes multiple copies of the cardioactive neuropeptide FMRFamide, another encodes the N terminal extended peptides GDP/SDPFLRFamide and the third exon encodes a common hydrophobic leader sequence. *In situ* hybridization data show that expression of the gene is limited to a discrete number of cells located throughout the major ganglia of the brain. The hydrophobic leader is expressed in all cells whereas the neuropeptide encoding exons are alternatively spliced. The neuropeptide encoding exon splicing pattern is mutually exclusive, such that a single neuron only expresses one class of neuropeptide.

As well as encoding the FMRFamide and GDP/SDPFLRFamide the neuropeptide precursor proteins encode a number of other potential peptides including EFLRIamide, EFFPLamide and a peptide ending in the C terminal sequence SEEPLY. Immunocytological studies will be presented showing that these peptides are syntheized in the brain of *Lymnaea*, and that in some cases they can account for the physiological activites of single identified neurons.

523.11

DISTRIBUTION OF THE NEUROPEPTIDE APGW-NH_2 IN THE CENTRAL NERVOUS SYSTEM AND MALE REPRODUCTIVE ORGANS OF *LYMNAEA STAGNALIS*. J. van Minnen, A.B. Smit* and R.P. Croll[1]. Faculty of Biology, Vrije Universiteit, 1081 HV Amsterdam, The Netherlands and [1]Department of Physiology & Biophysics, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4H7.

Recently, a gene has been cloned from neurons located in the anterior lobes of the cerebral ganglia of *Lymnaea stagnalis*.. The product of this gene is processed to become 10 copies of the neuropeptide APGW-NH_2 and a single copy of a 40 amino-acid polypeptide. The present study describes the distribution of neurons expressing the APGW-NH_2 gene by means of *in situ* hybridization and immunocytochemistry in the CNS and male reproductive organs. The majority of the neurons expressing APGW-NH_2 is located in the anterior lobes of the cerebral ganglia. The right lobe, which consists largely of motoneurons innervating the penis, contains 120-150 APGW-NH_2 neurons whereas the smaller left lobe, which possesses no penial motoneurons, contains only 15-20 APGW-NH_2 neurons. In addition, clusters of 4-6 small neurons are also found in the right parietal and visceral ganglia, and a total of 10-15 other cells are scattered elsewhere within the CNS. Neurites containing APGW-NH_2 can be traced throughout the CNS and into the periphery with particularly strong projections to the penial complex, vas deferens and prostate gland.

This study indicates a general, important role of APGW-NH_2 in the functioning of the gastropod nervous system. Furthermore, comparative studies on other molluscan species (*e.g.Helix, Aplysia, Mytilus*)indicate many similarities in APGW-NH_2 containing neurons and peripheral projections to male reproductive organs and suggest evolutionary conservation in the use of this neuropeptide.

524.1

THE ROLE OF HORMONES IN REGULATING THE MITOTIC ACTIVITY OF NEUROBLASTS IN THE MOTH, *MANDUCA SEXTA*. R. Booker and J. Kim Section of Neurobiology and Behavior, Cornell Univ., Ithaca, NY 14853.

The neurons generated by postembryonic neuroblasts (NBs) play an important role in shaping the nervous system of the adult *Manduca*. Each NB generates a discrete nest of progeny. The NBs are individually identifiable, and each NB generates a characteristic number of progeny, ranging from 10 to more than 100. We examined mitotic activity of the NBs, using immunocytochemical detection of the thymidine analog 5-Bromodeoxyuridine, in combination with a series of endocrine manipulations.

The mitotic activity of the NBs was not uniform throughout the larval stage. There was little change in the mitotic activity of the NBs throughout the feeding larval stage. However, at the onset of metamorphosis, which occurs on the first day of wondering (W-0), there was a 10 fold decline in the number of labeled cells counted within the nests. The next day (W-1) the mitotic rate of all the NBs rebounded.

A series of ligation experiments were used to isolate the NBs from the sources of hormones. The results suggest that the dramatic drop in mitotic activity on W-0 was induced by the small peak of ecdysteroids which occurs on the third day of the fifth larval instar (5-3) and marks the first time the ecdysteroids occur in the absence of the juvenile hormones (JH). The dramatic drop in NB activity was blocked by the application of JH mimics (JHM) to intact 5-3 animals. Instead of a rapid fall, the mitotic activity of the NBs dropped slowly over a period of 8 days in the JHM treated animals. The rebounding of NB mitotic activity on W-1 was correlated with the appearance of the large prepupal peak of ecdysteroids. Ligating abdomens on W-0 prevented this increase in mitotic activity. Replacing the ecdysteroids with an infusion of 20-hydroxyecdysone (20-HE) dramatically increased the mitotic activity of the NBs. However, infusion of 20-HE into animals treated for 14 days with JHM, which prevents the cessation of mitotic activty triggered by the small peak of ecdysteroids, did not result in an increase in mitotic activity. This suggests that the number of progeny a NB generates is determined by intrinsic cues. However, our endocrine manipulations suggest that hormones regulate the mitotic activtiy of the NBs at the onset of metamorphosis developmental.

524.2

CLONAL ANALYSIS SHOWS *NOTCH* IS REQUIRED FOR NORMAL SENSILLAR DEVELOPMENT IN THE WING OF *DROSOPHILA*. E.Rulifson and S.S.Blair. Neuroscience Training Program and Dept. Zoology, U. Wisconsin, Madison, WI 53706.

The techniques of mitotic recombination and enhancer-trap based cell marking were used to generate mosaic clones of a null allele of the *Notch* gene in the wing of *Drosophila*. It is thought that cell-cell interactions are required for the proper segregation of neural and epidermal lineages during embryonic and larval development. Clonal studies of *Notch* in the differentiation of bristles on the notum support the idea that *Notch* protein is a receptor for a lateral inhibitory signal; this signal is required to produce the normal number of correctly positioned sensilla within fields of competent cells. Our preliminary results showed that mutant clones of *Notch* in the wing produced supernumerary sensillar precursors surrounding the positions of normal campaniform sensilla. However, clones of mutant cells in positions not normally containing campaniform sensilla differentiated as epithelium. At later stages, after sensillar cells become immunoreactive for anti-HRP, clones of mutant cells were seen to contain clusters of supernumerary neurons. Thus, in the wing, as in the notum, *Notch* may be required by competent cells for the generation of the correct pattern of sensilla.

524.3

ANALYSIS OF A DROSOPHILA GENE CLUSTER EXPRESSED DURING CNS DEVELOPMENT. S. D. Zhang*, D. Mellerick-Dressler*, H. Gainer, W. Odenwald* and J. Kassis*[1]. Lab. of Neurochemistry, NINDS, NIH and [1]Ctr for Biologics Evaluation and Research, FDA. Bethesda, MD 20892

Enhancer-detection screening has led to the identification of a gene cluster located at 83c which is active during Drosophila neurogenesis. Thus far, two non-overlapping genes (*83c alpha* and *beta*) that are transcribed in converging directions have been partially characterized.

Genomic and cDNA analysis indicates the *83c alpha* gene may encode a transcription factor. The deduced protein sequence contains an acidic domain and four linked TF-IIIA -like zinc fingers. Whole mount *in situ* hybridizations reveal that the *83c alpha* gene is first activated at the time of neuroblast formation in clusters of cells within the cephalic neurogenic region. Simultaneously, *83c alpha* expression is also turned on in groups of three to four cells located in the ventral cord midline. Each group is positioned at the anterior portion of each parasegment. During ventral cord development the *83c alpha* gene displays successive anterior to posterior bilaterally symmetrical waves of activation in cells flanking the ventral midline. These cells may be neuroblasts. Late in CNS development there is an overall decrease in *83c alpha* gene expression in both the cephalic lobes and in the ventral cord. Except for four to six cells along the posterior boundary of the contracted ventral cord, no transcripts are detected in the abdominal ventral cord.

Sequence analysis of genomic and cDNA *83c beta* clones identifies an open reading frame of at least 2Kb. The encoded protein bears no homologies to previously described proteins. *83c beta* transcripts are detected in early embryos; suggesting this gene is maternally expressed. Late in embryonic development *83c beta* is expressed throughout the CNS. However, unlike *83c alpha*, expression of *83c beta* is not limited to the CNS.

524.4

POSTEMBRYONIC NEUROGENESIS AND DEVELOPMENT OF THE ANTENNAL LOBE IN THE TOBACCO HORNWORM *MANDUCA SEXTA*. K.A. Sorensen, N.T. Davis, and J.G. Hildebrand. ARL Division of Neurobiology, University of Arizona, Tucson, AZ 85721.

Manduca sexta exhibits dramatic changes in its appearance and behavior during its metamorphosis from larva to adult. These changes are accompanied by extensive reorganization of the nervous system, involving the death and remodelling of subsets of larval neurons as well as the postembryonic addition of new neurons. We are studying the origins of neurons in the primary olfactory center, the antennal lobe, in the brain of *M. sexta*. We previously identified 5 neuroblast nests (3 lateral, A-C, and 2 medial, D,E) that, based on their positions adjacent to the larval antennal center, appeared likely to contribute to the reorganization of this structure into the adult antennal lobe. We are now interested in determining how these 5 nests relate to the 3 groups of neurons (lateral, medial, and anterior) found in the adult antennal lobe.

To determine the contribution of these neuroblast nests to development of the adult antennal lobe, successive stages of prepupal and pupal development were examined in histological and histochemical preparations. Cell death is evident within the nests from stage W0-W2; nest-cell processes appear as prominent tracts leading from each nest to olfactory neuropil from stage W3 forward. The olfactory neuropil remains larval in character (each nest having its own discrete tract and characteristic point of entry into the olfactory neuropil) until stage P2, after which somata and tracts associated with each of the lateral nests (A-C) appear to coalesce, forming a characteristic adult lateral cell group and input tract by stage P4. Medial nests retain their orientations and input tracts relative to the neuropil during metamorphosis, with nest E appearing to contribute to the medial cell group of the adult antennal lobe and nest D, to the anterior cell group. [Supported by NIH grant AI-23253]

524.5

CRITICAL PERIOD OF INTERACTIONS LEADING TO THE BIRTH OF PERIPHERALLY INDUCED CENTRAL (PIC) NEURONS DURING LATE EMBRYOGENESIS IN THE LEECH , *HIRUDO MEDICINALIS*. Thomas Becker and Eduardo R. Macagno. Dept. of Biological Sciences, Fairchild Center, Columbia University, NY, NY 10027.

PIC neurons are present only in the sex ganglia (those in the fifth and sixth body segments) in *Hirudo*. They appear late in embryogenesis as a result of an inductive interaction between the male genitalia and the CNS (Baptista et al., Nature **346**, 855-858; 1990). The induction requires intact nerves connecting the male genitalia and the sex ganglia. Male organ removal prior to embryonic day 13 (E13) prevents the appearance of PIC's entirely. Conversely, at E16, the removal of the peripheral target no longer prevents the birth of PIC's (Baptista and Macagno, J. Neurobiol. **19**, 707-726; 1988). To explore the basis of this critical period of PIC induction, we performed a series of male organ ablations at E10-11, followed by back-transplantation of male organs at later stages from donors of the same age. PIC neurons only appeared in animals that received a back-transplant up to E16. To test whether this resulted from the loss of inductive capacity of male organs after E16, as opposed to a loss of CNS responsiveness, we performed heterochronic transplantations. Organs from animals up to E40 were able to induce PIC's in E13-16 hosts, but those from older donors appeared to have lost this ability. Back-transplantation of 11-day-old male organs into animals older than 16 days that had their male organ removed at E10 never resulted in the appearance of PIC cells. We conclude (1) that the interaction can take place in as short a time as one day and (2) that the sex ganglia lose their sensitivity to the inductive signal after day 16, although the male organ retains its ability to induce up to E 40.

524.6

REGULATION OF NEURONAL NUMBER AND SIZE IN FROGS DEVELOPING FROM HALF EMBRYOS. Francesca Mariani* and Margaret Hollyday. Department of Biology, Bryn Mawr College, Bryn Mawr PA 19010.

The developmental processes which determine neuron number and its relation to body size are not well understood. To further explore this issue, we studied embryos developing from fertilized eggs of Xenopus laevis which were divided at the two-cell stage. Single blastomeres were isolated using microsurgical techniques. Detailed observations were made of sets of experimental embryos and their matched controls to compare cleavage patterns, rate of development and growth. Embryos were raised until they showed signs of impending death; they were then processed for light microscopic analysis. Quantitative estimates of body size were estimated from stained serial sections using a camera lucida attachment and digitizing tablet; cells counts were made of selected sections to estimate total neuron number. The oldest embryos studied to date were at stage 48-49 of Nieuwkoop and Faber.

Experimental embryos showed altered early cleavage patterns; the isolated blastomere displayed an additional, vertically oriented cleavage division before cleaving horizontally. Nevertheless, both experimental and control embryos underwent neurulation at the same time, despite the diminished size of the experimental embryo. The experimental embryos were consistently smaller than their matched controls thoughout the observation period. Analysis of body size and total neuronal number in the oldest tadpoles revealed that experimental embryos were approximately half-sized compared to control embryos and that their nervous systems contained half the number of neurons as did controls. These preliminary results suggest that both body size and neuronal cell number are heavily influenced by the volume of cytoplasm in the egg.

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524.7

A TEMPORAL AND SPATIAL SHIFT IN THE REGULATION OF NEUROGENESIS. R.D.Heathcote and M.G.Larson. Department of Biological Sciences, University of Wisconsin, Box 413, Milwaukee, WI, 53201.

Environmental cues are known to influence the differentiation of neural crest cells and could also influence their genesis. We examined neurogenesis in a ganglion whose neurons continued to accumulate throughout a prolonged period of development. The cardiac ganglion of the frog *Xenopus laevis* contained a single type of neuron whose precursors continued to divide and differentiate long after neural crest migration had ceased. We located a source of these late neuronal precursors and determined that their proliferative ability differed from precursors that had just completed migration from the neural crest.

In order for new neurons to continue to be added to the ganglion, the source of neuronal precursors must shift from the neural crest to another site early in ganglion formation. When hearts were isolated in culture in the presence of ^{3}H-thymidine, some cells incorporated label into their DNA, divided and became neurons. Thus precursors to cardiac neurons were active in a germinal zone that included the heart. The neuronal precursors in the heart continue dividing long after precursors from the neural crest supply the first neurons to the ganglion. Regulation of proliferation in these 2 sets of precursors appeared to be different. Cytosine arabinoside (araC), blocked thymidine uptake (and cell division), but had different effects on the 2 types of neuronal precursors. When hearts from animals whose cells had just arrived from the neural crest were cultured in araC, they contained half the number of neurons of control hearts (cultured without araC). Following neural crest migration, araC had no effect on the number of neurons in cultured hearts. Thus, precursors from older hearts maintained in our culture conditions were not capable of the same amount of neurogenesis as precursors that had just arrived from the migrating neural crest. This difference likely reflected changes in the mechanisms regulating neurogenesis at these 2 different times and locations.

524.8

BIRTH AND MIGRATION OF CELLS IN THE CENTRAL POSTERIOR NUCLEUS OF WEAKLY ELECTRIC KNIFEFISH *(EIGENMANNIA SP.)* **DURING ADULTHOOD.** G.K.H. Zupanc[1,2] and M.M. Zupanc*[3]. [1]Scripps Institution of Oceanography, [2]Department of Neurosciences, and [3]Department of Biology, University of California at San Diego, La Jolla, CA 92093.

In *Eigenmannia sp.*, the central posterior nucleus (CP) is a bilateral diencephalic cell group, stretching from the third ventricle approximately 400 μm laterally. At its lateral extent, the CP fuses with the prepacemaker nucleus (PPn), which controls frequency modulations of the electric organ discharge. Recent morphological data have suggested that the PPn is much larger than assumed in earlier studies (e.g., M. Kawasaki *et al.*, J. Comp. Neurol. *276:* 113-131, 1988) and that it incorporates at least part of the traditionally defined CP (G.K.H. Zupanc and W. Heiligenberg, submitted). Furthermore, experiments employing 5-bromo-2'-deoxyuridine (a thymidine analogue which is incorporated into replicating DNA and which enables the detection of mitotic S-phase cells) in adult knifefish have revealed the generation of approximately 20-50 new cells per 12 hrs unilaterally in the periventricular zone of the CP (G.K.H. Zupanc, Soc. Neurosci. Abstr. *16:*128, 1990). In the present autoradiographic study, we examine the fate of these newborn cells.

Fish were injected with ^{3}H-thymidine and sacrificed after various lengths of survival time. While ^{3}H-thymidine labelling was highly concentrated in the periventricular zone after 12 hrs (m, median distance of labelled cells from ventricle = 8 μm; n = 135 cells examined), newborn cells appeared to migrate laterally on the following days (day 2: m = 17 μm, n = 106; day 4: m = 27 μm, n = 41). The median distance of labelled cells from the ventricle reached a plateau after about one week (day 5: m = 29 μm, n = 177; day 7: m = 29, n = 111; day 28: m = 29 μm, n = 38). Light and electron microscopic observations suggest that at least part of the newly generated elongated periventricular cells differentiate into round or ovoidal neurons of the CP during their lateral migration.

524.9

A NOVEL MARKER FOR CORTICAL SUBPLATE NEURONS AND SOME SUBVENTRICULAR ZONE CELLS IN EARLY POSTNATAL RAT BRAIN. Z-Y. Yu and J.E. Bottenstein. Marine Biomedical Institute, University of Texas Medical Branch in Galveston, Galveston, TX 77550

Our previous work has shown that conditioned medium (CM) from the B104 CNS neuronal cell line increases proliferation of O-2A glial progenitors and immature oligodendrocytes. We generated mouse monoclonal antibodies (mAbs) against a partially purified heparin-binding fraction of the CM from this cell line. One of these antibodies (AC3) was used in combination with mouse mAbs against 68 kD neurofilament protein (NF; a neuronal marker) or against G_{D3} ganglioside (a marker for immature neuroepithelial cells) to identify the cells expressing the AC3 antigen. A double immunoperoxidase method was used on 50 μm vibratome sections of early postnatal rat brain. Strong AC3 immunoreactivity was observed within cells of the **cortical subplate** in P0-1 and P7 (P, postnatal day) rat forebrain, but it disappeared by P14. The majority of these AC3-positive cells double stained with NF mAbs, indicating they were neurons. In the **subventricular zone (SVZ)**, especially in the medial portion beneath the lateral ventricle, strong AC3 immunoreactivity was detected at P0-1 and P7, which was weaker at P14. An apparent lateral migration of AC3-positive SVZ cells was seen at P7 and P14. Only a few AC3-positive cells in this region were double stained by G_{D3} mAbs. However, strongly G_{D3}-immunoreactive cells were observed in the cingulum, corpus callosum, and other parts of the SVZ. No double staining was detected for AC3 and G_{D3} in the cingulum, corpus callosum, or cortical subplate. Our results indicate that AC3 mAbs provide a novel marker for cortical subplate neurons and for some SVZ cells and may be useful in studies of the maturation and migration of cells in rat brain.

524.10

NOVEL cDNA CLONES DEFINE DEVELOPMENTAL SUBPOPULATIONS OF GRANULE CELLS IN MOUSE CEREBELLUM. S.G.Kuhar[1], L.Feng[1], S.Vidan[2], M.E.Ross[3], M.E.Hatten[2] and N.Heintz[1]. [1]Rockefeller Univ., New York, NY 10021, [2]Columbia Univ., New York, NY 10032,[3]Univ. of Minnesota, Minneapolis, MN 55455

The development of the mammalian cerebellum involves a complex system of cell:cell interactions which are controlled by both intrinsic genetic programs and epigenetic factors. To define the molecular events underlying cerebellar development, we have initiated a search for genes which mark specific stages in granule cell (GC) differentiation. Polyclonal antisera made in rabbits against purified postnatal days (P) 3-5 GC was adsorbed on PC12 cells and adult cerebellar tissue and used to screen a cDNA library made in λgt11 from purified P3-P5 GC (Ross et al., Soc. Neurosci. Abs., Vol.16 Pt.I p.151, 1990). Of 58 purified phage clones, 42 were found to be unique by restriction enzyme analysis of PCR amplified inserts. Search of all GenBank entries with 100-500 nucleotides of 5' sequence showed 36 novel clones. Northern blots of poly A(+) RNA made from cerebella of different aged mice and from various tissues have shown that six of the 26 clones analyzed thus far are developmentally regulated. Five of these six are brain-specific. In situ hybridization has shown localized expression of several of these subclones to cellular subpopulations in the mouse P8 cerebellum. For example, clones whose expression is restricted to superficial layers of the EGL, or to the outermost cells of the IGL have been identified. Our present efforts are directed toward identifying which of these novel genes require specific cell:cell interactions for expression, and toward understanding their role in cerebellar development.

524.11

MONOCLONAL ANTIBODIES AGAINST CELL NUCLEI IN THE DEVELOPING CHICK NERVOUS SYSTEM. A. M. Smith, L.C. Ellis* and T. N. Parks. Dept. of Anatomy, Univ. of Utah Sch. Med., Salt Lake City, UT 84132.

Recent studies have demonstrated that a subset of nuclear proteins which bind DNA are involved in the regulation of gene expression during development. It is our long-range goal to identify and characterize these proteins in the chick nervous system. Using both affinity chromatography to isolate DNA-binding proteins and hybridoma technology, we are producing monoclonal antibodies against cell nuclear proteins in the nervous system of the chick. We report here on four MAbs which were made against mouse brain nuclear protein (A60, A61, A18, F41; Smith et al., *Soc.Neurosci.Abstr. 16:* 642, 1990) and found to cross-react in the chick and two new MAbs (1F5 and 2D4) which stain chick neuronal cell nuclei. The new MAbs were produced by immunizing mice with protein isolated from chick brain nuclei. Total soluble nuclear protein was loaded onto a DNA-cellulose column and eluted with high molar salt. Silver stained gels of eluted fractions showed that most proteins were in the flow-through fraction, but that high salt elutions contained proteins predominantly in the 35-70 kDa range. Both 1F5 and 2D4 stain most neuronal nuclei in the chick brain as early as embryonic day (E) 19. Further immunohistochemical studies of several stages of development, as well as the results of immunoblot analyses will be presented. The four other MAbs all stain neuronal cell nuclei in the chick. The cognate antigens for A60 and A61 bind to a DNA cellulose column and are eluted with high molar salt, supporting our hypothesis that these proteins are DNA-binding proteins. A60 stains only neurons in the chick CNS and PNS and is detected as early as E2 in a few cells in the neural tube. With the exception of Purkinje cells, A60 staining is detected in most neurons by posthatching day (P) 5. A61 is detected as early as E1 and stains most cell nuclei at this stage. Later in development, A61 stains neurons and glia as well as several other cell types. Monoclonal antibodies A18 and F41 stain only neurons in the developing chick brain and are both detected as early as E10. Immunoblot analysis has been performed for these four antibodies in the chick and will be presented.

Supported by USPHS grant DC00144.

524.12

BIOCHEMICAL AND MOLECULAR CHARACTERIZATION OF THE 5A11 ANTIGEN, A NOVEL ADHESION MOLECULE OF THE NEURAL RETINA. P.J. Linser, J.M. Fadool and M.J Greenberg. The Whitney Laboratory and The Department of Anatomy and Cell Biology, University of Florida, St. Augustine, FL 32086.

The 5A11 antigen is a unique adhesion molecule identified in the neural retina that participates in neuronal-glial interactions. Three antibody probes have been prepared to this antigen to facilitate its characterization during retina development. Immunohistochemical analysis shows that the antigen is ubiquitous on retina cell plasma membranes early in development but later becomes restricted to Muller glial cells, photoreceptor cells and the pigmented epithelium. By Western blot analysis, all antibodies identify a doublet at 43-45kDa with and without reduction. An additional band at 68kDa is also recognized by the probes. Lectin and endoglycosidase analyses of the immunopurified antigen revealed several characteristics of the glycoprotein antigen: the antigen contains complex N-linked carbohydrates that comprise about 25% of the apparent molecular weight of the 43-45kDa doublet; the 43 and 45 kDa forms differ by the degree of sialation; differential digestions showed the presence of at least three biantennary carbohydrate moieties; the 68kDa band seems to be distinct from the others in its glycosilation and peptide core. Microsequencing of immunoisolated 43-45kDa material has produced sequences that show little homology with sequences in the available data bases. PCR was used to produce and then clone additional sequence from retina mRNA. Again the sequence appears to be unique.

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525.1

Delayed Phosphorylation Of GAP-43 During Neurite Outgrowth in a Mouse Neuroblastoma Line J.T. Megerian, K.F. Meiri[1], W.L. Klein, Northwestern Univ. Inst. Neurosci, Evanston, IL 60208; Dept. Pharmacology, [1]SUNY Health Science Center, Syracuse, N.Y. 13210.

GAP-43 (aka neuromodulin, B50, pp46, F1), originally identified as an 'axonal growth associated protein', is a protein kinase C substrate thought to be involved in synaptic plasticity, neurite outgrowth, and neurotransmitter release (reviewed by Coggins P.J. & Zwiers, H., J. Neurochem. 1991 56:1095-1106). Previous studies from this laboratory using cell lines and primary nerve cultures have indicated that GAP-43 expression is neither necessary nor predictive for neurite outgrowth (Megerian & Klein, Soc Neurosci Abs, 1990; 1989). Others have reached similar conclusions (Baetge & Hammang, Neuron 1991, 6:21-30). Recently, Meiri et al (J. Cell Biol. 1991, 112(5):991-1005) have developed a panel of phospho-specific antibodies and have used them in vivo to show that phosphorylation of GAP-43 is temporally/spatially restricted to distal neurites in proximity of their targets. Using these same antibodies, we show that, in vitro, phosphorylated GAP-43 is absent from newly differentiating mouse neuroblastoma cells, despite an abundance of unphosphorylated GAP-43 in both differentiated and undifferentiated cells. Phospho-GAP is observed only in more differentiated cultures, in those cells having very long neurites. In these cells, P-GAP is punctate, localized to the distal neurite segment and is most prominent in the growth cone. Many of the growth cones containing P-GAP are in contact with other neurites and/or growth cones, suggesting that contact triggers GAP-43 phosphorylation. These results, along with our previous findings that GAP-43 is absent in early neuritogenesis, indicate that the protein has a late occurring function in neurite elongation, consistent with a role in growth cone-neurite interaction.

525.2

POST-TRANSCRIPTIONAL REGULATION OF GAP-43 mRNA LEVELS DURING NERVE GROWTH. L. H. Lin, B. Costello*, J. A. Freeman and J. J. Norden, Dept. of Cell Biology, Vanderbilt University, Nashville, TN 37232.

Elevation of GAP-43 expression has been correlated with neuronal growth states. A number of agents, including NGF, FGF, EGF, dBcAMP, KCl and TPA were reported to stimulate GAP-43 expression in PC12 cells. The regulation of GAP-43 expression, however, is not well understood. Nuclear runoff assays were performed in order to determine if the elevated GAP-43 mRNA levels in stimulated PC12 cells and developing rat brain arise via an increased transcription rate. PC12 cells were treated with NGF, FGF, EGF, dBcAMP, KCl, TPA or DEX for 6 hrs. None of the agents tested had any effect on GAP-43 transcription rate. The same results were obtained from PC12 cells treated with 4-day-old rat brain extract. When the transcription rate of GAP-43 mRNA of 4-day-old rat brain was compared to that of adult, no differences were noted. We then measured mRNA levels in PC12 cells treated with NGF, FGF or dBcAMP for 1 day, followed by treatment with the transcription inhibitor actinomycin D, with or without effector, for a second day. NGF was found to increase message stability. FGF and dBcAMP had no effect. Our data indicate that GAP-43 mRNA levels are regulated post-transcriptionally, both in PC12 cells and in developing rat brain. NGF stimulation in PC12 cells involves message stabilization whereas FGF and dBcAMP stimulation involve other post-transcriptional mechanism(s). (Supported by NIH Grants NS25150 to JJN and NS18103 to JAF)

525.3

NEURON-SPECIFIC PROMOTER CONSTRUCTS FOR OVEREXPRESSION OF B-50(GAP43) IN TRANSGENIC MICE.
J. Verhaagen[1], J.J. Plaizier[1], J.A.G. van Roon[1], J. Oberdick[2], J. I. Morgan[2], S. Forss-Petter[3], F. L. Margolis[2] [1]Rudolf Magnus Institute, Univ. Utrecht, NL,[2]Roche 8Inst. Molec. Biol. Nutley, NJ,[3]Scripps Clinic, La Jolla, CA.

A fundamental question regarding the function of the growth associated protein B-50 is: Is B-50 a determining factor in nerve fiber formation during neurodevelopment and after nerve damage? As a first necessary step to address this question in vivo three neurons-pecific promoter constructs have been prepared to study the effects of overexpression of B-50 in transgenic mice: 1. The olfactory marker protein (OMP) coding sequence in a 11kb OMP genomic clone was replaced by the B-50 coding sequence. With this construct B-50 can be targeted to mature olfactory neurons, cells that do not normally express B-50. The effect of B-50 expression in mature olfactory neurons on the lifespan of these neurons will be studied. 2. The Purkinje cell/retinal bipolar neuron specific L7 protein promoter has been fused to the B-50 coding sequence. This enables the study of the effect of B-50 overexpression on the development of these particular neurons in an otherwise unchanged brain. 3. A neuron-specific enolase (NSE)-B-50 transgene was prepared to express B-50 in virtually all neurons during adulthood. Since B-50 levels normally decline as development of the nervous system proceeds, NSE-B-50 transgenic animals offer an opportunity to study regeneration and synaptic plasticity in a number of CNS and PNS structures containing higher levels of B-50 than in non-transgenic mice.

525.4

APPROACH TO STUDY GAP-43 FUNCTION BY HOMOLOGOUS RECOMBINATION-MEDIATED GENE TARGETING IN EMBRYONIC STEM CELLS. J.D. Pollock, S.J. Engle*, J.A. Tischfield*, and L. Yu. Dept. of Med. & Mol. Genetics, Indiana Univ. Sch. of Med., Indianapolis, IN 46202.

GAP-43 (also known as neuromodulin and F1) is a neural specific phosphoprotein enriched in growth cones and axons. GAP-43 synthesis is increased during neuronal outgrowth and during axonal regeneration. It is phosphorylated during long-term potentiation in the hippocampus, suggesting its possible association with learning. However, despite many studies, little is known about the physiological role of GAP-43.

One way to analyze GAP-43's function is to generate a null mutation of the endogenous GAP-43 gene in animals and to observe the effects of such mutation. The development of the technique of gene targeting in embryonic stem cells via homologous recombination by Capecchi, Evans, and Smithies's groups makes it possible to achieve this goal. Using a GAP-43 cDNA clone as probe, we isolated genomic clones of the mouse GAP-43 gene. Two targeting vectors were constructed which contain thymidine kinase genes flanking the second exon of the GAP-43 gene. In one vector the exon is interrupted by the insertion of a neomycin-resistance gene, and in the other a lacZ gene is fused to the neomycin-resistance gene. Mouse embryonic stem cells are transformed with these vectors to generate targeted null mutation in the GAP-43 gene.

525.5

LOCAL REGULATION OF GAP-43 PHOSPHORYLATION IN GROWTH CONES OF NEURITES REGENERATING IN CULTURE. E.W.Dent* and K.F. Meiri, Dept. Pharmacology, SUNY Health Science Center, Syracuse, NY13210.

The nervous-system specific protein GAP-43 is highly enriched in the membrane skeletons of growth cones where it is a major substrate for protein kinase C. Previously, we have shown that induction of GAP-43 phosphorylation is precisely regulated during axonogenesis *in vivo* (Meiri et al., J.C.B., 112, 991-1005, 1991). Here we have used embryonic DRG neurons *in vitro* to study the regulation of GAP-43 phosphorylation between and within individual growth cones. Two major findings emerge: First, when dissociated E16-20 DRGS are cultured, GAP-43 phosphorylation is initially switched off and only gradually reestablishes its prior *in vivo* levels by about 20 hours, by which time a dense plexus of neurites has already been generated. This down-regulation does not reflect either levels or activity of kinase C and cannot be ameliorated with excess NGF, phorbol esters or conditioned medium from long-term cultures. Second, Phosphorylated GAP-43 has a restricted distribution in individual growth cones: Phospho-GAP-43 colocalizes with actin at the ruffling edge of lamellae and is also highly concentrated in filopodia. Additionally, discrete phospho-GAP-43 'hot spots' may appear within the body of individual growth cones. These results, which show that the induction of GAP-43 phosphorylation seen during development is also recapitulated during regeneration, and further confirm that phospho-GAP-43 is not required for initial stages of axon outgrowth, also demonstrate the local regulation of GAP-43 phosphorylation in individual growth cones.
Supported by NS 26091 and the March of Dimes.

525.6

ABNORMAL NEURITE EXTENSION IN GAP-43 DEFICIENT PC12 CELLS STABLY TRANSFECTED WITH MUTATED (SER^{41}-ALA^{41}) GAP-43. E.E. Baetge, L.E.Bickerstaff,° K.M. Felsenstein, J.P. Hammang and K.F.Meiri° CNSMol. Neurobiol. Bristol-Myers Squibb, Wallingford, CT 06495. Dept. Pharmacology,° SUNY Health Science Center, Syracuse NY 13210.

The nervous-system specific kinase C substrate GAP- 43 is localized to the membrane skeleton of growth cones and has been linked to both axon outgrowth and neurotransmitter release. However, in the PC12 line, PC12(B) axon extension can occur without normal levels of GAP-43 (Baetge & Hammang Neuron 6:21, 1990). Here we show that these PC12(B) cells actually extend neurites more readily and more rapidly, and are also less adhesive to substrates than 'GAP- 43-normal' counterparts. The PC12(B)cells were transfected with a GAP-43 cDNA in which the kinase C phosphorylation site at ser^{41} had been mutated to alanine, preventing phosphorylation at that site. Clones that stably expressed mutant GAP- 43 were more adhesive to substrates and to each other than either the parent line or 'GAP-43 normal' PC12 cells. The transfected cells also extended fewer neurites more slowly than controls. Under highpower Nomarski optics, alterations to the morphology of live transfected cells were also apparent: Massive elaborations of filopodia arose from cell bodies and neurites as well as growing tips. The membranes of cell bodies and neurites often appeared distorted and accumulations of large vesicles were seen throughout the cells. The magnitude of these effects correlated with the amount of mutant GAP-43 expressed. These results, which extend previous observations that GAP-43 is not necessary for axon elongation, further suggest that over-expression of non-phosphorylatable GAP-43 may interfere with cytoskeletal organization and membrane vesicle transport.

525.7

ALTERED ORGANIZATION OF INTERMEDIATE FILAMENT PROTEINS IN GAP-43 DEFICIENT PC12(B) CELLS. [1]J.P. Hammang, [2]K.F. Meiri and [1]E.E. Baetge. [1]Cell and Molecular Neurobiology, Bristol-Myers Squibb Company, Wallingford, Ct. 06492, [2]Department of Pharmacology, SUNY Health Sciences Center, Syracuse, N.Y. 13210.

Intermediate filaments are tissue specific cytoskeletal proteins that influence cell shape and function. To determine whether the growth-associated protein, GAP-43 is involved in intermediate filament organization in PC12 cells, we compared the pattern of intermediate filament expression in GAP-43 deficient PC12(B) cells (Baetge and Hammang, Neuron 6 p. 21-30, 1991) with lines of "GAP-43- normal" PC12 cells. Furthermore, GAP-43 expression in the PC12(B) cells was restored by transfecting the cells with vectors containing a rat GAP-43 cDNA together with the genes for hygromycin B or neomycin resistance. Cells were treated with NGF to induce neurite expression for 3-7 days. All cells were processed for immunofluorescence using antibodies to the neurofilament proteins and to peripherin. GAP-43 containing and GAP-43 deficient PC12(B) cells exhibit neurofilament immunoreactivity within the cell body and extending out to the end of the neurites. The distribution of peripherin was markedly different. In PC12(B) cells peripherin immunoreactivity was confined to the cell body and was absent in the neurite processes, whereas in "GAP-43-normal" PC12 cells, or in the GAP-43 transfected PC12(B) cells, the peripherin was distributed within the neurites. The GAP-43 deficient PC12 cells will be useful for the investigation of the role(s) of GAP-43 in neuronal development and function.

525.8

GAP-43 MESSAGE LEVELS IN A HUMAN NEUROBLASTOMA CELL LINE BEFORE AND AFTER DIFFERENTIATION BY RETINOIC ACID. M.Weisenhaus*, J.P.E.Tokeson*, K.E.Rogers, S.Sullivan, M.F.D.Notter, P.D.Coleman. Dept. Neurobio. and Anat., Univ. of Rochester, Rochester N.Y. 14642.

It has been shown that neuroblastoma cells can be differentiated *in vitro* to acquire many neuronal phenotypes that are absent prior to differentiation. These include the expression of neuronal surface markers, electrical properties, cessation of cell division, and induction of morphological characteristics, including the extension of neurites with growth cones. This latter property was investigated by slot blot quantification of GAP-43 message, both before and after differentiation with 10uM retinoic acid. GAP-43, a phosphoprotein, is a marker of neuritic sprouting *in vivo* both during development and during synaptic reorganization. Differentiation of LAN-1 cells by retinoic acid induced a 3.5 fold increase in GAP-43 message levels as compared with controls, normalized for oligo dT slot blot hybridization. Northern blot analysis ensured intact RNA and confirmed the specificity of the GAP probe, which was made by random priming a full length GAP-43 cDNA. It is suggested that this paradigm can be used to study neurite plasticity and the regulation of GAP-43 *in vitro*. Supported by LEAD AG 09016, R01 AG 01121, and NIH NS25778.

525.9

POST-TRANSCRIPTIONAL MECHANISMS DURING PROCESS OUTGROWTH II. Stabilization of GAP-43 and other neuronal mRNAs in TPA induced PC12 cells. V.V. Cansino*, D. T. Kohn* and N. I. Perrone-Bizzozero. Department of Biochemistry, University of New Mexico, Albuquerque, NM, 87131-5221, U.S.A.

Previous work from our laboratory showed that the expression of the neuronal growth-associated protein GAP-43 is induced by nerve growth factor (NGF) via a selective change in the rate of degradation of the mRNA (Perrone-Bizzozero et al, (1990) Neurosci. Abtr. 16, 814). To study this further, we have examined the effects of NGF and other agents that modulate neurite outgrowth on the steady-state levels and on the relative rates of decay of GAP-43 and other neuronal mRNAs. By Northern blot analysis, we found that GAP-43 mRNA levels in PC12 cells were increased in response to the phorbol ester TPA, but not by either dibutyryl-cAMP or the Ca^{2+} ionophore A23187, suggesting that GAP-43 expression in these cells depends on the activation of protein kinase C. Furthermore, TPA had a much greater effect than NGF on both the onset and extent of process outgrowth and on the levels of the mRNAs for the neuronal proteins GAP-43, β-tubulin II (β-tub) and actin. In mRNA decay studies, TPA was found to increase 3-4 fold the half-lives of GAP-43, β-tub and actin mRNAs but had no effect on that of the housekeeping enzyme glyceraldehyde-3-phosphate-dehydrogenase (G3PD). The effect of TPA on the stability of GAP-43 and β-tub mRNAs was blocked by dexamethasone but not by cycloheximide, suggesting that post-translational modification of a neuronal factor is required for these effects. In conclusion, our results indicate that in addition to transcriptional control, post-transcriptional mechanisms seem to play an important role in the regulation of gene expression during neuronal differentiation. Supported by NSF (BNS-9011199) and the American Paralysis Association (PBI-9006) to N.P.B.

525.10

IDENTIFICATION OF *CIS*-ACTING ELEMENTS REGULATING GAP-43 PROMOTER ACTIVITY. E. Nedivi*, G. S. Basi, and J. H. P. Skene. Dept. of Neurobiology, Stanford University Medical School, Stanford, CA 94305

One of the best characterized examples of a gene that exhibits both neural-specific induction and later axon-dependent repression in neurons is the single-copy gene encoding GAP-43. In order to analyze regulatory elements controlling transcription of this gene, we have now isolated a functional GAP-43 promoter from rat, and 5 kb of DNA flanking this promoter. A 386 bp fragment containing the GAP-43 promoter fused to a gene for chloramphenicol acetyltransferase (CAT) drives expression of this reporter gene when transfected into dissociated cells from embryonic rat cortex or into NGF-treated PC12 cells, but not in several non-neuronal cell lines. This indicates that *cis*-acting elements in or near the core GAP-43 promoter can account for a substantial degree of the gene's neural-specific expression.

The promoter activity of the 386 bp fragment is modulated by additional elements lying on either side of it. Addition of an upstream DNA fragment containing the repeating sequence (GT)17 to the 386 bp fragment strongly inhibits CAT expression in cortical cultures. A downstream fragment containing the repeating sequence (GA)35 mildly depresses expression when added to the 386 bp fragment alone, but strongly counteracts the repressive effects of the upstream region when both flanking elements are present. The repeating sequences have the potential to adopt unusual DNA conformations. A 230 bp fragment located further downstream of the promoter appears to stabilize fluctuations in the level of promoter activity. On its own, this downstream fragment can act in an orientation-independent fashion to direct low-level expression of a marker gene in transient transfection assays, behaviour reminiscent of some types of non-promoter regulatory sequences from other genes (e.g. Cell 32, 503). The 1 kb DNA fragment containing all of these functionally characterized elements also contains upstream of the promoter, a sequence motif common to several neural-specific genes. Preliminary experiments indicate that an additional 4 kb of more upstream DNA may contain two 'silencer' elements, differing in strength and size, that cumulatively repress GAP-43 promoter activity. Supported by grant EY 07397.

525.11

FUNCTIONAL CONSERVATION OF GAP-43 GENE REGULATORY ELEMENTS BETWEEN MAMMALS AND FISH. E. Reinhard*, E. Nedivi, J. Wegner*, J.H.P. Skene, and M. Westerfield. Inst. of Neurosci., Univ. of Oregon, Eugene, OR 97403 and Dept. of Neurobiol., Duke Univ., Durham, NC 27710.

Expression of the single-copy GAP-43 gene is confined to the nervous system, where GAP-43 is widely expressed during development and then decreases drastically as synapses are formed. Distal axon interruption reinduces GAP-43 expression in the CNS of fish and amphibians, but not in the mammalian CNS. To explore the phylogenetic conservation of *cis*-acting elements responsible for early neural-specific expression and the later repression of this gene, we have introduced the rat GAP-43 promoter and surrounding DNA into transgenic zebrafish.

Three DNA fragments from the 5' end of the rat GAP-43 gene, a 386 bp fragment containing a neural-selective GAP-43 promoter (Nedivi et al., this volume), and larger fragments of 1 kb or 5 kb encompassing the smaller piece, were fused to a gene for β-galactosidase and injected into one-cell zebrafish embryos. Expression patterns were analyzed at several developmental stages. The constructs were strongly expressed in the developing nervous system at stages when extensive neurite outgrowth takes place (20-60 h after fertilization). At 50 h, for example, 85% of the cells expressing the 1 kb construct were neurons, 6% pigment cells, 1% muscle, and 8% other cells. The 386 bp fragment alone also directed expression preferentially in neurons; more than 80% of cells expressing the transgene were in the nervous system. In contrast, an RSV-promoter directed β-galactosidase expression in muscle cells (38%), skin (31%), and various other cells (31%). In embryos older than 35 h, the 386 bp and 1 kb promoter fragments were active almost exclusively in the brain, whereas the 5kb fragment was activated also in more posterior regions of the nervous system. Therefore, the 5 kb fragment may contain additional regulatory elements.

Our results demonstrate that *cis*-acting control elements from the rat GAP-43 gene are specifically activated in zebrafish neurons, suggesting that elements controlling the neural-specificity of GAP-43 gene expression have been functionally conserved during vertebrate evolution. Supported by grants HD22486 and EY07397.

525.12

GAP-43 mRNA BINDING PROTEINS RELATED TO NEURAL DIFFERENTIATION. N. Irwin, N. I. Perrone-Bizzozero and L. I. Benowitz. Dept. Neurosurgery, Children's Hospital, Harvard Med. School, Boston MA 02115; Dept. Biochem., U. New Mexico.

Expression of the membrane phosphoprotein GAP-43 is closely linked to the development and reorganization of neuronal connections[1,2]. Although growth-related changes in GAP-43 are generally paralleled by changes in levels of the mRNA, nuclear run-on studies indicate that these mRNA changes do not reflect altered rates of gene transcription[3,4]. For a number of other mRNAs, regulation involves the binding of cytosolic proteins to defined nucleotide sequences, which either enhance or retard degradation by ribonucleases[5]. Using band-shift assays, we found that the migration of a 3' fragment of GAP-43 mRNA on non-denaturing gels was retarded when the mRNA was incubated with cytosolic factors from cells undergoing neurite outgrowth. Factors from undifferentiated cells caused the mRNA fragment to migrate to a different position. To identify these binding proteins, cellular proteins were separated by SDS-PAGE, transfered to nitrocellulose, and probed with a radiolabeled fragment of the 3' end of GAP-43 mRNA. NGF-stimulated PC12 cells contained a 17 kDa protein with high GAP-43 mRNA binding activity; this protein did not bind control RNA and was barely detectable in unstimulated PC12 cells. Another GAP-43 RNA binding protein was visualized by similar methods in the neonatal rat cortex. We are currently characterizing the identity and biological activity of these proteins. *Support: NIH EY 05690.*

[1]L. I. Benowitz & A. Routtenberg (1987) Trends Neurosci. **10:** 527-532. [2]J. H. P. Skene (1989) Ann. Rev. Neurosci. **12:** 127-156. [3]H.J.Federoff, E.Grabczyk, M.C. Fishman (1989) J. Biol. Chem **263:** 19290-19295. [4]N.I. Perrone-Bizzozero, N. Irwin, S.E. Lewis, I. Fischer, R.L. Neve, L.I. Benowitz (1990) Neuroscience Abstracts **16:** 814. [5]R.J. Bandziulus, M.S. Swanson, G. Dreyfus (1989) Genes Devel. **3:** 431-437.

525.13

POSSIBLE ROLE OF CALMODULIN BINDING TO B-50 (GAP-43) IN THE MECHANISM OF NORADRENALINE RELEASE.

P.N.E. DeGraan, J.J.H. Hens*, M. De Wit*, P. French*, A.B. Oestreicher* and W.H. Gispen*. Div. Mol. Neurobiol., Rudolf Magnus Inst. and Inst. Mol. Biol., Univ. of Utrecht, Padualaan 8, 3584 CH Utrecht, The Netherlands.

B-50 (also known as GAP-43, F1 and neuromodulin) is a neuron-specific protein kinase C (PKC) substrate and is thought to be involved in the mechanism of noradrenaline (NA) release (Dekker et. al., *Nature* 324, 74, 1989). In purified systems and in native synaptosomal plasmamembranes it has been shown that B-50 binds calmodulin in the absence but not in the presence of calcium. In this study we investigated the relevance of B-50/calmodulin interaction in the mechanism of NA release. Calcium-induced NA release from Streptolysin-O permeated synaptosomes was measured using ^{3}H-NA. B-50/calmodulin interaction was measured using the homobifunctional crosslinker disuccinimidylsuberate (De Graan et al., *J.Neurochem.* 55, 2139, 1990). Calcium induces a dose-dependent dissociation of the B-50/CaM complex (EC_{50} 10^{-6} M) in the presence or absence of 150 mM K^+). The EC_{50} for complex dissociation and calcium-induced NA release are identical. Both calcium-induced NA release and B-50/CaM complex formation (at 10^{-6} M calcium) can be inhibited by polymyxin B, but not by calmodulin inhibitors such as W7 and trifluoperazine. The sensitivity of these two parameters for polymyxin B is at least 10-fold higher than PKC-mediated B-50 phosphorylation. Our data show that the inhibition of NA release by polymyxin B is not due to inhibition of PKC. These results are in line with the hypothesis that calmodulin-binding to B-50 plays a role in the regulation of NA release.

525.14

TWO FORMS OF GAP-43 DISTINGUISHED BY MIGRATION ON SDS-PAGE CORRELATE WITH THE PALMITOYLATED AND NON-PALMITOYLATED ISOFORMS. Sean I. Patterson and J. H. Pate Skene. Department of Neurobiology, Box 3209, Duke University Medical Center, Durham, North Carolina 27710.

An abundant protein component of growth cone membranes, GAP-43, has been shown to undergo covalent, post-translational addition of fatty acid residues concurrent with its attachment to cellular membranes (J. Cell Biol. 108, 613-624). Incorporation of 3H-palmitate into GAP-43 by isolated growth cones suggests that cycles of acylation and de-acylation of the protein may occur *in vivo*. Using SDS-PAGE at high voltage (300V), we have resolved two closely migrating forms of GAP-43 (apparent molecular weights 48 and 50 kDa, respectively, on 12% acrylamide gels), which can be detected on Western blots with mAb 9-1E12. Under similar conditions, 3H-palmitoylated GAP-43 migrated as a single band detected by fluorography, corresponding in migration to the upper band detected by Western blotting. Sub-cellular fractionation of growth cone preparations shows that the lower, non-acylated form is found predominantly in a cytosolic fraction. The upper, acylated form of GAP-43 found in washed membrane preparations can be converted to the soluble, lower form by treating the membranes with hydroxylamine at pH 10, which removes the fatty acid from the protein. This is consistent with a role for covalenlty bound fatty acid in the binding of GAP-43 to growth cone membranes. It is not yet clear whether the lower isoform is produced by de-acylation alone, or by additional modification that follows de-acylation of GAP-43. The lower, non-acylated form produced by chemical treatment is highly hydrophilic and exhibits a substantially reduced binding to nitrocellulose membranes. One technical implication of this finding is that standard Western blotting of tissue samples will systematically underestimate the occurence of this form of GAP-43 *in vivo*, relative to the upper acylated form. With appropriate calibration, however, these results provide an assay for assessing the relative proportions of these two forms of GAP-43 under changing biological conditions *in vivo*. Supported by NIH grant NS 20178.

525.15

IDENTIFICATION OF THREE PHOSPHORYLATION SITES IN THE GROWTH-ASSOCIATED PROTEIN GAP-43. S.A. Spencer and M. Willard. Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

GAP-43, a protein that is concentrated in neuronal growth cones and certain presynaptic terminals, has been suggested to perform functions in axon outgrowth, transmitter release, and synaptic plasticity. It is a substrate for protein kinase C, which phosphorylates it at a single site, serine 41, and thereby changes its affinity for calmodulin. Previous experiments indicated that in cultured neurons, GAP-43 is phosphorylated at additional sites by other protein kinases. To identify these additional sites, dispersed superior cervical ganglion cells from rat were grown in culture for five days and then labeled with ^{32}P-orthophosphate for four hours. GAP-43 was immunoprecipitated from these cultures and then proteolyzed with trypsin or endoproteinase Asp-N; the resulting radiolabeled peptides were purified by reverse-phase HPLC. Sequential Edman degradation of these phosphorylated fragments produced three sequences corresponding to amino acids 38-43, 90-106, and 167-176 of GAP-43. The first peptide, IQASFR, was phosphorylated at serine 41, the site recognized by protein kinase C. ^{32}P-orthophosphate was released from the second and third peptides at the cycles of Edman degradation corresponding to serine 96 and threonine 172 respectively, demonstrating that these two sites, in addition to serine 41, are phosphorylated in cultured neurons. The similarity of the amino acid sequences surrounding these two sites, i.e. APAT S^{96a} PKAE, and AAAT T^{172} PAAE, suggests that they may be phosphorylated by the same enzyme. These sequences resemble those recognized by a recently described enzyme designated proline-dependent proline kinase. (Supported by NIH grant EYO2682.)

525.16

GAP-43 mRNA EXPRESSION IN THE RAT HIPPOCAMPUS. H.M.Chao and B.S. McEwen. Laboratory of Neuroendocrinology, The Rockefeller University, New York, NY 10021

GAP-43 (growth associated protein 43) is a neuron-specific phosphoprotein localized in growth cones, whose expression is closely correlated with axonal growth. GAP-43 is abundant throughout the brain during development, but in the adult brain its expression is greatly diminished and anatomically restricted. The hippocampus is an area in which GAP-43 persists in the adult, a reflection perhaps of the synaptic plasticity known to occur in this brain region. We have analyzed the expression of the GAP-43 mRNA in different subfields of the rat hippocampus by in situ hybridization. Our results indicate that there is a significantly higher level of GAP-43 mRNA in the CA3+4 region than in CA1+2, and an intermediate level of expression in the dentate gyrus. Since CA3 pyramidal cells have a dense projection to CA1 pyramidal cells, the relative abundance of GAP-43 mRNA in the CA3+4 region may account for the prominent immunostaining for GAP-43 observed in the CA1 subfield. In addition, in rats that have been bilaterally adrenalectomized for 7 days, there is an increase in the level of GAP-43 mRNA in several subfields of the hippocampus relative to sham-operated animals. These findings are consistent with previous reports describing negative regulation of the GAP-43 gene by glucococorticoids in vitro. We are currently investigating the effects of steroid replacement in adrenalectomized rats and of steroid treatment in adrenal-intact rats on the hippocampal expression of GAP-43 mRNA. (Supported by MH41256 and NS07080.)

525.17

ULTRASTRUCTURAL DISTRIBUTION OF B-50/GAP-43 IN CULTURED HIPPOCAMPAL NEURONS DURING VARIOUS DEVELOPMENTAL STAGES. M. Van Lookeren Campagne, C.G. Dotti[1], E.R.A. Jap Tjoen San, W.H. Gispen and A.B. Oestreicher. Rudolf Magnus Institute, University of Utrecht, Utrecht, NL, and [1]European Molecular Biology Laboratory, Heidelberg, FRG

The distribution of B-50/GAP-43 in hippocampal neurons *in vitro* has been studied at different stages of development by light and electron microscopy. Axons and dendrites are distinguished by morphological criteria and marker antibodies, using immunocytochemical detection by silver enhanced 1 nm gold particles. Particle density is quantitated by video imaging. Directly after plating the neurons extend several filo- and lamellipodia. Ultrastructurally, B-50 is mainly present in the cytosol of lamellipodia and associated with small clear vesicles in the perinuclear area. After 2 days *in vitro* (2 DIV), neurons have several neurites of equal length or one long axon and several short dendrites. B-50 is homogenously distributed at the plasma membrane of all neurites, their growth cones and the cell body. After 7 DIV the spacial distribution of B-50 varies. Where synaptic contacts are scarce or absent, B-50 is detected at the plasma membrane of axons and dendrites. In neurons receiving synaptic contacts, B-50 at the plasma membrane of dendrites is reduced compared to axons. In this developmental stage MAP2 is exclusively present in the microtubules of dendrites. In neurons maintained in culture for 14 to 21 days, cell density appears to play a crucial role in the plasma membrane distribution of B-50. In high density cultures the cell body and dendritic plasma membrane is almost devoid of B-50. The plasma membrane of axons and axon terminals is rich in B-50. In low density cultures of neurons without synaptic contacts, B-50 is present at the plasma membrane of axons, dendrites and the cell body. At all developmental stages B-50 is detected at vesicles of 100 nm, which are mainly present in growth cones. These results suggest that synaptogenesis, rather than functional polarization, determines the restricted localization of B-50 in the axonal domain.

525.18

THE PATTERN OF GAP-43 IMMUNOREACTIVITY IN POSTNATAL RAT SPINAL CORD. D.J. Stelzner and J.A. Strauss*. Dept. of Anatomy and Cell Biology, SUNY Health Science Center, Syracuse, New York 13210.

Monoclonal antibodies to a phosphorylated form of GAP-43 (2G12/c7) or to both phosphorylated and non-phosphorylated forms of GAP-43 (5E7/G2) (J. Cell Biol. 112:991) were used to study the distribution of GAP-43 during postnatal development within rat spinal cord (0,3,6,12,15,18,24,48,90d; N=4). Tissue was lightly fixed in Bouin's and embedded in paraffin. Blocks were transversely sectioned at 10 um, and tissue prepared for immunohistochemistry using HRP and avidin-biotin amplification. A similar distribution of immuno-staining was seen with both antibodies, although denser and somewhat broader immunoreactivity was seen with the 5E7 antibody in 15d and older tissue. Heavy staining filled much of the white matter and the neuropil of the intermediate and ventral gray matter at birth. Lamina I was labeled from birth on. At 3,6,and 12d, regions containing corticospinal (CS) axons became the heaviest labeled areas of the white matter as CS axons extended through the spinal cord and immuno-reactivity increased in dorsal horn. Gracile fasc. remained lightly labeled until 12d although little staining was found in the cuneate fasc. Axons in other areas of white matter became progressively less heavily labeled. Some axons remained immunolabeled in CS pathways until 24d. At 15d the upper laminae of the dorsal horn, CS projection areas in the medial dorsal horn, and a medial zone surrounding the central canal remained heavily labeled. Less immunostaining was seen in 18-24d material; little was seen at 48 and 90d, particularly with 2G12. (Supported by grant NS14096).

525.19

AUTORADIOGRAPHIC PATTERNS OF GAP-43 mRNA EXPRESSION IN ADULT RAT BRAIN. L. Kruger, Dept. of Anatomy and Cell Biology, UCLA Medical Center, Los Angeles, CA; C. Bendotti, R. Rivolta*, and R. Samanin*, Lab. Neuropharmacology, Mario Negri Institute, Milan, and M. Bentivoglio, University of Verona, Italy.

The neuron-specific protein associated with axonal outgrowth during development and nerve regeneration, GAP-43, is expressed in highly selective neuronal populations in the adult rat brain. We previously reported high levels of GAP-43mRNA expression in mature brainstem monoaminergic neurons (Bendotti et al., '91). A survey of the distribution of GAP-43mRNA throughout the adult CNS revealed highly specific localization patterns that differ from the distribution of immunoreactivity reported by others.

High mRNA levels are evident in the primary olfactory and in the secondary gustatory pathways. Low levels are found in the main visual, auditory and somatosensory relays, although "secondary," midline and intralaminar thalamic nn. exhibit high levels. In cerebral cortex, insular and parastriate fields exhibit highest levels. Other regions of high density include inferior olive and cerebellar granular layer, hippocampus CA_3, entorhinal cortex, amygdala, hypothalamic nn., bed n. of stria terminalis, n. basalis, lateral reticular, periaqueductal gray, and substantia gelatinosa. The variety of anatomical substrates provides a new basis for examining the propensity for synaptic "plasticity" in mature neurons.

525.20

GAP-43 RESPONSES TO NERVE INJURY AND PERIPHERAL INFLAMMATION. R.L. Nahin, M. DeLeon and M.A. Ruda, Neurobiology and Anesthesiology Branch, NIDR, NIH Bethesda MD 20892.

Dorsal root ganglia (DRG) levels of GAP-43 have been shown to increase after axotomy. In the present study we compare GAP-43 mRNA and peptide levels after: 1) complete sciatic transection; 2) a chronic constriction injury (CCI) that results in behavioral hyperalgesia (Bennett and Xie, Pain 33 (1988) 87-107); 3) peripheral inflammation and accompanying hyperalgesia produced by two unilateral hindpaw injections of complete Freund's adjuvant (CFA), 5 and 10 days prior to sacrifice. Total mRNA was extracted from L4-5 DRG, 7 days after nerve transection or CCI, and 10 days after induction of inflammation in a hindpaw. RNA blots were hybridized with a ^{32}P-labeled cDNA probe for rat GAP-43. For cellular localization, fixed DRG were processed for in situ hybridization using a ^{35}S-labeled cDNA probe, or for immunoreactivity using a monoclonal antibody to GAP-43. Despite the fact that nerve transection destroys all fibers while the chronic constriction injury spares 60-70% of unmyelinated fibers, RNA blot analysis revealed at least a 2-fold increase in GAP-43 mRNA after both nerve transection and CCI. Using in situ hybridization, we localized this increase in mRNA to both large and small DRG neurons. In contrast, peripheral inflammation did not produce any alteration in GAP-43 mRNA or peptide. We conclude that 1) complete axotomy is not necessary for induction of GAP-43 synthesis; 2) increased primary afferent activity following inflammation and hyperalgesia is not sufficient to alter GAP-43 levels.

NERVE GROWTH FACTOR VI

526.1

SPECIFICITY OF NGF-INDUCED CEREBROVASCULAR SPROUTING. L.G. Isaacson[1] and K.A. Crutcher[2]. [1]Dept of Zoology, Miami University, Oxford, OH 45056; [2]Dept of Neurosurgery, University of Cincinnati College of Medicine, Cinci OH 45267.

We have previously shown that intraventricular infusion of nerve growth factor (NGF) into the adult rat brain elicits sprouting from mature cerebrovascular axons associated with the intracranial internal carotid artery (ICA). A 3-fold increase in the total number of perivascular axons was found at the ultrastructural level (Isaacson et al.1990). Since the ICA receives innervation from sympathetic, sensory, and parasympathetic sources, we undertook the present study to determine: 1) to what extent the sprouted axons are sympathetic in origin and 2) whether non-sympathetic fibers respond to NGF in the absence of sympathetic fibers. One group of rats received bilateral superior cervical ganglionectomy after a 14-day infusion of either NGF (NGFsx) or cytochrome C (VEH sx) and was sacrificed 3 days later. The other group received ganglionectomy 3 days prior to the onset of the infusion and was sacrificed at the end of the 14-day infusion period (sxNGF or sxVEH). The total number of perivascular axons associated with the ICA was determined using electron microscopy. Bilateral ganglionectomy resulted in a significant (73%) reduction in the number of perivascular axons indicating that the majority of sprouted fibers are sympathetic in origin. Both sxVEH and sxNGF rats exhibited a significant decrease compared to nonganglionectomized VEH rats, but the decline was greater in the sxVEH rats (67%) than in sxNGF rats (47%) indicating that some non-sympathetic axons may be responding to NGF in the absence of sympathetic fibers. These results suggest that mature cerebrovascular innervation can be modulated by NGF infusion and that sympathetic axons apparently are more responsive than non-sympathetic fibers.

526.2

CHANGES IN NGF RECEPTOR AND GAP 43 mRNA ASSOCIATED WITH COLLATERAL SPROUTING AND REGENERATION OF DORSAL CUTANEOUS NERVES IN THE RAT. K.M. Mearow, G.M. Ross*, A. Gloster, M. Holmes* and J. Diamond. Dept. Biomedical Sciences, McMaster University Medical Centre, Hamilton, Ontario, Canada.

Previous studies have demonstrated that collateral sprouting of intact nociceptive nerves is NGF-dependent, while regeneration of the damaged fibres shows no such requirement (Diamond et al, 1987, PNAS 84:6596). In our approach to explain this difference, we have begun to look at the expression of NGF-R and GAP 43.

DRGs of regenerating, collateral sprouting and control dorsal cutaneous nerves were isolated and used for analysis. In situ hybridization (ISH) was used to examine changes in mRNA distribution at the cellular level. These changes were quantified by solution hybridization using total RNA and PCR amplification of the specific messages.

The results indicate that the mRNAs for NGF-R and GAP 43 are elevated in the DRGs of sprouting nerves at 1 day post-op, decreasing to control levels by 1 week post-op. The ISH results show that these changes are specific to the small DRG neurons. We are presently using the same approach to study "precocious sprouting" (e.g. Doucette & Diamond, 1987, J. Comp. Neurol. 261:592), a dramatic shortening in the latency of nociceptive collateral sprouting induced by impulse activity. (This work is supported by the Centres of Excellence for Neural Regeneration and Functional Recovery).

526.3

ANTI-NGF TREATMENT BLOCKS THE COLLATERAL SPROUTING OF CHOLINERGIC FIBERS IN THE HIPPOCAMPUS. E.E.M. Van der Zee, J. Fawcett, J. Stanisz, R. Racine and J. Diamond. Departments of Biomedical Sciences and Psychology, McMaster University, Hamilton, Ontario, Canada, L8N 3Z5

Entorhinal cortex lesion leads to sprouting in the dentate gyrus of fibers from NGF-sensitive cholinergic neurons of the basal forebrain. We have now tested whether this sprouting is NGF-dependent. Eight days after a unilateral electrolytic lesion of the entorhinal cortex, the density of AChE positive fibers in the ipsilateral and contralateral outer molecular layers was compared. Either anti-NGF or non-immune serum was injected daily via a permanently implanted cannula in the ipsilateral lateral ventricle. In separate studies antibodies were shown to penetrate into predominantly ipsilateral brain tissue. The ipsilateral cholinergic sprouting was totally prevented by anti-NGF treatment; however the widening of the inner molecular layer (attributed to sprouting of commissural/associational fibers) seemed to be unaffected. The 8 d. anti-NGF injections did not reduce the density of AChE positive fibers in the contralateral dentate gyrus or in unlesioned brains. The findings thus implicate NGF, or a molecule that is recognised by the polyclonal anti-NGF, such as BDNF or NT-3, in the collateral sprouting of the cholinergic septohippocampal fibers. (This McMaster Node is a member of and supported by the Centres of Excellence for Neural Regeneration and Functional Recovery).

526.4

DEVELOPMENT AND CHARACTERIZATION OF CHICKEN POLYCLONAL ANTIBODIES TO NERVE GROWTH FACTOR RECEPTOR (NGFR) THAT INHIBIT LIGAND BINDING. A.A.Zupan, J.C.Pryor*, I.Leung*, L.Coussens* and E.M.Johnson,Jr. Dept. of Pharmacology, Washington Univ. Sch. of Med., St. Louis, MO 63110; Chiron Corp., Emeryville, CA 94608.

In this report we describe the development and characterization of a polyclonal chicken antibody against the recombinant extracellular domain of the human NGFR. This paradigm offers several distinct advantages. First, the evolutionary differences of antigen source and antibody producer enhance antigenic potential. Second, egg-yolk immunoglobulins are readily accessible, produced in large quantities, and conveniently packaged for facile storage. The purified antibody is novel in its ability to recognize receptor from a variety of species (including human, rat, mouse, and guinea pig), as determined by immunocytochemistry, immunoprecipitation and/or in vivo retrograde transport assays. Furthermore, this antibody has the capacity to block ligand binding to these receptors. To date this has only been demonstrated for the anti-human NGFR monoclonal antibodies, e.g., ME20.4-IgG. Because of the broad range of species reactivity we propose that this antibody will be useful in determining the role of the low-affinity NGFR in signal transduction, as well as contributing to improved receptor immunoassays (e.g., electron microscopy and immunoblotting.

526.5

INTRACEREBROVENTRICULAR (ICV) ADMINISTRATION OF NGF TO NEWBORN RATS INDUCES AN INCREASE OF ACETYLCHOLINESTERASE (AChE) ACTIVITY IN FOREBRAIN REGIONS. G. Vantini, N. Schiavo*, C. Cosi, N. Gabellini* A. Leon and M.A. Tria*. Fidia Research Labs, 35031 Abano Terme, Italy.

Forebrain cholinergic neurons respond in vivo to icv administration of NGF with a prominent and selective increase of choline acetyltransferase activity. We here report that, in the septal area and striatum, NGF administration also affects the enzymatic activity of AChE. A significant increase (about 50%) of AChE activity was observed in both septum and striatum of 10-day-old rats treated with NGF (10ug/injection) on postnatal days 2,4,6, and 8. In septal area this effect was associated with an increase of AChE mRNA. In adult animals, a modest increase (about 15%) of striatal AChE activity was observed after continuous icv infusion of NGF (100ug over two weeks). Since in forebrain regions AChE is largely associated with cholinergic neurons, and in view of the multiple roles that the molecular forms of AChE can exert in neuronal development and function (see for example Small D.H., TIBS, 15:213-216), the present observations confirm and extend the notion that NGF (or related molecules) acts in vivo by modulating both functional and structural properties of forebrain cholinergic neurons.

526.6

ACETYLCHOLINE RELEASE IN VIVO IN LESIONED ANIMALS GRAFTED WITH GENETICALLY MODIFIED CELLS IN THE CORTEX. PREVENTION OF NEURONAL DEGENERATION IN THE NUCLEUS BASALIS MAGNOCELLULARIS (NBM). D. Maysinger, P. Piccardo*, L. Lamarre*+, M. Goiny*+ and A.C. Cuello. Department of Pharmacology and Therapeutics, McGill University, Montreal, Canada H3G 1Y6. +Department of Pharmacology, Karolinska Institute, Stockholm, Sweden S-104-01.

Degenerative changes in the nucleus basalis magnocellularis (NBM) can be prevented by chronic treatment with trophic factors such as nerve growth factor (NGF). The aim of the our study was to investigate whether neuronal degeneration of the NBM could be prevented by grafting genetically modified fibroblasts in the cortex at the site of the lesion. Male Wistar rats (n = 26) were unilaterally lesioned by disrupting the blood vessels in the area of the fronto-parietal cortex. Following devascularization, fibroblasts (both secreting and non-secreting NGF) were placed onto the surface of the cortex. Cell suspension ($3.0 \times 10^5 - 3.0 \times 10^6$ cells per animal) was introduced into sterile, biodegradable gel-foam (0.8 x 0.8 x 0.1 cm; Upjohn) and layered over the damaged cortex. Thirty days following surgery, animals were anaesthesized and subjected to microdialysis. Our results demonstrated that, in animals grafted with NGF-secretor cells, there was a diminished decrease in ChAT enzymatic activity in the NBM and less neuronal cell shrinkage and loss of processes. Furthermore, microdialysis studies also showed an increased release of ACh in the remaining cortex proximal to the devascularizing lesion. Thus, fibroblasts, genetically modified to produce and secrete NGF and grafted to the CNS, prevented retrograde cholinergic degeneration, demonstrating a functional and morphological response to the grafted cells. (Funded by the Medical Research Council of Canada and the Centres of Excellence Network for Neural Regeneration and Functional Recovery.)

526.7

NERVE GROWTH FACTOR PRODUCING PRIMARY FIBROBLASTS GRAFTED INTO THE BASAL FOREBRAIN OF BEHAVIORALLY CHARACTERIZED AGED RATS. K. S. Chen and F. H. Gage. Dept. of Neurosciences, 0624, U.C.S.D., La Jolla, CA. 92093.

Rats exhibit behavioral and anatomical alterations as a function of aging. Previously, we have demonstrated that grafts of fetal basal forebrain cells into the hippocampus, cortex or nucleus basalis, as well as chronic unilateral infusions of nerve growth factor (NGF) into the lateral ventricles can partially reverse some of the age-related behavioral changes and basal forebrain cholinergic neuronal atrophy. In this study we grafted primary fibroblasts, genetically modified to produce NGF, bilaterally into the area of the nucleus basalis of aged (22 mos) Fischer 344 rats. The primary fibroblasts were obtained from Fischer 344 rats and infected with a retroviral vector containing the cDNA for mouse NGF. Either NGF-producing or control non-infected fibroblasts were grafted into aged and adult rats that had been behaviorally characterized on the Morris water maze. The aged animals, as a group, were impaired on the water maze compared to the adult animals, although not all aged rats exhibited deficits. Upon retesting following grafting, the aged rats that received grafts of NGF-producing cells had significantly improved their retention of the location of a hidden platform, though not to the level of the initially unimpaired aged animals, whereas the aged rats that received non-infected control cells did not show an improvement in their performance. In contrast, grafts of NGF-producing fibroblasts to adult rats resulted in a detriment in performance on this task. Grafts of both NGF-producing and control fibroblasts survived in the aged and adult brains. Immunohistochemical staining with an antibody to NGF-receptor (NGF-R) revealed the presence of NGF-R-positive processes within the NGF-producing grafts but not within the control grafts. NGF-R-positive process density appears greater within the grafts into the adult rats compared to the aged rats. Quantitative morphometric analysis of NGF-R-positive cells in the basal forebrain will be presented for each animal. These findings show that grafts of NGF-producing cells into the basal forebrain region of aged rats can survive and partially ameliorate observed behavioral deficits.

526.8

NGF ENHANCES CHOLINERGIC TRANSMISSION BETWEEN NUCLEUS BASALIS AND BASOLATERAL AMYGDALA IN RAT H.C. Moises, M.D. Womble, M.S. Washburn, and L.R. Williams+. Dept. of Physiology, Univ. of Michigan, Ann Arbor 48109 and + The Upjohn Co., Kalamazoo, MI., 49001.

Although it well-documented that administration of NGF stimulates transmitter-related functions in basal forebrain cholinergic neurons (BFCN), it is unknown whether this facilitates neurotransmission between BFCN and their postsynaptic target cells. To examine this possibility, we compared the ability of nucleus basalis (NBM) stimulation to elicit a muscarinic slow depolarization (slow EPSP) in pyramidal cells in basolateral amygdala (BLA) in slice preparations obtained from control and NGF-treated rats. Young adult Fisher 344 rats were treated i.c.v. with vehicle or NGF (1.2 μg/day) for 3 weeks. When recording in normal medium, NBM stimulation (30 Hz for 500 ms) elicited an atropine-sensitive slow EPSP in 15 of 24 neurons in slices (n=14) from NGF-treated rats, compared to only 5 of 27 cells in control slices (n=16). Although eserine (2 μM) increased the number of cholinergic responses that were recorded in both control (22/27) and NGF preparations (22/24), the frequency-response relationship for eliciting the slow EPSP in NGF-treated slices showed a significant leftward shift, compared to that of controls. In contrast, no differences in neuronal responsiveness to bath applications of carbachol were observed between control and NGF-treatment groups. These data suggest that NGF treament can enhance forebrain cholinergic transmission, possibly by increasing acetylcholine release.

526.9

NGF LEVELS, CHOLINERGIC SEPTAL NEURONS, AND SEPTAL CYTOSKELETAL MARKERS ARE PRESERVED FOLLOWING EXCITOXIC LESIONS OF THE HIPPOCAMPUS: M.A. Burke, J. Roback, B.H. Wainer and J.H. Kordower, Dept. of Anatomy, Univ. Illinois Sch. Med., Dept. Pharmacology, Univ. Chicago Sch. Med., Dept. Neurological Sci., Rush Presbyterian Med.Ctr., Chicago Ill. 60612 USA.

Cholinergic septal neurons persist following elimination of hippocampal neurons, their normal source of endogenous nerve growth factor (NGF; Sofroniew et al, *Science*, 247: 338-342, 1990). This suggests that either NGF is not required for the maintenance of basal forebrain neurons or that basal forebrain neurons utilize NGF from other sources following this lesion. To further characterize this phenomenon, rats received injections of ibotenic acid into the hippocampus and were sacrificed 2 weeks-7 months later. As seen previously, no reductions in ChAT-immunoreactive neurons were found ipsilateral to the lesion at any time point studied. Furthermore, antibodies which recognize phosphorylated neurofilaments that can normally label perikarya of axotomized neurons, did not stain septal perikarya ipsilateral to the lesion. RNA blot analysis revealed that despite a comprehensive loss of hippocampal neurons on the side of the injection, the hippocampal remnant expressed NGF mRNA levels comparable to the contralateral side. Since excitotoxic lesions primarily affect neurons, these data suggest that either hippocampal glia or residual hippocampal neurons upregulate NGF synthesis in response to the death of neighboring neurons and may still play a role in sustaining cholinergic basal forebrain neurons following hippocampal damage. (Support: AG 09468 (JHK) NS25787 (BHW) 5-T32HD070009 (JR))

526.10

DISTRIBUTION AND RELATIVE DENSITY OF NERVE GROWTH FACTOR RECEPTORS (NGFR) IN THE RAT BRAIN AS A FUNCTION OF AGE AND ANTI-NGF TREATMENT. B.A. Urschel and C.E. Hulsebosch. Dept. of Anat. and Neurosci., Marine Biomed. Inst., Univ. of Texas Med. Br., Galveston, TX 77550.

It is clear that Nerve Growth Factor (NGF) has a role in the central nervous system (CNS). In order to begin to determine the possible roles of NGF in the CNS, we gave neonatal rats daily subcutaneous injections of antibodies to NGF (ANTI-NGF) for a period of up to one month. By utilizing a monoclonal antibody (192-IgG), which recognizes the NGFR, and standard immunohistochemical techniques we have localized NGFR in various aged ANTI-NGF treated animals and compared the anatomic localization and relative density of the NGFR immunoreactive regions to same age untreated and preimmune sera treated littermates. We confirm previously reported localizations of NGFR immunoreactivity (NGFR-I) in the rat brain. In addition, we demonstrate that NGFR-I levels of ANTI-NGF treated rats in the molecular and Purkinje cell layers of the cerebellum, vestibular nuclei, spinal tract of V and cochlear nuclei remain at lower concentrations compared to same aged control animals. We also demonstrate that NGFR-I levels in the granular layer of the cerebellum are decreased and that NGFR-I in the basal nucleus is completely lost with ANTI-NGF treatment. We hypothesize that ANTI-NGF biologically inactivates NGF, which over a period of 30 days results in decreased NGFR-I. These results may be due to neuronal loss in these regions following ANTI-NGF treatment. Supported by Sigma Xi, NS11255, NS25450, NS01217, RR03979, Bristol Myers.

526.11

DIFFERENTIAL EFFECTS ON SENSORY NERVE PROCESSES AND BEHAVIORAL ALTERATIONS IN THE RAT AFTER TREATMENT WITH ANTI-NGF. C.E. Hulsebosch, P.N. Brown, and B.A. Urschel. Dept. of Anat. and Neurosci., Marine Biomed. Inst., Univ. of Texas Med. Br., Galveston, TX 77550.

Published work on the effects of antibodies to Nerve Growth Factor (ANTI-NGF) treatment on rats has shown an increase in the number of unmyelinated central processes of dorsal root ganglia (DRG) neurons. This increase is interpreted to be sprouting of the central projections of the DRG neurons. To test for sprouting of the peripheral DRG projections, we quantitated the number of peripheral DRG processes in the peripheral nerves of ANTI-NGF treated compared to untreated rats, following selective surgery to eliminate motor and sympathetic nerve fibers. We report the numbers of peripheral processes in an NGF deprived environment decrease by 48% compared to untreated controls and the decrease is selective for the unmyelinated fiber population. Since the majority of the unmyelinated population is nociceptive, two nociceptive behavioral measures, one reflexive (tail flick) and one nonreflexive (paw or skin pinch), were performed and demonstrated decreased response times in the ANTI-NGF treated compared to untreated and preimmune treated rats. These data suggest a directional effect primarily on the unmyelinated sensory population which results in altered nociceptive behavior, induced by the suppression of one endogenous factor, NGF. Supported by NS11255, NS01217, HL07615, Bristol Myers, Hall Endowment, Sigma Xi.

526.12

IN SITU LOCALIZATION OF NERVE GROWTH FACTOR AND ITS RECEPTOR IN THE DEVELOPING ORGAN OF CORTI AND *IN VITRO* EFFECT OF ANTI NERVE GROWTH FACTOR ANTIBODIES. T.R. Van DeWater, P.P. Lefebvre*, V. GalinovicSchwartz*, T. Weber*, G. Moonen. Depts. of Otolaryngology and Neuroscience, Albert Einstein College of Medicine, Bronx, N.Y. 10461 and Depts. of Human Physiology-Pathophysiology and Otolaryngology*, University of Liege, 4020 Liege, Belgium.

Nerve growth factor (NGF) and nerve growth factor receptor (NGF-R) were immunolocalized in the peripheral auditory system of 5 day post partum (PP)rat pups. NGF protein was detected in both the inner (IHC) and outer (OHC) hair cells of Corti's organ, the somas as well as both peripheral and central axons of spiral ganglion neurons and in the sheath cells of the VIIIth cranial nerve. NGF-R antibody was not immunolocalized over the somas of either the IHC or OHC, however intense immunolabel was present over the neurons that compose the spiral ganglion and nerve endings at the bases of both the IHC and OHC. Explants of 5PP rat organ of Corti with attached spiral ganglion cells showed that antibodies directed against 2.5S NGF can disrupt the normal pattern of innvervation (detected by anti-neurofilament antibodies)when compared to normal serum control explants of Corti's organ. These findings strongly support a role for NGF or a member of the NGF family (i.e BDNF or NT3) in development of the peripheral auditory system.

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526.13

NGF INCREASES THE SOMA PROFILE AREA AND THE COMPLEXITY OF DENDRITIC TREES OF CHOLINERGIC INTERNEURONS IN ORGANOTYPIC STRIATAL CULTURES OF NEWBORN RATS. C. Spenger+, K. Rufener+*, L. Studer* and H.-R. Lüscher. Dept. of Neurosurgery, Inselspital, CH-3010 Bern+; Dept. of Physiology, CH-3012 Bern Switzerland.

The influence of NGF on the development of cholinergic striatal interneurons in terms of cell number, cell size and complexity of dendritic tree was studied in vitro. The striatum was taken from newborn Wistar rats and chopped in 250 μm thick slices. From these slices identical circular pieces of 1.1 mm in diameter were punched out. 4 series of 20 cultures each were grown simultaneously by means of the roller tube method. The medium of the 4 culture series contained 5 ng/ml NGF, no NGF, 20 ng/ml anti-NGF antibody (AB) or 5 ng/ml NGF plus 20 ng/ml anti-NGF-AB respectively. After 13 days in vitro all cultures were fixed and stained for AChE. The number of AChE positive interneurons in cultures grown with NGF, without NGF, with anti-NGF-AB or with NGF plus ani-NGF-AB was 12.7 ± 10.2, 6.9 ± 7.8, 6.5 ± 9.7 and 4.4 ± 4.5, respectively. The soma profile area of the cholinergic neurons in cultures grown with NGF,without NGF and with NGF plus anti-NGF-AB were 698 μm^2 ± 228, 480 μm^2 ± 151 and 441,8 μm^2 ± 158, respectively. 5 cells each of cultures grown with and without NGF were reconstructed and the dendritic trees analyzed. The mean combined dendritic length of the cells grown with and without NGF was 5751 μm ± 2342 and 3737 μm ± 935, respectively. The mean number of stem dendrites was 5.6 ± 1.5 and 4.2 ± 0.4, respectively and the mean total number of branch points per cell was 47 ± 29 and 28 ± 5, respectively. The areas covered by the dendritic tree of the neurons grown with and without NGF were not different. These data demonstrate first, that NGF promotes survival and growth of the cholinergic striatal interneurons in culture and second, that cells of the same age in vitro grown with NGF possess a more complex dendritic tree than those grown without NGF.

526.14

NGF ATTENUATES NBM LESION-INDUCED CHOLINERGIC NEUROCHEMICAL MARKER DEFICITS IN YOUNG, BUT NOT IN AGED, F344 RATS. A.C. Santucci, P.D. Kanof, and V. Haroutunian. Psychiatry Service, Bronx VAMC & Dept. of Psychiatry, Mt. Sinai School of Medicine, New York, NY 10468.

This study's aim was to determine whether nerve growth factor (NGF) administration would enhance central cholinergic activity in aged animals that have sustained experimentally-induced cholinergic lesions. Accordingly, 14 young (3-4 months) and 13 aged (22-23 months) F344 rats were prepared with NMDA-induced unilateral lesions of the NBM. During the same surgical session, subjects were implanted with a subcutaneously placed osmotic pump filled either with 6 μg of ß NGF (young, n=7; aged, n=6) or artificial cerebrospinal fluid (young, n=7; aged, n=7) and connected to an indwelling cannula directed at the lateral ventricle. Animals were sacrificed and their brains dissected two weeks after surgery. Concentrations of CAT and AChE within the caudate, cortex and hippocampus were measured. Results indicated a significant lesion-induced depletion of CAT and AChE within the frontal cortex of both young and aged subjects ($p<.001$). Nerve growth factor attenuated these depletions by approximately 35%. However, this effect of NGF was observed only in young animals ($p<.05$). This finding contrasts a previous report from our laboratory indicating NGF's ability to increase cholinergic neurochemical markers in aged non-lesioned animals. Taken together, these data suggest that NGF's effect on cholinergic activity in the aged brain may depend upon the neurological integrity of the tissue.

Supported by a grant from the American Federation for Aging Research.

526.15

TRANSCRIPTIONAL CROSSTALK IN THE STIMULATION OF GALANIN GENE EXPRESSION BY NERVE GROWTH FACTOR. S.C. Hooi, D.R. Abraczinskas* and L.M. Kaplan. Gastrointestinal Unit, Mass. General Hospital and Program in Neuroscience, Harvard Medical School, Boston, MA 02114.

Nerve growth factor (NGF) is an potent neurotrophic agent required for the differentiation and maintenance of neural crest-derived neurons. The PC12 rat pheochromocytoma cell line provides a useful model for examining mechanisms by which NGF induces cellular differentiation. NGF exposure causes these cells to adopt a neuron-like phenotype. This trans-differentiation is associated with stimulated expression of several neuropeptide genes including galanin. The induction of galanin expression in these cells is rapid, peaking 4 hours after NGF addition, with mRNA increased more than 100-fold. To study the transcriptional regulation of the galanin gene by NGF, galanin-luciferase reporter constructs were examined by transient expression in PC12 cells. Deletion analysis revealed 2 regions in the galanin 5'-flanking sequence that conferred NGF responsiveness to the transfected reporter gene. Dose-response analysis, transfections in protein kinase A- and C-deficient PC12 cells, and experiments using inhibitors of protein kinases indicate that the NGF response cannot be fully accounted for by either the cAMP-dependent or protein kinase C pathway alone. Rather, NGF acts cooperatively with agents that stimulate these activities. The simultaneous addition of forskolin, TPA and NGF caused a 50-fold increase in luciferase activity over untreated controls. Gel mobility shift analysis using the proximal NGF-responsive region revealed several discrete DNA-protein complexes. A major site of protein binding was located between bases -22 and +4, a GC-rich region with several GCGGC repeats. Methylation interference analysis confirmed protein binding in close proximity to G residues in this region. Protein binding was not competed by sequences known to bind previously characterized transcription factors including AP1, AP2, AP3, NF1, SP1 and zif-268. Efforts are underway to isolate and further characterize the protein that binds to this NGF-responsive element.

526.16

NERVE GROWTH FACTOR REGULATES THE EXPRESSION OF THE CALCIUM-BINDING PROTEIN S100α. D.B. Zimmer, R.H. Cochran and S.J. Strada. Dept. Pharmacol., Univ. S. Alabama, Mobile, AL 36688.

One mechanism by which nerve growth factor (NGF) induces differentiation in neuronal cells is to increase the expression of specific proteins such as 42C, a member of the S100 family of calcium-binding proteins. This study utilizes PC12 cells to examine the effect of NGF on the expression of other members of this protein family, S100α and S100β. Northern blot analysis revealed that PC12 cells express S100α and not S100β mRNA. In addition, the steady state level of S100α mRNA increased with NGF treatment. This mRNA increase was accompanied by a 2-fold increase in S100α protein in NGF-treated cells. While, NGF treatment resulted in increased S100α protein levels, it did not alter the S100α-binding protein profile or subcellular distribution of S100α. In both treated and untreated cells, S100α exhibited a punctate staining pattern in the cell body with no detectable staining in the cell processes. Altogether, these results demonstrate that NGF increases S100α expression and suggest that S100α may mediate cellular responses to NGF in neuronal cells.

526.17

NERVE GROWTH FACTOR SIGNAL TRANSDUCTION IN RAT SEPTAL NEURONS GROWN IN BILAMINAR CULTURE. L. Mudd*, M. Downen, J. Roback, J. Zucker, M. Wert*, B. Wainer and H.C. Palfrey*. Dept. Pharmacol. & Physiol. Sciences, University of Chicago, 947 E. 58th Street, Chicago, IL 60637.

We have developed a method for the preparation of primary neuronal cultures from rat septal nuclei dissected on embryonic day (E)16. Neurons are grown in defined medium in a bilaminar culture system consisting of a post-natal glial cell layer 1 mm away from the neuronal plane. Greater than 99% of the cells in the neuronal plane stain positively for neurofilament proteins (NFP), but less than 1% for glial fibrillary acidic protein (GFAP). A nerve growth factor receptor (NGFr) is expressed in 24 ± 8% of these cells. We have studied the mechanism of NGF signal transduction in these cultures and have demonstrated that NGF rapidly increases expression of the protooncogene c-fos, tyrosine phosphorylation, microtubule associated protein (MAP) kinase activity. Nerve growth factor transiently increases tyrosine phosphorylation in a subset of neurons with maximal stimulation 2 min after NGF treatment, followed by a return to basal levels within 10 min. A similar time course is observed for MAP kinase activation with phosphorylation of myelin basic protein (MBP) increasing to a maximum within 2 min and declining to basal levels within 10 min. The increase in fos expression shows a maximum in 17.8 ± 3.6% (mean ± SEM) of cells 30 min following NGF administration as demonstrated immunocytochemically. Fos levels decline to those of untreated cells within 4 hours. Thus NGF signal transduction in these post-mitotic neurons shows significant similarities to events previously demonstrated after NGF administration to PC12 cells. Preliminary investigations of long term effects of NGF in these neuronal cultures suggest that NGF alone cannot entirely account for the survival characteristics provided by the presence of a glial plane. These results indicate that other trophic factors released by glial cells support the long-term viability of the neuronal population. The bilaminar culture system should be a valuable tool for the evaluation of septal responses to neurotrophic agents.

526.18

CELL LINES DERIVED FROM INTESTINAL EPITHELIAL CELLS SYNTHESIZE NERVE GROWTH FACTOR IN CULTURE. G.W. Varilek*, G.A Neil*, B. Antisdel* and N.J. Pantazis. Depts. of Internal Medicine and Anatomy, Univ of Iowa College of Medicine, Iowa City, IA 52242.

Although nerve growth factor (NGF) has been detected in the gastrointestinal tract of adult rats (Weskamp and Otten, J. Neurochem., 48:1779, 1987), the cell(s) responsible for NGF synthesis in the tract have not been identified. Since intestinal epithelial cells are a major cellular component of the gastrointestinal tract, the aim of this study was to determine whether intestinal epithelial cells are a potential source of NGF. Two cell lines, Caco-2 and IEC-6, were selected for study since they are derived from intestinal epithelial cells and they display many characteristics of epithelial stem cells. Conditioned medium (CM) was obtained from confluent cultures of Caco-2 and IEC-6 and examined in two NGF bioassays. CM from both cell lines induced neurite outgrowth from rat pheochromocytoma (PC12) cells and sensory neurons (dorsal root ganglia). This outgrowth was similar to that induced by purified NGF and was inhibited by polyclonal anti-NGF antibody. In addition, an NGF-ELISA (Mobley et al., Neuron 3:655, 1989) detected NGF in CM from Caco-2 and IEC-6 cells. These data demonstrate that Caco-2 and IEC-6 cells synthesize and release NGF into the culture medium. This suggests that intestinal epithelial cells are a potential source of NGF in the gastrointestinal tract. Supported by the CCFA, Univ. of Iowa College of Medicine Grant, and Univ. of Iowa Aging Research Seed Grant.

526.19

RECAPITULATION OF THE DEVELOPMENTAL PATTERN OF NGF RECEPTOR IMMUNOREACTIVITY IN REGENERATING RAT OLFACTORY NERVE. [1,2]H. Vickland, [1,2]L.E. Westrum and [3]M.A. Bothwell. Depts. of [1]Biol. Struct., [2]Neurol. Surg., and [3]Physiol. & Biophys. University of Washington, Seattle, WA 98195.

We have previously described a differential pattern of expression of nerve growth factor receptor (NGFR) in the young and adult rat olfactory system (Vickland, et al., 1991, *Brain. Res.*, in press and Neurosci. Abstr. 1989). We found NGFR immunoreactivity (IR) along the young olfactory nerve (ON), possibly indicating the growth state of axons before they reach their targets and NGFR-IR in the glomeruli of the olfactory bulb (OB) of the adult, perhaps indicative of synaptic maintenance. Because many investigators have found NGFR-IR in regenerating peripheral nerve, we sought to determine if the regenerating rat olfactory system would show a recapitulation of the developmental pattern of NGFR-IR. Lesions to the ON were performed on adult rats and the olfactory tissue was taken after survival times of 3, 6, 10, 14, 20, 45 and 90 days. The shorter survival times showed a recapitulation of the developmental pattern: NGFR-IR began to be expressed along the regenerating ON and it disappeared in the OB glomerular layer. In longer survival times we observed a reestablishment of the typical adult pattern. As in our previous study, electron microscopy shows that NGFR-IR is localized to glial cells. These findings clearly indicate a role for NGFR in the regenerating rat olfactory system similar to its role during development. (Supported by NIH Grants NS09678, NS23343 and 2T32HD07183-11A1. L.E.W. is a research affiliate of the CDMRC).

526.20

NGF RECEPTORS IN THE OLFACTORY SYSTEM.
Mary S. Bailey, Qizhi Gong, Sarah K. Pixley, Michael T. Shipley. Dept. of Anatomy and Cell Biology, Univ. of Cincinnati, Cincinnati, OH 45267.

To address the possible role(s) of NGF in development and regeneration in olfactory system, we have assessed the cellular localization of NGFrs in adult and developing rats using two monoclonal antibodies directed against NGFrs (217C and 192).

NGFr-immunoreactivity (NGFr-IR) was observed on fibers in deep layers of the olfactory bulb late in development but was not apparent before E18. NGFr-IR was also observed in neurons of the basal forebrain and their axons coursing into the deep layers of the olfactory bulb. In the adult, NGFr-IR remains abundant in olfactory bulb glomeruli and in fibers in deeper layers of the bulb. These results suggest that NGFrs in the adult olfactory bulb glomeruli and deep layers is located on the cholinergic centrifugal afferents from the basal forebrain. This will be tested by lesioning the basal forebrain cholinergic neurons with ibotenic acid; we predict that this manipulation will eliminate NGFr from the adult olfactory bulb.

In developmental tissue there was high NGFr-IR in the epithelium and olfactory nerve which may be localized to Schwann cells. However, in contrast to perinatal animals, only a few streaks of NGFr-IR were present in the adult olfactory nerve. These "streaks" of NGFr seen in the adult nerve may represent fascicles transiently "denervated" by normal olfactory neuron turnover and replacement. Consistent with this hypotheses, NGFrs were robustly re-expressed in the olfactory nerve following lesion of the olfactory epithelium. [Supported by NIDCD DC00347 and NINDS NS29218]

HORMONES AND DEVELOPMENT: STEROID RECEPTORS

527.1

ONTOGENY OF THE HIPPOCAMPAL BINDING CAPACITY OF THE GLUCOCORTICOID AND MINERALOCORTICOID RECEPTOR.
D.M.Vázquez, M.I.Morano and H. Akil. Mental Health Research Institute and Department of Pediatrics, University of Michigan, Ann Arbor, MI
The developing HPA axis is hyporesponsive to stressful stimuli during early life. Once the adrenocortical response is established, failure to terminate the corticosterone rise is evident following certain stressors. The hippocampal (HC) glucocorticoid (GR) and mineralocorticoid (MR) receptors have been implicated in the modulation of glucocorticoid negative feedback. In order to investigate the emergence of this mechanism, we have studied the ontogeny of GR and MR binding capacity (Bmax) in the HC. Adrenalectomy (ADX) was performed on day: 6, 10, 14, 18, 22, 28, 35, 45 and adult animals. GR and MR Bmax HC analysis was done 12 hours after ADX using saturating amounts of ^{3}HCorticosterone. RU26938, a GR agonist, and corticosterone were used as ligands to calculate specific GR or MR Bmax. GR Bmax reached adult levels on day 10 and remained steady. In contrast, MR Bmax was greater than adult from day 6 until day 45; when it decreased and approached adult concentrations. Thus, GR:MR Bmax ratios increased with age (d6=1:1 to adult=6:1). Looking at the total HC, it was evident that the greatest absolute increase for both receptors occurred between days 22 and 45, at a time when failure to terminate the adrenocortical response is described. This suggests that an impaired glucocorticoid modulation during development is not the result of lower GR and MR hippocampal binding as has been reported in the aged animal. The difference in MR to GR ratio during this time may contribute to this phenomena. Alternatively, immature circuitry between HC and hypothalamus may explain the lack of feedback. Supported by MH422251.

527.2

DEVELOPMENTAL VS. ACTIVATIONAL EFFECTS OF GONADAL STEROIDS ON THE BINDING PARAMETERS OF CORTICOSTEROID RECEPTORS IN HIPPOCAMPUS OF MALE VS. FEMALE RATS.
B.B. Turner, M.S. Ansari*, T. Ansari*, L.I. Holtsclaw*, and X. Chen. Department of Physiology, James H. Quillen College of Medicine, East Tennessee State University, Johnson City, TN 37614-0002.

We have previously reported that female rats have fewer hippocampal Type I and Type II corticosteroid receptors than do males, and that the in vitro affinity of the Type I receptor from females is several fold less than that from males. In this study, we asked whether the presence of the gonads during development contributes to these differences or whether the short term presence of gonadal steroids is solely responsible. Male and female pups were gonadectomized either on day 1 or as adults. Each male group was injected (s.c.) with either vehicle alone or 250 ug testosterone propionate for 10 days. Likewise, females were injected with vehicle or 2.5 ug estradiol benzoate. Animals were adrenalectomized 12 hr before sacrifice. For each hippocampus, Type I binding was measured using a saturation plot (^{3}H-dexamethasone + RU 28362) and total binding was measured with a single, saturating concentration of ^{3}H-dexamethasone. Three-way analysis of variance showed a main effect of sex steroids on the Bmax of Type I receptors ($p < 0.01$): estrogen treated females had fewer Type I receptors than did other groups. No significant main effects were found with respect to the Kd of the Type I receptor; values were similar to those previously reported for males. Type II receptor number showed a main effect of sex ($p < 0.02$): female groups had fewer Type II receptors than did male groups. These results indicate that the presence of estrogen down-regulates the Type I receptor but does not influence its affinity. In contrast, expression of the Type II receptor may be developmentally influenced by gonadal steroids. Supported by NS 22158.

527.3

POSTNATAL DEVELOPMENT OF CORTICOSTEROID RECEPTOR IMMUNOREACTIVITY IN THE RAT CEREBELLAR CORTEX. A. Lawson*, R. Ahima, Z. Krozowski* and R. Harlan. Dept. Anatomy, Tulane Med. School, New Orleans, LA 70112 and Baker Med. Inst. Prahan, Australia 3181 (ZK).

Glucocorticoids are required for normal CNS development. High levels are neurotoxic and reduce myelination. The actions of glucocorticoids are mediated via either a high affinity Type I (mineralocorticoid) receptor or a low affinity Type II (glucocorticoid) receptor. MRNA and immunoreactivity (ir) of both receptors have been localized in the adult rat cerebellar cortex, a region which undergoes most of its development during postnatal life. We have studied the development of Type I-ir and Type II-ir in the postnatal rat cerebellar cortex using MINREC4 antiserum and BUGR2 monoclonal antibody, respectively. Type I-ir Purkinje cells were first observed at postnatal day 5 (P5) in contrast to Type II-ir cells which were first observed at P15. In the Purkinje cell layer, both receptor systems developed rapidly to adult levels by P20. Type I-ir and Type II-ir in the granular and molecular layers were first observed at P5 and P15 respectively; however, the rate of development was slower. By P30 the density of immunoreactive cells in these layers was still less than in the adult. The earlier development of the Type I receptor system suggests it may mediate most of the actions of corticosteroids in the cerebellum during early postnatal life. Supported by NS24148.

527.4

NEONATAL PRAZOSIN TREATMENT REDUCES CYTOSOLIC ESTROGEN RECEPTOR LEVELS AND OVARIAN WEIGHT IN ADULT FEMALE RATS. R. H. Fitch† and H. Feder^. †Center for Molecular and Behavioral Neuroscience, ^Biological Sciences Department, Rutgers University, Newark, NJ, 07102.

Exposure of primed adult female guinea pigs to the alpha-1 antagonist prazosin has been shown to reduce estrogen receptor levels in the hypothalamus and preoptic area. To further investigate this interaction between the noradrenergic and neuroendocrine axes, newborn female rat pups (<2 hours old) received a s.c. implant of prazosin (.0125 mg/day for 5 days) or placebo. After establishing the presence of regular estrus cycles by daily vaginal smears, subjects were sacrificed by perfusion with 30cc cold DMSO on the morning of proestrus. The ovaries and uterus were removed and weighed. Brains were removed and frozen, and later the medial preoptic area (POA), the corticomedial amygdala (AMG), mediobasal hypothalamus (HYP), and a cortical sample (CTX) were dissected out and analyzed for cytosolic estrogen receptors. Results showed that receptor levels in the POA, HYP, and AMG were reduced by 32%, 44%, and 35% respectively for the prazosin group ($p<.05$). Cortical receptor levels were low and showed no group differences. Ovarian weight was also significtly reduced in the prazosin group ($p<.0001$), although uterine weight was unaffected. Interestingly, prazosin treated females showed a post-pubertal increase in body weight characteristic of ovariectomized females, while controls showed no such increase. These results indicate that neonatal prazosin exposure affected ovarian development and reduced, either directly or indirectly, cytosolic estrogen receptor levels in the POA, HYP, and AMG.

527.5

COCAINE DECREASES HYPOTHALAMIC ESTROGEN RECEPTOR mRNA LEVELS IN NEONATAL FEMALE RATS. C. Benton*, H. Najmabadi*, W.J. Raum*, S. Bhasin* and D.H. Olster. Division of Endocrinology, Harbor/UCLA Medical Center, Torrance, CA 90509.

Neonatal exposure to testosterone or estradiol (E) androgenizes the rat brain. We have previously shown that cocaine interferes with this process, and decreases hypothalamic nuclear estrogen receptor (ER) binding. To examine if these effects of cocaine are mediated via changes in hypothalamic ER mRNA levels, 3 day old female rats were left untreated, received E (0.2 μMol/kg) or E plus cocaine (10 mg/kg). ER mRNA levels were quantified by hybridization of northern blots with a human ER cDNA probe. E treatment increased levels of hypothalamic ER mRNA; this effect was blocked by cocaine. Thus, cocaine antagonizes E-stimulation of ER mRNA in the hypothalamus, perhaps by influencing ER mRNA transcription or stability. This may result in fewer ERs available for interaction with DNA-responsive elements, and in this manner interfere with activation of E-responsive genes involved in androgenization of the brain. (Supported by NIH DK 07571, DA 04490 and HD 23483.)

527.6

ONTOGENY OF ESTROGEN RECEPTOR-LIKE IMMUNOREACTIVITY IN THE BRAZILIAN SHORT-TAILED OPOSSUM BRAIN. C. D. Jacobson, L. R. Ross* and C. A. Fox. Department of Veterinary Anatomy, Iowa State University, Ames, IA 50011.

In this study, we have used the Brazilian short-tailed opossum (*Monodelphis domestica*) as a model to study the ontogeny of estrogen receptors in the mammalian brain. *Monodelphis* is a small, pouchless marsupial which breeds well under laboratory conditions and whose young are born in an immature sexually undifferentiated state. The Abbott H222 monoclonal rat estrogen receptor antibody (gift of Abbott Laboratories) was utilized in an indirect immunohistochemical procedure to detect estrogen receptors in the developing opossum brains. Estrogen receptors were first expressed in the dorsomedial and ventromedial hypothalamus of the opossum ten days after birth (10PN). Most regions that contained estrogen receptor immunoreactivity (ER LI) in the adult opossum contained ER LI at 15PN. These areas include the lateral septum, medial preoptic area, bed nucleus of the stria terminalis, periventricular preoptic area and hypothalamus, amygdala, dorsomedial and ventromedial hypothalamic nuclei, arcuate nucleus, ventral premammillary nucleus, and the midbrain central grey. The number of ER LI cells increased from 15PN-60PN in all regions of the brain that contain ER LI cells in the adult opossum. The time course of expression indicates that estrogen receptors are present in early development of the *Monodelphis* brain and may mark the beginning of a critical period for sexual differentiation of the opossum brain.

527.7

POST-NATAL DEVELOPMENT OF TARGET REGIONS FOR GONADAL HORMONES IN THE BRAIN OF THE CYNOMOLGUS MONKEY. R.P. Michael and R.W. Bonsall. Department of Psychiatry, Emory University School of Medicine and the Georgia Mental Health Institute, Atlanta, Georgia 30306.

To determine if the adult pattern of testosterone uptake and metabolism by brain develops before or after birth, 4 male and 5 female neonatal cynomolgus monkeys, gonadectomized 3 days earlier, were injected subcutaneously at 5-8 days of age with 500 μCi ^{3}H-testosterone (^{3}H-T). 60 min later, brains and other tissue samples were removed. Purified nuclear pellets and supernatants were prepared and analyzed by HPLC. The aromatized metabolite ^{3}H-estradiol (^{3}H-E_2) was found only in nuclear pellets from hypothalamus and amygdala where it represented 40-55% of the radioactivity. ^{3}H-T was the major androgen present in nuclear pellets from all 8 brain regions studied, and levels in both nuclear pellets and supernatant fractions were significantly higher in females than in males ($P<0.005$), suggesting a difference in clearance rates. Comparisons with orchidectomized fetuses at 122 days gestation and with fully adult male castrates showed that the largest developmental increase occurred in the amygdala after birth: nuclear levels of ^{3}H-E_2 were 4-fold higher in adults than in neonates. Nuclear concentrations of ^{3}H-T increased markedly during development in most brain regions except the cerebellar cortex where they were initially high and later declined. Major differences were also noted in brain supernatants: metabolic conversion of ^{3}H-T was much faster in fetuses and neonates than in adults. The development of the hormone-sensitive neural systems regulating behavior in adult primates may depend on changes in enzyme activities and receptor levels occurring after the neonatal period. Supported by USPHS grant MH 40420 and by the Georgia Department of Human Resources.

527.8

THE OCCUPATION OF NUCLEAR ESTROGEN BINDING SITES IN THE FETAL MACAQUE HYPOTHALAMUS BY STEROIDS OF TESTICULAR ORIGIN. R.W. Bonsall, D. Zumpe* and R.P. Michael. Department of Psychiatry, Emory University School of Medicine and the Georgia Mental Health Institute, Atlanta, Georgia 30306.

Previous results with macaque fetuses given ^{3}H-testosterone (^{3}H-T) at around 120 days of gestation demonstrated a sex difference in nuclear levels of the aromatized metabolite ^{3}H-estradiol (^{3}H-E_2) in hypothalamus. To determine if this might be due to prior occupation of receptors by endogenous steroids in the male, we have now compared the uptake of ^{3}H-T and its metabolites in intact male fetuses (plasma T 571.2 ± 215.5 ng/100 ml, N=5), sham-gonadectomized female fetuses (33.8 ± 25.2 ng/100 ml, N=5) and male fetuses orchidectomized *in utero* about 7 d earlier (14.6 ± 5.7 ng/100 ml, N=5). Fetuses were given 500 μCi ^{3}H-T s.c. and, 60 min later, brains were dissected to obtain the hypothalamus and 7 other samples. Purified nuclear pellets and supernatant fractions were analyzed by HPLC to identify radioactive metabolites. Hypothalamic nuclear concentrations of ^{3}H-E_2 in intact males (847 ± 195 dpm per mg DNA) were significantly lower than those in females (2,147 ± 542 dpm per mg DNA) and in orchidectomized males (2,233 ± 345 dpm per mg DNA) ($P<0.05$), but nuclear and supernatant concentrations of ^{3}H-T did not differ in the 3 groups. Thus, fetal orchidectomy eliminated the difference between males and females, supporting the original hypothesis that occupation of estrogen receptors is greater in intact male than in intact female fetuses at this stage of gestation. Aromatized metabolites of T acting via estrogen receptors during fetal life could, therefore, account for some of T's organizational effects on primate physiology and behavior. Supported by USPHS grant MH 40420 and by the Georgia Department of Human Resources.

527.9

ONSET OF ANDROGEN RECEPTOR mRNA EXPRESSION IN THE CNS OF *XENOPUS LAEVIS*; LOCALIZATION USING *IN SITU* HYBRIDIZATION. M.A. Cohen, L.M. Fischer, D.B. Kelley, Dept. Biol. Sci., Columbia Univ., NY, NY 10027

In the clawed frog, *Xenopus laevis*, male mate calling is controlled by testicular androgen secretion. Nuclei in the CNS calling circuit have been mapped; all have been shown to concentrate radiolabeled androgen, as does the effector organ, the larynx. Previous studies using tritiated dihydrotestosterone detected CNS androgen concentrating cells from tadpole stage 64 on; no labelled cells were detected at stage 60. The absence of labeled cells at stage 60 could be due to lack of androgen receptor (AR) expression or to competition for labelling by endogenous hormones. To determine the onset of AR expression in the calling nuclei, we localized AR transcripts in developing tadpole brains using *in situ* hybridization. A cloned AR cRNA probe, isolated from *X. laevis* laryngeal cDNA, was used. AR mRNA expression in neurons was detected as early as stage 56, when the gonads differentiate. At this and subsequent stages, transcripts were localized to motor nucleus of cranial nerves IX-X, medullary reticular formation, a presumed sensory nucleus of cranial nerve V, pretrigeminal nucleus of the dorsal tegmental area of the medulla, laminar nucleus of the torus semicirculus, anterior pituitary, ventral thalamus, and anterior spinal cord. The earliest stages of receptor expression and the hormonal control of this developmental program are under investigation.

Supported by NS 23684

527.10

DEVELOPMENTALLY REGULATED ECDYSTEROID BINDING AND IMMUNOCYTOLOGICALLY IDENTIFIED INSECT NEURONS. H.J. Bidmon,* W.E. Stumpf and N.A. Granger. Dept. of Cell Bio. and Anat., UNC, Chapel Hill, N.C. 27599.

To study the action of 20-hydroxyecdysone (20-HE) on the CNS of larval Manduca sexta, high affinity ecdysteroid binding sites ($K_D \leq 4nM$) in cerebral ganglia were labelled *in vitro* with ^{3}H-ponasterone A (PNA, a 20-HE agonist) or ^{125}I-PNA. Certain autoradiograms were immunostained for tyrosine hydroxylase or the prothoracicotropic hormone (PTTH). Analysis of autoradiograms revealed stage-specific expression of ecdysteroid receptors (ER) in the CNS during the 4th and 5th larval stadia. During the 4th stadium, the number of cerebral neurons with ER peaked on day 2 and decreased steadily until day 0 of the 5th stadium. In the 5th stadium, the number of target neurons peaked at the times of the two ecdysteroid peaks. ER during both stadia were present in neurosecretory centers of the protocerebrum, including the prothoracicotropes, and in certain neurons of the subesophageal ganglion (SEG). The prothoracicotropes exhibited nuclear ER only during periods of active ecdysteroid synthesis by the prothoracic glands. Similar developmental fluctuations in numbers of neurons with ER occurred in the SEG, where several target neurons stained for tyrosine hydroxylase. The results demonstrate stage-specific ecdysteroid binding in neurons with identified peptide and neurotransmitter functions.

HORMONES AND DEVELOPMENT: CNS

528.1

ESTROGEN INCREASES PROENKEPHALIN mRNA EXPRESSION IN THE VMN OF JUVENILE MALE AND FEMALE RATS. Annabell C. Segarra, Jesus A. Angulo and Bruce S. McEwen. The Rockefeller University, Laboratory of Neuroendocrinology, 1230 York Ave., New York, N.Y. 10021.

Opioids have been implicated in sexual differentiation of the brain and in the regulation of reproductive behavior in adult rats. Previous studies indicate that estrogen administration in adults increases proenkephalin mRNA in the VMN of female, but not male, rats (Romano et al., Brain Res.1990; 536:63). We have determined proenkephalin mRNA expression in estrogen-treated juvenile male and female rats to investigate the developmental pattern of estrogenic regulation of enkephalinergic neurons in the VMN. Rats were gonadectomized at 16 days of age and treated with estradiol benzoate (40 μg/kg) or oil from day 21-23. Sections of the ventromedial nucleus (VMN) and preoptic area (POA) were studied by in situ hybridization histochemistry at the single cell level and quantified with the assistance of an image analysis system. Estrogen caused an upward shift in the amount of mRNA expressed per cell in the VMN of male and female juvenile rats. No significant sex difference was observed in oil-treated animals nor in the response to estrogen treatment. Taken together, these results indicate that there is a difference in the response of VMN enkephalinergic neurons of male rats according to the developmental stage studied. A similar situation has been reported in estrogen induction of spine density (Segarra & McEwen, 1991; Neuroendocrinol., in press) and of cytosol progesterone receptors (Bogic et al., 1988; Endocrinol. 112:2735) in the VMN of juvenile vs adult male rats. It is possible that the testosterone surge during puberty renders the male VMN unresponsive to estrogenic induction of enkephalinergic messsage (WS 07080 and F34 GM13001-01).

528.2

LOCALIZATION AND HORMONAL REGULATION OF THE c-raf-1 PROTO-ONCOGENE IN DEVELOPING RAT HYPOTHALAMUS. R.C. Whorf and S.A. Tobet, Dept. of Biochemistry, E.K. Shriver Center, Waltham, MA 02254 & Prog. in Neuroscience, Harvard Medical School, Boston, MA 02115.

In studying sexual differentiation of the brain, we examined the expression of the protein product of the proto-oncogene c-*raf*-1 (Raf-1). We have immunocytochemically and biochemically determined the localization of Raf-1 in embryonic rat brain regions and its regulation by hormonal stimulation using an affinity-purified anti-peptide antiserum specific for Raf-1 (NH-44). Western blot analysis revealed a 77 kD polypeptide isolated in the cytosol of developing rat brains. Female embryonic day 22 (E22) hypothalami (HYP) contained the highest levels of Raf-1, 2-fold (1.5-2.3) higher than male HYP by semi-quantitative western blot and quantitative protein dot blot analyses. HYP levels were greater than brainstem or cortex in western blots. To test hormonal regulation of Raf-1, testosterone propionate (TP) was administered to pregnant rats on E17; male and female fetuses were examined on E22. This treatment significantly decreased Raf-1 levels in female HYP, but not in male HYP, as determined by western blot analysis. No significant sex differences or response to hormone treatments were observed in either the brainstem or cortex. Administration of TP 3h after birth did not change Raf-1 levels examined 24h later. In summary, Raf-1 was localized within selective regions of the rat brain and its expression altered by exogenous prenatal hormonal stimulation. Raf-1 signal transduction may act to delimit hormonal critical periods in sexual differentiation of the brain.

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528.3

IMMUNOCYTOCHEMICAL AND HISTOCHEMICAL ANALYSES OF GONADOTROPIN RELEASING HORMONE (GnRH), TYROSINE HYDROXYLASE (TH), AND CYTOCHROME OXIDASE (CO) ACTIVITY WITHIN THE SEPTAL, HYPOTHALAMIC, AND THALAMIC AREAS OF CHICKS SHOWING EARLY SEXUAL MATURATION. G. S. Fraley*, W. M. Saidel, and W. J. Kuenzel of University of Maryland at College Park, MD 20742.

A modified, retractable Halasz knife was used to produce parasagittal cuts isolating the hypothalamus from lateral connections in chicks two weeks of age. By five weeks of age, operated chicks which showed advanced development of secondary sex characteristics were selected, matched with sham-operated controls and prepared for immunocytochemical analysis of brain tissue. Three nuclei showed significant differences between knife-cut birds and controls: the bed n. of the pallial commissure (nCPa), the paraventricular n. (PVN), and the anterior lateral thalamic n. (LA). A greater number of both TH and GnRH perikarya were found in the nCPa of respondant birds compared to sham-operated controls. A greater number of GnRH neurons were also found in the LA of knife-cut birds. The PVN of experimental birds had densely staining cells when reacted with Cytochrome Oxidase indicating increased metabolic activity. These brain nuclei may be important for the control of gonadal development in birds.

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528.4

CASTRATION AFFECTS THE GnRH NEURON SYSTEM OF MALE DJUNGARIAN HAMSTERS WITH DELAYED PUBERTY. S.M. Yellon and C.P. Haase*. Div. Perinatal Biology, Depts. Physiology, Pediatrics, and Anatomy, Loma Linda Univ., Sch. of Med., Loma Linda, CA 92350.

Puberty in the Djungarian hamster (age 25 d) is characterized by peak gonadotropin secretion and increased numbers of unipolar but not bipolar GnRH-immunoreactive (GnRH-i) cell bodies in the medial preoptic area (MPOA) and diagonal band of Broca (DBB), regions that contain the majority of GnRH perikarya. The pubertal increase in GnRH-i cell number can be blocked by exposure to short days (*Endo 124 Suppl:257A, 1989*). To determine whether the testes or testosterone (T) contribute to this photoperiod effect, males reared in short days (10L:14D) were castrated at 12 d (n = 10) and implanted with a blank or T-filled silastic capsule (5 mm s.c.). Testis-intact males (n = 5), as well as comparably treated males reared in long days (16L:8D; n = 15) served as control. Brain sections (60 μm) from the MPOA and DBB were processed for GnRH immunocytochemistry (LR1 from R. Benoit). In long-day reared males, regardless of treatment more unipolar GnRH-i soma were present at 25 d (71 ± 4) compared to that at 15 d (42 ± 3; $p < 0.05$, ANOVA). At each age (15 or 25 d), GnRH-i cell numbers were the same in castrates, blank or T-treated, as in testis-intact controls. Thus, the testes or T do not contribute to the pubertal increase in GnRH-i neuron number. In short days, similar numbers of unipolar GnRH-i cells were found in both 15 and 25 d testis-intact males and 15 d old castrates, comparable to that in 15 d males in long days. By contrast, castrate males in short days at 25 d (blank and T-treated) had significantly more unipolar GnRH-i cells (vs 15 d; $p < 0.05$), numbers equivalent to that in long-days males at 25 d. Bipolar GnRH-i cell numbers were the same regardless of age or treatment. Findings indicate that the testis but not testoster-one contribute to the mechanism by which short days block pubertal development of the GnRH neuron system in the Djungarian hamster.

HD22479

528.5

SEX DIFFERENCES IN SYNAPTIC NUMBERS IN THE BINOCULAR AREA OF THE VISUAL CORTEX OF THE RAT. Silvia N. M. Reid and Janice M. Juraska. Neuroscience Program and Department of Psychology, University of Illinois, Champaign 61820.

We have previously found that male rats have more neurons in the binocular area of the primary visual cortex (Oc1B) and a larger Oc1B than female rats (Reid & Juraska, Neurosci. Abst., 1990). In the present study we investigate sex differences in synaptic connections. Synaptic density of layer II-III of the Oc1B was estimated from 7 littermate pairs of 90 day old Long-Evans hooded rats. The number and the length of synapses were obtained from 12 electromicrographs (40,000X) for each hemisphere of each animal. Synaptic length was used as a correction factor in estimation of synaptic densities.

Our preliminary data show that synaptic densities, the average synaptic length and the synapse-to-neuron ratio were not significantly different between sexes. Because layer II-III of Oc1B in the male rat was larger, it contained more asymmetrical synapses and total synapses (20%) than that of the female rat. More animals will be added. Supported by NSF BNS 89-09164.

528.6

EFFECT OF PERINATAL ESTROGEN ON THE CORPUS CALLOSUM OF ADULT FEMALE RATS. C. M. Mack*, R. H. Fitch, P. E. Cowell, L. M. Schrott, and V. H. Denenberg. Biobehavioral Sciences Graduate Program, University of Connecticut, Storrs, CT 06269 and Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ 07102.

To determine the contribution of perinatal estrogen on adult callosal size, female rats were either ovariectomized (OVX) on postnatal day 12 (P12) or were OVX followed by chronic implantation of a 10mm silastic tube containing B-estradiol (E) on P25. Unmanipulated males and sham females served as controls. The corpus callosum was examined at 110 days of age. The software package Stereology yielded callosal parameters of area, perimeter, length and 99 widths taken perpendicular to the longitudinal axis. Seven regional width factors, derived from a prior factor analysis of the rat callosum, were used to analyze group differences. The factors are widths 1-5 (W1-5), W6-17, W24-38, W46-57, W62-72, W79-95 and W96-99. Males had a larger callosal area than females and were also larger for regions W1-5, W6-17, W62-72 and W79-95. OVX females had a larger callosal area than control females and were also larger for W46-57 and W79-95. These results confirm our prior findings. In addition, the callosal area of the OVX + E females was smaller than that of the OVX group. Regional analyses revealed a smaller callosum in W6-17, W24-38, W46-57, W62-72, W79-95. These findings indicate that the presence of perinatal estrogen contributes to the feminization of the rat callosum. In addition, the critical period of estrogenic effects upon the adult female callosum extends at least through P25.

528.7

ONTOGENY OF A SEXUALLY DIMORPHIC PREOPTIC NUCLEUS IN JAPANESE QUAIL (Conturnix japonica). R. Thompson and E. Adkins-Regan, Department of Psychology, Cornell University, Ithaca, NY 14851.

The nucleus preopticus medianus (POMn) is a sexually dimorphic nucleus that is critically involved in the hormonal activation of male copulatory behavior. The larger volume observed in adult males appears dependent upon circulating testosterone (Panzica, Viglietti-Panzica, Calcagni, Anselmetti, Schumacher & Balthazart, 1987). The present study is an attempt to determine exactly when during normal development this nucleus becomes dimorphic. POMn was drawn under a projecting microscope in Nissl stained coronal sections (40 um) from animals sacrificed at two weeks of age (n=3 per sex) and three weeks of age (n=3 per sex). Areas were measured by computerized planimetry from consecutive serial sections (n=10 per animal) immediately anterior to and including the final section in which the anterior commisure (CA) was observed, so chosen because the nucleus gradually increases in size in sections moving caudally towards CA, appears at its largest immediately ventral to CA, and quickly dissappears in sections more posterior. POMn volume was derived from these area measurements. No significant sex differences in POMn volume were observed at either developmental age Our results indicate that the sexual dimorphism of POMn volume observed in adult birds is not a simple function of embryonic hormone exposure, but instead likely involves a dependence upon the pubertal increases in testosterone that begin around 3 weeks of age (Ottinger & Brinkley, 1979). Currently, identical measurements are being taken from birds 4,5,6,7 and 8 weeks of age, and sample sizes are being increased to n=6 per sex for all developmental ages, including those reported above.
[NSF #BN5 88-09441]

528.8

DEVELOPMENT OF SEX DIFFERENCES AMONG HVC NEURONS BORN DURING ZEBRA FINCH SONG LEARNING. E.J. Nordeen, M.J. Burek, and K.W. Nordeen. Dept. Psych., and Neuroscience Program, U. Rochester, Rochester, N.Y. 14627.

The higher vocal center (HVC), a region necessary for song production in zebra finches, contains more neurons in adult males (who sing) than in females (who do not sing). This dimorphism is regulated by early estrogen exposure and arises because new HVC neurons are added in greater numbers in juvenile males than in females. It is not known when these late-generated HVC neurons first become sexually dimorphic and thus, whether estrogen promotes their production or survival. We gave males and females 3H-thymidine on days 15 and 16 after hatching to label HVC neurons born on these days. Labeled neurons within HVC were then counted at 2, 13, and 39 days after the 3H-Thy regime. Neuronal labeling was not seen in HVC 2 days after 3H-Thy, implying that longer is needed for migration and/or neuronal differentiation. But, by 13 days after their birth new HVC neurons were already sexually dimorphic; the number of labeled HVC neurons being 4-5X higher in males than in females. This sex difference enlarged only slightly between 13 and 39 days after cell birth because a larger proportion of labeled HVC neurons was lost in females than in males during this time. However, this neuronal loss was not significant in either sex. Currently, we are counting labeled HVC neurons at other timepoints to determine if sex differences in their number emerge before these cells incorporate into HVC and interact with sexually dimorphic afferent or efferent targets.

HORMONES AND DEVELOPMENT: MOTOR NEURONS

529.1

DEVELOPMENT OF NEURONAL NUMBER IN PRIMARY AFFERENTS OF A SEXUALLY DIMORPHIC NEUROMUSCULAR SYSTEM IS ANDROGEN-DEPENDENT. A. Mills and D.R. Sengelaub. Program in Neural Science, Indiana University, Bloomington, IN 47405.

The spinal nucleus of the bulbocavernosus (SNB) in the rat contains many more motoneurons in males than in females. Androgens produce this sex difference by influencing normally occurring motoneuron death, but the site of androgenic action is unclear. Primary afferents have been previously demonstrated to be important in the normal development of spinal motor nuclei (Davis et al., 1983; Okado and Oppenheim, 1984). To investigate the potential role of afferents in the regulation of SNB motoneuron numbers, we studied the development and androgen dependence of the dorsal root ganglia (DRGs) which innervate SNB target musculature.

Male rats injected with WGA-HRP or Fluorogold into an SNB target, the bulbocavernosus muscle, showed extensive labeling of the L6 and S1 DRGs. Neuronal counts were therefore restricted to these DRGs, and made in normal males (M) and females (F) from embryonic day (E)18 through postnatal day (P)10, the period during which SNB motoneuron number is established. Counts were also made in females treated with testosterone propionate (TP) on E16-E22, P1, P3, and P5, a regimen which results in complete masculinization of SNB motoneuron number. Counts of DRG neurons declined from E18 through P10, by which time males had more DRG neurons than females [L6/S1 total: M (N=3), mean=8650 ± 220; F (N=3), mean=7030 ± 310]. Androgen treatment of females masculinized DRG neuron number at P10 (N=2; mean=8000 ± 470). These results suggest that the DRG can be masculinized by TP, a finding consistent with the hypothesis that primary afferents may be important in establishing the androgen-dependent sex differences in SNB motoneuron number.
(Supported by NIH NS08917 to AM)

529.2

HORMONAL CONTROL OF DENDRITIC DEVELOPMENT IN SEXUALLY DIMORPHIC RAT SPINAL NUCLEI. L.A. Goldstein and D.R. Sengelaub. Program in Neural Science, Dept. of Psychology, Indiana University, Bloomington, IN 47405.

Dendritic development of motoneurons in the spinal nucleus of the bulbocavernosus (SNB) is biphasic and androgen dependent. During normal postnatal development, SNB dendrites grow exuberantly and then retract to mature lengths. In adult males castrated at one week of age, SNB dendrites fail to grow beyond pre-castration lengths, but grow exuberantly in castrates treated with testosterone (T). To determine if dendritic growth involves the conversion of T to dihydrotestosterone (DHT) or estrogen (E), male rats were castrated on postnatal day (P) 7 and given daily injections of either DHT or E. Dendritic length and soma size of all SNB motoneurons retrogradely labeled with HRP were measured at P28 or P49. Results show that DHT, but *not* E, is as effective as T in supporting dendritic growth of SNB motoneurons, lending further support for the role of androgens but not estrogen in the masculinization of sexually dimorphic spinal motor nuclei.

The dendritic development and androgen regulation of motoneurons in the dorsolateral nucleus (DLN) were also examined. Using HRP, dendritic length and soma size of DLN motoneurons in normal males and in castrates treated with either T or oil vehicle were measured at P7, P28, P49 and P70. Like the SNB, dendritic development in the DLN is androgen dependent. Unlike the SNB, dendritic growth in the DLN is monotonic; the dendritic length of motoneurons increases more than 500% between P7 and P70. These results suggest that in addition to androgens, other factors may play a part in regulating dendritic development. Given that SNB and DLN motoneurons occupy different spinal locations and have different dendritic geometries, one factor may be the differential distribution of afferents to the two nuclei.
(Supported by NIH NS24877)

529.3

ROLE OF GONADAL STEROIDS IN THE DEVELOPMENT OF LAMINA X MOTONEURONS THAT INNERVATE PERINEAL MUSCLES IN MONGOLIAN GERBILS. T.B. Decker, C. Ulibarri, and T.R. Akesson. Dept of Veterinary and Comparative Anatomy, Physiology, and Pharmacology, College of Veterinary Medicine, Washington State University, Pullman, WA 99164-6520.

The bulbocavernosus (BC), levator ani, and anal sphincter muscles of gerbils are innervated by a sexually dimorphic, androgen-sensitive motoneuron pool located above the central canal in lamina X of lumbosacral spinal cord. This research investigated the role of postnatal gonadal steroids in the development of this unique motor system.

On the day of birth (P1) male gerbils were castrated or given sham surgeries. Female gerbils were injected with 100 μg dihydrotestosterone propionate and 20 μg diethylstilbestrol in 0.05 ml safflower oil or vehicle. As adults (P60) gerbils were castrated as necessary and implanted with Silastic capsules of testosterone propionate (10 mm). Four weeks later gerbils received injections of 1 μl of cholera toxin-HRP (0.2% CT-HRP) in each side of the BC. After one week gerbils were perfused and alternate sections were processed for visualization of CT-HRP (Mesulum, *J Histochem Cytochem 26*:106) or stained for Nissl substance.

Neonatally castrated males, steroid-treated females and vehicle-treated males had clearly visible BC muscles. Vehicle-treated females did not. Virtually all of the CT-HRP labelled motoneurons were located in lamina X above the central canal (213/214). Neonatally castrated males had fewer motoneurons than did vehicle-treated males (171 ± 5 vs 190 ± 9, respectively). Steroid-treated females had fewer motoneurons than did males (104 ± 4.9), but many more than vehicle-treated females (30 ± 2). These data suggest that the BC-spinal cord system develops under the control of gonadal steroids. Additionally, exposure to steroids either prenatally, or as a single low dose postnatally is sufficient to masculinize the BC and its innervating motoneurons. Supported by HD 22869 (TRA).

529.4

MOTONEURONS IN LAMINA X INNERVATE PERINEAL MUSCLES IN MONGOLIAN GERBILS. C. Ulibarri, P. Popper, and P.E Micevych. Dept of Veterinary & Comparative Anatomy, Physiology, and Pharmacology, College of Vet Med, Wash State Univ, Pullman, WA 99164 & Dept of Anatomy & Cell Biol, Lab of Neuroendocrinol, Brain Res Institute, UCLA Sch Med, LA, CA 90024.

The spinal nucleus of the bulbocavernosus innervates the bulbocavernosus (BC), levator ani, and anal sphincter (AS) muscles in several rodent species. Onuf's nucleus innervates these same muscles as well as the ischiocavernosus muscle and bladder sphincters in primates and carnivores. The purpose of this research was to define the location of the spinal motoneuron pool innervating the perineal muscles of gerbils and to determine if these motoneurons are sexually dimorphic and/or sensitive to adult castration effects.

Fluorogold (4%) or cholera toxin-HRP (0.2%) was injected into the BC, AS or IC in male and female gerbils. After IC injections labelled motoneurons were present in the dorsolateral nucleus of ventral horn in lumbar 5 and 6, as described in rats. The motoneurons labelled after BC or AS injections were found in lamina X above the central canal in lumbar 5 and 6 of spinal cord. We believe that this is the first report of motoneurons in lamina X of the spinal cord.

The motoneuron pool in lamina X was sexually dimorphic. Male gerbils had six times as many motoneurons as female gerbils, and motoneurons of males were significantly larger. Intact males had 192 ± 12 motoneurons (cell surface area 642.2 ± 10.5 μm^2) in the lamina X motoneuron pool while intact females had 31.9 ± 3 motoneurons (cell surface area 437.2 ± 19.3 μm^2).

Adult castration decreased the size of these motoneurons, but had no effect on the number. Castrated males had significantly smaller motoneurons (540.1 ± 15.1 μm^2) than intact males or males implanted with Silastic capsules of testosterone propionate at castration (658.8 ± 16.4 μm^2). Supported by NS 21220 to PEM.

529.5

PRENATAL FLUTAMIDE ALTERS SEXUALLY DIMORPHIC SPINAL NUCLEI. **W.Grisham, J.M.Casto, M.L.Kashon, I.L.Ward, and O.B.Ward. Department of Psychology, Villanova University, Villanova, PA 19085**

The dorsolateral nucleus (DLN) and spinal nucleus bulbocavernosus (SNB) of the lumbar spinal cord are sexually dimorphic. Males have more neurons than females in both nuclei and males have larger neurons in the SNB. The spinal cords of 16 male and 9 female offspring of rats which received 5 mg of the anti-androgen, flutamide, on days 11 through 21 of gestation and 16 male and 16 female controls were examined.

Flutamide males had fewer DLN neurons ($\bar{X}$=301) than did control males ($\bar{X}$=488), but flutamide females ($\bar{X}$=258) were not different from control females ($\bar{X}$=280). Flutamide males also had significantly fewer and smaller SNB neurons than did control males. Flutamide did not significantly alter the SNB of females. These results support the hypothesis that the sexually dimorphic characteristics of the DLN and SNB are androgen dependent.

529.6

DELAYED EXPRESSION OF CALCITONIN GENE RELATED PEPTIDE (CGRP) IN SNB MOTONEURONS. Nancy G. Forger, Lynn Hodges and S. Marc Breedlove, Dept. Psychology, Univ. California, Berkeley, CA 94720.

Motoneurons of the spinal nucleus of the bulbocavernosus (SNB) innervate sexually dimorphic perineal muscles. The development of this neuromuscular system is androgen dependent and differs temporally from that in other motoneuronal pools: 1) the normal period of motoneuron death is later in the SNB, with significant loss occurring postnatally and 2) neuromuscular synapse elimination occurs later in SNB cells. CGRP expression has been correlated with the period of motoneuron death in chicks and has recently been shown to be androgen regulated in rat SNB motoneurons in adulthood (Popper & Micevych, '89). We therefore examined CGRP-like immunoreactivity (CGRP-LI) in motoneurons of the lumbar cord of rats from postnatal day 3 to adulthood. In accord with previous findings, many lateral horn motoneurons were clearly positive for CGRP-LI on day 3 and all subsequent ages examined. In contrast, no CGRP-LI was detected in SNB cells on day 3 and only faint staining was observed on days 9-10. By day 16 occasional heavily labeled cells were found in the SNB and the adult pattern of CGRP expression was present by day 33. CGRP expression therefore appears to be significantly delayed in SNB motoneurons, suggesting an association with the events of cell death and/or synapse elimination.

529.7

ANDROGEN REGULATES GAP JUNCTION mRNA EXPRESSION IN THE MOTONEURONS OF LUMBAR SPINAL CORDS IN ADULT MALE RATS. A. Matsumoto (1), Y. Arai* (1), A. Urano (2) and S. Hyodo* (3). (1) Dept. Anat., Juntendo Univ. Sch. Med., Hongo, (2) Ocean Res. Inst., Univ. Tokyo, Nakano, (3) Fac. Art & Sci., Univ. Tokyo, Meguro, Tokyo, Japan.

Gap junctions are considered to play an important role in metabolic and electrical coupling between neurons. We studied androgenic influence on the expression of the mRNA for gap junction protein in the androgen-sensitive motoneurons in the spinal nucleus of the bulbocavernosus (SNB) by using in situ hybridization. Adult male rats (Wistar) were castrated and implanted with silastic tubes containing testosterone (T) or nothing. Animals were sacrificed 4 weeks later. A complementary DNA specific for the mRNA for rat liver gap junction protein (connexin 32)(a gift from Dr. D.A. Goodenough) was applied to in situ hybridization on cryostat sections of the lumbar spinal cords. Autoradiographic signals for connexin 32 mRNA were found to be localized on the somata and proximal dendrites of SNB motoneurons. The number of signals per motoneuron in castrates was significantly smaller than that in controls. The value in castrates given T was not significantly different from that in controls. These evidence suggests that androgen may regulate the expression of gap junction gene in SNB motoneurons. (Supported by grant from the Ministry of Education, Culture and Science of Japan).

529.8

ANDROGENIC MODULATION OF THE EXCITABILITY OF LUMBAR NEURONS IN THE RAT BULBOCAVERNOSUS REFLEX. J. Tanaka & A.P. Arnold. Dept. Psychology, and Brain Research Institute, UCLA, Los Angeles CA 90024-1563.

Since motoneurons in the spinal nucleus of the bulbocavernosus (SNB) are sensitive to androgens, we compared the bulbocavernosus (BC) reflex, recorded in BC motor nerve in response to electrical stimulation of the contralateral pudendal afferents, in intact (n=16), castrated (n=14) and testosterone-treated (n=12) male rats under urethane anesthesia. No group differences in reflex latency, sensory or motor conduction velocity, or central delay were observed. We also examined the effects of stimulation of the contralateral pudendal sensory or BC motor nerves on extracellular antidromic responses of SNB motoneurons evoked by stimulation of the BC motor nerve. Stimulation of the contralateral pudendal afferents 7-35 msec prior to stimulation of the BC motor nerve reduced the amplitude of averaged antidromic potentials recorded in the SNB nucleus, whereas contralateral BC motor nerve stimulation had no effect. The magnitude of this suppression was much greater in intact and testosterone-treated castrated males than in castrates, indicating that androgen modulates the excitability of SNB motoneurons or other neurons involved in the BC reflex. Supported by NIH grant HD15021.

529.9

ANDROGENS DIFFERENTIALLY AFFECT SYNAPTIC EFFICACY WITHIN MOTOR UNITS OF A SEXUALLY DIMORPHIC MUSCLE. N. Nagaya-Stevens and A. A. Herrera, Dept. of Biological Sciences, University of Southern California, Los Angeles, CA 90089.

The flexor carpi radialis (FCR) is a sexually dimorphic forelimb muscle used by male frogs during mating. Preliminary data indicate that motor units (MUs) in the shoulder and elbow regions of the FCR are innervated by motor neurons in spinal segments 2 and 3, respectively. Studies comparing castrated males (C) with castrates given testosterone (CT) have shown that FCRs from CT males contract more slowly and have larger fiber cross sectional areas (CSAs). The effects of testosterone on CSA and contraction time are more pronounced in shoulder MUs. Using intracellular recording, we found that many shoulder fibers in CT muscles are innervated by subthreshold junctions. Only 63 % of shoulder junctions in CT muscles triggered fiber action potentials with a single nerve stimulus compared to 97 % of shoulder junctions in C muscles. Elbow junctions were found to be mainly suprathreshold in both CT and C muscles (80 and 98 %). These results suggest that testosterone may preferentially affect the synaptic efficacy of neuromuscular junctions within motor units in the shoulder region. The larger CSAs of shoulder fibers from CT muscles suggest that lowered synaptic efficacy may result from a decrease in input impedance (R_o). Mean R_o of shoulder fibers in CT muscles was significantly less than in C muscles (0.29 vs. 0.44 MΩ; $p < 0.0002$). We are currently investigating whether changes in neurotransmitter release may also contribute to altered synaptic efficacy.

Supported by NIH grant NS 27209.

529.10

CELLULAR SPECIFICITY OF STEROID INFLUENCES ON PROCESS OUTGROWTH OF IDENTIFIED MOTONEURONS IN CULTURE. J.L. Witten and R.B. Levine, ARL, Division of Neurobiology, U. Arizona, Tucson, AZ 85721.

Dramatic remodeling of the central nervous system (CNS) occurs during insect metamorphosis to accompany the changing lifestyle of the animal. In the moth, Manduca sexta, identified motoneurons exhibit cell-specific changes in morphological and biochemical properties during this transition and these changes are mediated by the steroid hormones, the ecdysteroids. To determine whether these changes result from the direct action of the steroid hormone on the neurons and to explore mechanisms underlying the cellular specificity of these effects, we have developed methods to culture identified motoneurons. We compared the effects of ecdysteroid on neurite outgrowth in two sets of identified motoneurons that have different developmental fates. Leg motoneurons display extensive growth of their central arborizations during adult development in vivo (Kent and Levine, '88) and have been shown previously to exhibit enhanced neurite outgrowth in the presence of ecdysteroids in vitro (Griffin and Levine, '89). In contrast to the leg motoneurons, the motoneurons that innervate the abdominal intersegmental muscles (ISM) do not exhibit significant dendritic growth during adult development in vivo, although they do alter the expression of a neuropeptide (Witten and Truman, '90). Accordingly, the ISM motoneurons do not show a steroid-mediated enhancement of neurite outgrowth in culture. Thus, these experiments confirm the specificity of steroid hormone action on process outgrowth.

529.11

LARYNGEAL NERVE AXON NUMBER IN PRE-METAMORPHIC *XENOPUS LAEVIS* IS ANDROGEN SENSITIVE. J. C. Robertson, J. T. Watson and D. B. Kelley, Dept. of Biol. Sci., Columbia Univ., New York, NY 10027.

Sexual dimorphism in the number of axons in the laryngeal nerve of the clawed frog (*Xenopus laevis*) originates before metamorphosis. At stage 56, males and females have equal numbers of laryngeal nerve axons; at later stages through adulthood, males have more axons. To investigate possible influence of androgen on laryngeal nerve axon number, tadpoles were maintained from stage 54 to 62 in water treated with dihydrotestosterone (DHT, Sigma), the anti-androgen flutamide (Schering Corp.) or ethanol (vehicle). Axons were counted on transmission electron micrographs of laryngeal nerves. Treatment with DHT (0.001 mg/L) increased axon number in both sexes (males=860, females=865) as compared with controls (male=675, female=460). At a concentration of 0.2 mg/L, axon number of flutamide treated males (578) was lower than that of control males, but not as low as control females. Number of axons in females from this flutamide group (498) was not significantly different from control females. With a flutamide dosage of 1.0 mg/L, male (588) and female (498) axon numbers did not significantly differ from the lower flutamide treatment. Hypermasculinization of axon number with DHT treatment may involve recruitment of additional vocal motor neurons or axon sprouting. The similar effect on male axon number of two flutamide concentrations could indicate that androgen sensitivity of this system begins prior to stage 54, that conversion of flutamide to an active form may be incomplete, or that other androgens may be involved.
Supported by NS 23684.

HORMONES AND DEVELOPMENT: THYROID HORMONE

530.1

ROLE OF THYROID HORMONES ON OLFACTORY NEUROGENESIS IN THE POSTNATAL RAT: A QUANTITATIVE 3H-THYMIDINE STUDY. M. Paternostro and E. Meisami Physiol. Dept., Univ. of Illinois, Urbana, IL 61801

We have previously shown that the marked increase in surface area and total neuronal number occurring in the olfactory epithelium(OE) of growing rats is severely reduced in rats made hypothyroid (HT) from birth, while rats allowed to recover from hypothyroidism at 25d showed complete recovery in these parameters by 90d. To better understand these effects, a quantitative 3H-thymidine study was undertaken. Normal(N) and HT rats were injected with 3H-thymidine(5uCi/gbw) at 25d and 75d, while recovery rats were injected at 75d. Rats were sacrificed 1, 5 or 15d post injection(pi). Autoradiographs from septal OE were studied by measuring the density of labeled cells in both the basal cell zone(bcz) and the receptor zone(rz). Results from the 25d injection group revealed at 1d pi, a 25% reduction in the density of labeled cells in the bcz of HT rats(p=0.003). In both groups at 5d and 15d pi, the density of labeled cells was markedly decreased in the bcz and proportionally increased in the rz. The density of labeled cells in the rz at 15d pi was not significantly different between the two groups. Thus early thyroid deficiency reduces the rate of basal cell proliferation, but does not seem to significantly affect the survival and migration of the developing neurons. Work is currently being carried out on the 75d injection group.

530.2

THYROID HORMONE DEFICIENCY DISRUPTS DEVELOPMENT OF β-ADRENERGIC SIGNAL TRANSDUCTION IN THE NEONATAL RAT HEART. J. B. Pracyk and T.A. Slotkin, Dept. Pharmacol., Duke Univ. Med. Ctr., Durham, NC. 27705. (EPA CR-813769, USPHS HD-09713, CIIT fellowship)

In mature animals, thyroid hormone regulates cardiac β-adrenergic receptor numbers and the efficiency of receptor transduction. Exposure to excess thyroid hormone during early neonatal periods promotes the development of post-receptor transduction components independently from alterations in receptors; the current study determined whether normal development of β-adrenergic transduction requires endogenous thyroid hormone. To achieve neonatal hypothyroidism, pregnant rats were given propylthiouracil 20 mg/kg s.c. beginning on gestational day 17 through postnatal day 5 and their offspring were given the same dose on postnatal days 1 through 5. We evaluated receptor binding capabilities and adenylate cyclase activity in membrane preparations. Hypothyroidism produced an initial inhibition of [^{125}I]pindolol binding to cardiac membranes, followed by a return to control levels after discontinuing treatment. During the first two weeks, isoproterenol-stimulated adenylate cyclase activity displayed subsensitivity, corresponding to the deficits in β-receptor binding. Basal and forskolin-stimulated cyclase activities were initially unaffected, implicating a specific role for thyroid hormone in early stages of coupling of enzymatic responses to β-receptors. Thyroid induced deficiencies in basal and forskolin-stimulated adenylate cyclase activity eventually emerged, suggesting that, at later stages, normal development of adenylate cyclase requires prior establishment of a euthyroid state.

530.3

DISTINCT INDUCTION PATTERNS OF c-fos AND OTHER EARLY RESPONSE GENES BY INSULIN, HYDROCORTISONE, AND THYROID HORMONE IN GLIAL CELLS.

J. Cheng*, A. T. Arenander and J. de Vellis. Mental Retardation Research Center, Department of Anatomy and Cell Biology, Department of Physiology, and the Brain Research Institute, UCLA, Los Angeles, CA. 90024.

Recent evidence suggest various extracellular stimuli, such as growth factors and hormones, induce the cellular immediate early, or early response genes (ERGs) in neural cells. As such, ERGs may participate in the signal transduction processes that coordinate CNS development. However, whether the ERG products can confer the intracellular specificities of the extracellular signals is not clear.

In this study, we examined the induction of ERG mRNAs by insulin, hydrocortisone, and thyroid hormone in glial cells. We asked whether ERG expression could confer intracellular specificity by examining the patterns of ERG induction by these ligands individually or in combination. RNAs from treated astrocytes or C6 glioma cells were examined by northern analysis using ^{32}P-labeled cDNAs encoding for c-fos, c-jun, junB, egr1, NGF1B, or TIS11. Results showed that insulin, hydrocortisone, and thyroid hormone induced ERGs. The amounts and kinetics of ERG induction, however, differed in each treatment. Differences in the levels and time courses of mRNA expression were also observed when cells were treated with combinations of the ligands. Analysis of the ERG induction kinetics by various treatments showed that a specific pattern of ERG expression exists for a specific extracellular stimulus. These results suggest specific phenotypic responses to insulin, hydrocortisone, and thyroid hormone could be conferred by differential patterns of ERG expression. (Supported by NIH grant HD-06576 and DOE)

530.4

PERINATAL HYPOTHYROIDISM ELEVATES HIPPOCAMPAL MU–OPIOID RECEPTOR BINDING SITE DENSITY IN 31–DAY–OLD SPRAGUE–DAWLEY RATS. C.F. Sanchez,* K.A. Ortiz,* L.L. Paxton* and D.D. Savage. Department of Pharmacology, Univ. of New Mexico School of Medicine, Albuquerque, NM , 87131.

We have reported long–lasting reductions in kainate receptors and mossy fiber zinc in the hippocampal CA_3 region of rats made hypothyroid during the perinatal period using propylthiouracil (PTU). Perinatal hypothyroidism has been reported to elevate mu–opioid receptor binding in whole hippocampal homogenate preparations. To determine more precisely the anatomical location of the perinatal hypothyroidism–induced elevation in hippocampal mu–opioid receptors, we measured ^{3}H–Tyr–D–Ala–Gly–N–Methyl–Phe–Gly–ol (DAMGO) binding site density in 31–day–old PTU–treated rats using *in vitro* autoradiography techniques.

Timed pregnant Sprague–Dawley rat dams were given water containing either 0.002% or 0.02% PTU or untreated water from Gestational Day 18 until their litters were weaned at 31 days of age. Beginning on Postnatal Day 2, half of the offspring in each group received daily thyroxine (T_4) injections (1 microgram / 100 g body wt., sc) and the other half received saline. Offspring were sacrificed at 31 days of age. Serum T_4, T_3, body and brain weight data indicated that both groups of saline–injected PTU–treated rats were hypothyroid, whereas the T_4 supplemented PTU–treated rats were not different from euthyroid control rats.

Compared to untreated controls, specific ^{3}H–DAMGO binding site density was elevated significantly in both dorsal and ventral hippocampal formation of both 0.002% and 0.02% PTU–treated rats. In the dentate granule and hippocampal pyramidal cell body layers of PTU–treated rats, ^{3}H–DAMGO binding was increased 129% to 157% of control. In the apical dendritic field regions, binding was increased 212% to 263% of control. ^{3}H–DAMGO binding was increased also in the posterior neocortex of PTU–treated rats compared to controls. In contrast, ^{3}H–DAMGO binding was reduced to 20% of control in the deep cell region of the presubiculum. PTU treatment did not affect ^{3}H–DAMGO binding in the amygdala, entorhinal cortex, lateral or medial geniculate nuclei or dorsal lateral thalamic nucleus. T_4 supplementation reversed all of the PTU–induced mu–opioid binding site alterations noted above. (Supported by AA06548, RR08139 and GM08222)

530.5

NEONATAL HYPERTHYROIDISM SELECTIVELY DELAYS THE DEVELOPMENT OF RELATIONAL CUE USE AND INDUCES A SEXUALLY DIMORPHIC IMPAIRMENT OF RATS' SPATIAL NAVIGATION.

N.L. Dallal[1], M.E. Kleiderman[2], W.H. Meck[1], and C.L. Williams[3]. Departments of Psychology, Columbia University[1], New York, NY 10027, Brown University[2], Providence, RI 02912, and Barnard College[3], New York, NY 10027.

Both the short- and long-term behavioral consequences of early postnatal treatment of male and female Sprague-Dawley rats with 3,3', 5-triiodothyronine (T3 -- 0.5 µg/g body wt.) were examined by using water maze and radial arm maze tests of navigational ability at different stages of development. Because the hippocampus is one of several brain structures which selectively takes up T3, the deiodinated form of thyroxine, it was expected that behavioral tasks requiring hippocampal processing might be modified by this manipulation. A comparison of water maze performance at three time-frames spanning postnatal days (PD) 18-28 demonstrated that while all rats could learn the location of the escape platform using a single proximal cue as early as PD 18, the development of navigation using relational cues appeared on PD 24 for control rats but not until PD 28 for T3-treated rats. There were no effects of sex in either cue condition. Some of the cognitive effects of neonatal hyperthyroidism persist well into adulthood. When these same rats were trained as adults on a 12-arm radial maze with 8 baited and 4 unbaited arms, T3-treated rats required more choices to complete the task than did controls. This behavioral deficit was particularly pronounced in the T3-treated females suggesting a difference in the timing of developmental effects of T3 exposure in males and females.

530.6

SEX DIFFERENCES IN THE EFFECT OF NEONATAL HYPERTHYROIDISM ON HIPPOCAMPAL CELL MORPHOLOGY AND INTERVAL TIMING PROCESSES IN ADULT RATS.

M.E. Kleiderman, W.H. Meck, and C.L. Williams. Departments of Psychology, Brown University, Providence, RI 02912, Columbia University, New York, NY 10027, and Barnard College, New York, NY 10027.

The long-term behavioral consequences of neonatal treatment with 3,3',5-triiodothyronine (T3 -- 0.5 µg/g body wt.) administered on postnatal days 1, 2, and 4 were examined by training adult male and female Sprague-Dawley rats on a discrete-trials peak-interval timing procedure to discriminate between visual signals paired with 20-sec and 40-sec intervals. Signals were presented in a double-alternation pattern and response rate functions were analyzed separately for each of the four trial types. Analysis of the discrimination index for peak functions showed that T3-treated males were impaired in their ability to discriminate both within and between signal conditions relative to untreated males. In contrast, T3-treated females were improved in their ability to discriminate both within and between signal conditions relative to untreated females. No sex differences were apparent in control subjects. These results can be related to the sexual dimorphisms in basal forebrain and hippocampal cell morphology and the timing of T3 sensitivity of CA1 and CA3 pyramidal cells. Sexual dimorphisms and T3-induced cellular alterations are quantified using metrical and topological analyses (e.g., Sholl's method of concentric spheres) of 3D tracings of individual neurons obtained from Golgi-stained sections.

SYNAPTIC STRUCTURE AND FUNCTION II

531.1

A FAST ALGORITHM FOR SYNAPTIC CONNECTIVITY ESTIMATION.

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The conventional method for the synaptic connectivity estimation is to use correlation histograms. Such a histogram estimate needs sufficient observation data to assure its accuracy. Often, a stable recording session is too short to collect enough neural spikes. To tackle this difficulty, we discuss a minimum mean square error (MMSE) estimation of the synaptic connectivity in biological neural networks. The recursive algorithm is developed using a Kalman filter and a general neuron model. The mean square error can be made arbitrarily small as long as sufficient spikes are collected. We also show that this method estimates what cross-interval histograms estimate with the advantage that it converges at a faster rate, and hence it requires less data to reach the same accuracy. It is illustrated to estimate the synaptic connection strength and integration time of networks of both inhibitory and excitatory neurons. In contrast to the conventional correlation histogram in which minutes of data are required for the estimation, the MMSE estimation in these simulations needs only 10 sec. of data with an acceptable accuracy.

531.2

NETWORK ACTIVITATION LEVEL INFLUENCES SINGLE-CELL PROPERTIES

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The standard one-dimensional Rall cable model of nerve cells assumes that their electrotonic structure do not change in response to synaptic input. This model is used in a great number of both theoretical and anatomical-physiological structure-function studies. In particular, the membrane time constant τ_m and the somatic input resistance R_{in} are used to characterize single cells. However, these studies do not take into account that neurons are embedded in a network of spontaneously active neurons. These synapses will make up a large part of the membrane conductance, especially if recent evidence of very high specific membrane conductance, $R_m = 100\ k\Omega cm^2$, is taken into account. We numerically simulated the electrical behavior of a morphologically identified layer V cortical pyramidal cell receiving input from 4000 excitatory and 1000 inhibitory cells firing spontaneously at 0–2 Hz. We found that over this range of neuronal background activity τ_m and R_{in} can change by more than a factor 10 (7–80 ms, 18–153 $M\Omega$) and the electrotonic length of the cell by a factor 3. Thus the global activity of the network controls how individual cells perform spatial and temporal integration. This provides possible mechanisms for gain control, coincidence detection, and dynamic control of tuning curves.

531.3

EFFECTS OF MICROENVIRONMENT COMPOSITION ON CIRCUIT FUNCTION USING PUSH-PULL PERFUSION WITH MICROELECTRODE RECORDING IN THE CAT CUNEATE NUCLEUS. V.R. Roettger and M.D. Goldfinger, Dept. of Physiology & Biophysics, Wright State University, Dayton, OH 45435.

In Nembutal-anesthetized adult cats, cisternal CSF (cCSF) was withdrawn (4-6 ml/6 hr) with no change in arterial blood pressure (1). The Push-Pull Cannula (PPC) [previously described - (1)] was placed onto the surface of the medial Cuneate Nucleus and was not moved; perfusion rate was 20 µl/min. Pulled perfusate was collected in continuous 5-min periods and stored (-70°C) for subsequent amino acid analysis (2). During perfusion with cCSF, pulled glutamate (Glu) concentration was consistently higher than in cCSF, suggesting a cCSF/interstitial concentration difference. To assess circuit function during perfusion, 20 evoked potentials were elicited every 5-min by constant amplitude 0.1 ms pulses @ 1/sec to the ipsilateral forepaw skin. During control cCSF perfusion (30-60 min), P1, N1, and P2 component amplitudes and latencies remained constant.

I. Perfusion with artificial CSF (aCSF): Following perfusate switch to aCSF, P1 amplitude increased and N1 amplitude decreased during the 60 min perfusion period; latencies and P2 amplitude remained unchanged. These data may indicate that aCSF perfusion causes hyperpolarization of 1° afferent terminals and reduced EPSP amplitude in cuneothalamic relay neurons.

II. Perfusion with high Glu: Following perfusate switch to high-Glu cCSF (10-100x), all component amplitudes and latencies remained unchanged during the 60 min perfusion period.

Ref: (1)V.R.Roettger & M.D.Goldfinger. Soc. Neurosci. Abst. 16 (1990): 224. (2)V.R. Roettger et al. Soc. Neurosci. Abst. 15 (1989):857.

531.4

CALCIUM INFLUX AT THE PROXIMAL REGION OF A MOTONEURON INFLUENCES TRANSMITTER RELEASE FROM DISTANT MOTOR TERMINALS. S.J. Hong and G.A. Lnenicka. Dept. of Biol. Sci., State Univ. of New York, Albany, NY 12222.

Tonic *in vivo* stimulation of a crustacean phasic motoneuron alters the transmitter releasing properties of the motor terminals so that they become more similar to those of a tonic motoneuron (J. Neurosci. **5**:459-467, 1985). The activity-dependent synaptic changes include a long-term reduction in initial transmitter release. We have recently demonstrated that local depolarization of the proximal region of a crayfish phasic motoneuron (abdominal motoneuron, F-3) *in vitro* is sufficient to produce this reduction in initial transmitter release from motor terminals (Soc. Neurosci. Abstr. **16**:1162, 1990). In order to examine the role of Ca^{++} influx in the induction of this synaptic change, the proximal region of F3 was depolarized in Ca^{++}-free saline containing the Ca^{++} channel blocker Mn^{++} (6 mM). While conditioning in normal saline resulted in a significant 30% decrease in initial EPSP amplitude ($p<.01$, t-test; $n=12$), blocking Ca^{++} influx during conditioning prevented this reduction in EPSP amplitude ($p>.10$, $n=10$). These results demonstrate that Ca^{++} influx at the proximal region of a motoneuron can influence transmitter release from distant terminals.

To examine the role of protein synthesis, the protein synthesis inhibitor, cycloheximide (CHX), was bath applied during conditioning. CHX (0.6 mM) applied 2 hr prior to conditioning prevented the reduction in EPSP amplitude. However, CHX application at the onset of stimulation did not block the effect as the conditioned axon showed a significant 25% decrease in initial EPSP amplitude ($p<.05$, $n=5$). These results are consistent with previous *in vivo* findings which suggest that a pool of preexisting proteins is essential for the induction of this activity-dependent change in initial transmitter release (J. Neurosci. **10**:1099-1109, 1990). (Supported by NSF grant BNS-8720135.)

531.5

ENZYMATICALLY DISSOCIATED NMJs FUNCTION ALMOST NORMALLY AFTER MANUAL REATTACHMENT OF TERMINAL TO ENDPLATE. R.S. Wilkinson and S.D. Lunin*. Department of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, MO 63110.

NMJs are precisely organized at the submicron level, e.g., the close proximity of pre- and postsynaptic elements and the alignment of active zones opposite postsynaptic gutters. The functional importance of these features has been assumed but not directly tested. To address this we 'reconstructed' NMJs by manually positioning enzymatically-detached nerve terminals over vacant endplates in the snake transversus abdominis muscle. Reconstructed NMJs functioned immediately (<0.1 s). Mepps were of normal amplitude, slightly greater duration and lower frequency than controls. PSPs evoked in low Ca^{++} solution comprised fewer quanta than controls, but were of sufficient amplitude to elicit muscle action potentials (normal solution). Lifting the terminal ~0.1 µm above the endplate abolished mepps and PSPs. In contrast, deliberate lateral misalignment of terminal and endplate had no effect on mepp amplitude, but diminished mepp frequency and PSP amplitude in proportion to synaptic area lost. We conclude that basic synaptic function requires close contact between terminal boutons and endplate, and that area of overlap (number of boutons contacting the endplate) determines PSP amplitude. Precise alignment of active zones and gutters is evidently not required for basic function, or, alternatively, such alignment occurs spontaneously when terminal and endplate are placed in contact. Supported by NIH grant NS24752 and the MDA.

531.6

A POSSIBLE MECHANISM FOR BLOCKAGE OF SYNAPTIC TRANSMISSION BY Mg^{++}. J.H. Koenig and K. Ikeda. Division of Neurosciences, Beckman Research Institute of the City of Hope, Duarte, CA 91010.

The relationship between synaptic vesicles and the presynaptic dense body at the neuromuscular junction of *Drosophila* was studied under various conditions of activity, rest, or exposure to substances known to affect synaptic transmission. The synapses of the coxal muscle were exposed to a particular condition, then instantly fixed and prepared for observation by electron microscopy. It was observed that under conditions of inactivity with the nerve cut, moderate activity by stimulation, or more severe activity using veratridine, the vast majority of the active zones showed no sign of exocytosis. Furthermore, the synaptic vesicles were located above the dense body plate, at least 50 nm from the presynaptic membrane. In a few cases, synaptic vesicles were observed lying under the dense body plate, next to the plasma membrane. These appeared attached to the presynaptic membrane by 2 or 3 thin filament-like structures. In synapses exposed to 35 mM Mg^{++}, 1 mM Ca^{++} saline for 20 min prior to fixation, the relationship between the synaptic vesicles and the dense body was dramatically different. Now, a build-up of vesicles was observed under almost all of the dense body plates. Furthermore, the presynaptic membrane showed numerous exo/endo-like pits. Many of these appeared as if they could have been exocytosing vesicles, but others were much larger than a vesicle, or were attached to the presynaptic membrane by a long thin neck. Since transmitter release is blocked by high Mg^{++}, it is not possible that these images represent exocytosis at the moment of fixation. It is possible, however, that Mg^{++} blocks the removal of membrane after exocytosis, so that no further release may take place.

531.7

MUSCARINIC SYNAPTIC TRANSMISSION IN CANINE INTRACARDIAC GANGLIA. X. Xi, W.C. Randall*, and R.D. Wurster. Physiology Department., Loyola Univ. Med. Ctr., Maywood, Il 60153.

The role of muscarinic mechanisms in mediating slow excitatory and slow inhibitory postsynaptic potentials (s-EPSP & s-IPSP) was investigated in the canine intracardiac ganglion with intact synaptic connections. The right atrial fat pad containing these ganglia which provided vagal innervation to the SA node, were removed from anesthetized dogs. Following dissection in a superfused tissue bath, ganglion cells were recorded using conventional intracellular microelectrode recordings techniques (JANS 32:177-182, 1991). Repetitive orthodromic synaptic activation was accomplished using a suction electrode applied to interganglionic nerves (0.1 ms, < 1s, <40Hz) in order to evoke s-EPSPs and s-IPSPs. Of 85 cells studied, 35 cells exhibited only s-EPSPs (3-4 mV amplitude) accompanied by an increased input resistance. Of 16 cells with s-IPSPs, 8 cells had only a s-IPSP while the other 8 cells had a s-IPSP followed by a s-EPSP. The remaining 34 cells had no slow potentials. All slow potentials were blocked by the nonspecific muscarinic blockers atropine (10^{-7} M, N=25) or by a synaptic blocking solution containing low Ca^{2+}/ high Mg^{2+} (N=5). Physostigmine (10^{-7} M) enhanced all slow potentials (N=38). The reversal potentials for the s-EPSP and for the muscarinic agonist bethanechol induced depolarization was -90 to -100 mv, suggesting the involvement of potassium conductance changes. The M_1 receptor antagonist pirenzepine (10^{-8} M, N=6), but not the M_2 receptor antagonist 4-DAMP ($5x10^{-7}$ M, N=5), blocked the s-EPSPs and the bethanechol evoked depolarizations but not the s-IPSPs (N=3). In conclusion, canine intracardiac ganglion cells exhibit s-EPSPs which involve M_1 receptor and s-IPSPs which are mediated by a distinct muscarinic receptor.
(Supported by HL 27595)

531.8

SINGLE CHANNEL PROPERTIES OF ACETYLCHOLINE RECEPTORS COEXPRESSED WITH THE 43K PROTEIN IN OOCYTES AND COS-7 CELLS. L.P. Henderson, C. Brennan*, P. Scotland, S.C. Froehner. Depts. of Physiology and Biochemistry, Dartmouth Medical School, Hanover, NH 03756

The nicotinic acetylcholine receptor (AChR) is a pentameric protein comprised of four subunits in the stoichiometry of $\alpha2\beta\delta\gamma$ or $\alpha2\beta\delta\varepsilon$ which mediates synaptic transmission at the skeletal neuromuscular junction. Clustering of these receptors at a high density in the postsynaptic membrane has been attributed to their association with a peripheral membrane protein of M_r 43,000 (43K protein) (Froehner, 1991). We have addressed whether the association of the 43K protein with the receptor can affect channel function, and thus neuromuscular transmission, by expressing AChR ($\alpha2\beta\delta\gamma$) with and without the 43K protein in *Xenopus* oocytes and COS-7 cells. Analysis of recordings made in the cell-attached configuration demonstrated a primary class of ACh-induced events with a conductance of ~60pS and a biexponential distribution of open durations with a fast component, τ_1 =~0.5 ms, and a slower component, τ_2 =~ 7.0 ms for the apparent mean open time and ~11.0 ms for bursts (i_{avg}=6.0pA). Coexpression of the 43K protein did not produce significant differences in either the conductance or the open state kinetics of the AChR. This was true in oocytes and also in COS cells where it was possible to identify 43K-induced receptor clusters under fluorescence optics and to record directly from clustered receptors. These experiments support previous studies in intact muscle cells which have demonstrated that the single channel properties of clustered synaptic receptors associated with the 43K protein and diffuse nonsynaptic receptors are not significantly different (2,3).

1. Froehner, S.C. (1991). *J. Cell Biol.*, in press.
2. Brehm, P. and Kullberg, K. (1987). *Proc. Natl. Acad. Sci USA* 84: 2550.
3. Henderson. L.P.and Brehm, P. (1989). *Neuron* 2: 1399.

531.9

MINIATURE END-PLATE POTENTIALS FROM FROG SARTORIUS MUSCLE EXPOSED TO PEREZONE. E. Gijón, X. Garcia, and G. Alcántara*. Dept. of Physiol. and Dept. of Pharmacol. Sch. of Med. Universidad Nacional Autónoma de México. Ap. P. 70-250, México, D.F. 04510. MEXICO.

Perezone has an anticholinergic action in rat intestinal smooth muscle. It interrupts spontaneous activity, it blocks acetylcholine response, and it causes relaxation of an induced acetylcholine contraction. Perezone action in neuromuscular preparations shows that it also blocks neuromuscular transmission. The anticholinergic action of perezone is probably due to an interaction with acetylcholine receptors. To test perezone action we recorded intracellularly spontaneous miniature end-plate potentials from frog sartorius muscle. Perezone added to the bath induces a decrease in number and amplitude of the miniature end-plate potentials, and a depolarization of the superficial muscle fibers while the resting potential of less superficial fibers shows higher values, and miniature end-plate potentials of higher amplitude and frecuency. These results support that perezone interacts with acetylcholine receptors and probably decreases spontaneous acetylcholine release.

531.10

FLUCTUATION ANALYSIS OF SINGLE FIBER EPSPS IN THE SPINAL CORD OF THE BULLFROG. Ch. Stricker and H.-R. Lüscher, Dept. of Physiol., Univ. of Bern, Switzerland.

Synaptic transmission from muscle afferents to motoneurons in the frog spinal cord is both electrical and chemical. By comparing the fluctuations of the electrically and chemically evoked components, it should be possible to determine whether fluctuations are a result of failure to release transmitter or of uncertainty of potential propagation in afferent branches. We have shown that the method of variance analysis is not sufficiently sensitive to detect an expected variance of the electrotonic component because of contaminating noise. Therefore, we used a different approach. For comparison of the two components we have measured single fiber EPSPs in motoneurons elicited by tapping the fore limb triceps muscle (5 Hz). The animals were anesthetized with ice and the spinal cord and muscle rapidly dissected. The spinal cords were hemisected along the mid line and the preparation superfused with oxygenated Ringer (14°C). Motoneurons of the triceps muscle were impaled with bevelled microelectrodes (2 n K-citr., 30-70MΩ) and identified by antidromic stimulation. EPSP and noise measurements of only those cells were included in the analysis if the membrane potential during recording was <-55 mV and did not vary more than 5 mV. Afferent action potentials of the triceps nerve were used for spike triggered averaging of the EPSPs and noise records. The components of the EPSP were identified by shape and latency. The average and variance time courses of the EPSP were calculated and the latter corrected for noise variance. Amplitudes of the EPSPs were then grouped into two classes differing by at least two standard deviations of the noise. EPSPs belonging to the same amplitude class were averaged again and the results analyzed for shape and latency. The electrotonic component of the EPSP in the small amplitude group is often smaller in size than in the large amplitude group and that the quotient of electrotonic to chemical amplitude is always greater in the smaller group. The results suggest that the probability of transmitter release at this synapse is determined by two factors: impulse propagation and release process.

531.11

CALCIUM REGULATION IN RAT POSTERIOR PITUITARY NERVE TERMINALS. A.O. Johnson* & S.A. DeRiemer. Dept. of Biological Sciences, Columbia Univ., NY NY 10027.

The posterior lobe of the pituitary gland is composed primarily of neurosecretory axons and their terminals. This makes the gland a unique preparation for the study of synaptic mechanisms, specifically calcium regulatory mechanisms. We have used intact posterior pituitary glands or vibratome slices (60-80 μm) incubated with the membrane permeant calcium indicator dye, fluo-3/AM, in order to characterize the calcium regulatory mechanisms in these terminals. Data from standard fluorescent microscopy in similarly stained tissue is complicated by signals from surrounding structures. By using a confocal microscope we have been able to circumvent this problem. The initial phase in this project involved dealing with three technical problems. The first was to determine a range of laser intensities which gave reproducible signals without significant bleaching during experiments that lasted over forty minutes. Second, the application of secretogogues during intitial experiments caused tissue movements which interferred with data collection. We overcame this problem in the intact preparation by tacking the tissue down with minuten pins. The third was the calibration of changes in fluo-3 fluorescence with changes in intracellular calcium concentrations. This included the determination of whether the dye was primarily cytosolic and whether the concentration changed significantly over time. We have used the non-fluorescent calcium ionophore, 4-bromo-A23187, to determine the range of fluorescent responses to changes in the concentration of extracellular calcium and the accessibility of the dye. Data from endocrine cells and neuronal soma suggest that there are two major sources of calcium; extracellular calcium entering through ion channels and intracellular calcium released from stores. Posterior pituitary nerve terminals possess voltage gated calcium channels, however the presence of releaseable intracellular stores remains open. We are currently examining this question as well as the effects of neurotransmitters on calcium regulation in these nerve terminals. Supported by a Klingenstein Fellowship.

531.12

THREE DIMENSIONAL STRUCTURE OF NEUROSECRETORY AXONS ACCOMPANYING THE PERIPHERAL MOTOR AXONS IN MANDUCA LARVAE. M.B. Rheuben and D.M. Autio*. Dept. of Anatomy, Michigan State University, E. Lansing, MI 48824.

Insects have elaborate neurosecretory systems whose products are involved in development, ecdysis and modulation of muscle function. Nerve branches innervating skeletal muscles in Manduca include small axons of a neurosecretory (ns) type, but whose function is unknown. We have examined the ultrastructure of these ns axons in the vicinity of the neuromuscular junctions using serial sections. One ns axon accompanies a single motor axon to each muscle fiber. The ns axon is loosely wrapped in the outermost glial processes investing the motor axon, may at times be directly exposed to the hemolymph, and forms varicosities ranging from 0.24-1.1um in diameter and 0.6-6.7um in length, and with inter-varicosity distances averaging 5.5um. Within varicosities, dense- and clear-cored vesicles cluster around an electron dense presynaptic "specialization", many of which are in direct apposition to an ensheathing glial cell process. The ns axon itself, though close, does not appear to come into direct contact with the muscle fiber nor to enter into the neuromuscular junction. The results suggest that the ns axon could have a "distant" neuromodulatory influence on the neuromuscular junction or the muscle fiber, but also that it might interact in some direct way with the glial sheath.

531.13

SYNAPTIC ULTRASTRUCTURE OF TRIGEMINAL MOTOR NEURONS IN CATS. N. Capra, V. Hubbard*, and J. Bernanke*. Dept of Physiol., Univ. of MD, Baltimore, MD 21201; Dept. of Anatomy Univ. of MS, Jackson, MS 39216.

This study compares the synaptic ultrastructure along the soma and proximal dendrites of masseter and digastric motor neurons in cats. Horseradish peroxidase (HRP) injections were made into the digastric muscle in 2 cats and in the masseter muscle in 2 cats. Vibratome sections were reacted with diaminobenzidine to identify cells containing retrogradely transported HRP. Labeled neurons were trimmed from the sections, osmicated, processed into plastic, and prepared for ultrastructural study. Ten masseter and 10 digastric neurons, sectioned through the nucleolus, were studied. Terminal boutons in both cell populations contained either small clear spherical vesicles, a mixture of clear vesicles and large dense-core vesicles (LDCV), or small-flattened vesicles. Terminals containing flattened vesicles were more numerous along digastric neurons while spherical vesicles were more common in terminals on masseter neurons. Synaptic density along digastric somata was greater than along masseter somata (38% vs. 28%). In both populations, the largest terminals were associated with extensive subsynaptic cisterns and postsynaptic accumulations of granular endoplasmic reticulum. Other terminals contained typical symmetric or asymmetric contacts. Occasionally, boutons along the soma and proximal dendrites of masseter neurons were associated with prominent subsynaptic bars. In spinal cord motor neurons, these structures have been shown to be of dorsal root origin. Since this type of specialization is not seen on digastric neurons, it is possible that boutons with subsynaptic bars represent sites of monosynaptic input from the trigeminal mesencephalic nucleus. Supported by NIH, DE06027.

531.14

G_2-ACETYLCHOLINESTERASE IS LOCATED ON PRESYNAPTIC TERMINALS OF TORPEDO ELECTRIC ORGAN. L. Anglister, J. Eichler*, I. Silman* and M.K. Gentry*. Anatomy Dept., Hebrew Univ. Med. Sch., Jerusalem, Neurobiology Dept., Weizmann Inst., Rehovot, ISRAEL and Walter Reed Army Inst. Res., Washington, DC 20307

In Torpedo electric organ much of the acetylcholinesterase (AChE) is a globular dimer (G_2), anchored to the plasma membrane via covalently attached phosphatidylinositol and solubilized by a bacterial phosphatidylinositol-specific phospholipase C (PIPLC). While the structure of G_2-AChE is well-established, the localization of this form of the enzyme remains unclear. We have previously used selective solubilization by PIPLC combined with immunocytochemistry at the light microscope level, and shown an accumulation of G_2-AChE at synaptic sites. In order to define more precisely the subcellular location of G_2-AChE in Torpedo electric organ, a similar strategy was applied at the electron microscope level. Thin sections of electric organ were labelled with various antibodies to Torpedo AChE followed by gold-conjugated second antibodies, before or after exposure to the phospholipase. To test the resolution of the technique, antibodies against Torpedo acetylcholine receptors were employed. As expected, they bound to the postsynaptic face of electric organ synapses. Immunolabelling of AChE was detected in the synaptic clefts concentrated on both pre- and postsynaptic faces. Prior exposure to PIPLC significantly diminished labelling of the presynaptic nerve terminals. The results support our previous assignment, based on biochemical evidence and immunofluorescence, for a neuronal synaptic localization of G_2-AChE in Torpedo electric organ. (Supported by grants from BSF and Bruno Goldberg Foundation to L.A., Minerva Foundation to I.S. and Israel Acad. Sci. to L.A. and I.S.).

532.1

QUANTAL ANALYSIS OF ENDOGENOUS EXCITATORY POST-SYNAPTIC CURRENTS (EPSCS). Guosong Liu & J.L. Feldman. System Neurobiology Laboratory, Dept. Kinesiology, UCLA, Los Angeles, CA 90024-1527.

Quantal analysis can reveal important aspects of synaptic transmission. However, it is difficult to do quantal analysis in CNS neurons due to poor signal/noise ratio and lack of understanding of quantal variance. To solve these problems, we obtained whole cell patch clamp recordings in spinal cord motoneurons in the *in vitro* brainstem-spinal cord of neonatal rat (Liu & Feldman, Neurosci. Abs. 16:1184 '90), which allowed very low noise recording of EPSCs. This noise was further reduced by a novel filtering technique which allowed the removal of almost all background noise. In order to insure a complete space clamp of the EPSCs, only those recordings in which EPSCs had a very fast rise time (~0.5 ms) were selected for analysis. Under these conditions, we observed a quantal amplitude distribution of EPSCs consistent with an underlying quantal release process. The amplitude, location and variance of quantal peaks were determined by maximum likehood estimate procedure. The interval between the peaks in the amplitude distribution was ~10 pA. The mean EPSC amplitude ranged from 25 to 100 pA.

In order to analyze the origin of quantal current variations, synaptic currents were simulated based on channel kinetics associated with excitatory amino acid receptors. The experimentally observed quantal amplitude variations appear largely due to statistical properties of the channels. Therefore, we applied non-stationary noise analysis to each group of quantal currents to determine single channel conductance, number of channels, and probability of channel opening under endogenous EPSCs associated with synaptic drive to phrenic motoneurons. We found the smallest quantal synaptic current was due to activation of ~20 channels with single channel conductance ~30 pS. In summary, our results represent a novel approach for quantal analysis in CNS neurons and new insights into quantal release mechanisms *in situ*. Supported by NIH Grant NS 24742.

532.2

THREE POTASSIUM CHANNELS IN RAT NERVE ENDINGS. K. Bielefeldt, J.L. Rotter, M.B. Jackson. Dept. of Physiology, University of Wisconsin, Madison, WI 53706.

Voltage-dependent K channels determine the shape and duration of action potentials. Presynaptically, they influence transmitter release by limiting depolarization and, thereby, Ca influx through voltage-sensitive Ca channels. We used single channel techniques to study the K channels in cell-attached patches of nerve endings from rat posterior pituitary slices. In nominally symmetrical potassium, 3 K channels could be distinguished on the basis of channel conductance and kinetics. The most frequently observed K channel had a conductance of 21 pS, and inactivated rapidly (tau: 18 ms). A second K channel had a larger conductance of 110 pS, and inactivated more slowly (tau: 82 ms). A third K channel with a slope conductance of 16 pS activated slowly and did not inactivate during a 300 ms depolarization. An excellent correspondence was exhibited between the properties of these three K channels and the various components of macroscopic K current recorded in tight-seal whole-terminal experiments. Thus, the biphasic inactivation kinetics and complex voltage dependence of activation in macroscopic current can be explained in terms of the underlying behavior of three different K channels. The importance of voltage dependent K current inactivation in action potential broadening and frequency dependent facilitation suggests a physiological role for these K channels in synaptic plasticity.

532.3

CALCIUM CURRENTS RECORDED FROM PRESYNAPTIC TERMINALS IN THE CHICK CILIARY GANGLION. H. Yawo and A. Momiyama*. Department of Physiology, Kyoto University Faculty of Medicine, Kyoto 606, Japan.

The properties of calcium currents of vertebrate presynaptic nerve terminals were studied under whole-cell voltage clamp. Large calyciform presynaptic terminals of the chick ciliary ganglion were identified by fluorescence, dextran-tetramethylrhodamine applied to the cut end of the presynaptic oculomotor nerve 5 hrs prior to the experiment. When whole-cell recordings were made simultaneously from both the presynaptic terminal and postsynaptic ganglion cell, an EPSP (or EPSC) could be elicited by activation of presynaptic calcium currents. There was no evidence for the existence of low-threshold calcium currents in the presynaptic terminal. The high-threshold calcium current was potentiated by a dihydropyridine agonist, Bay K 8644. The potentiated calcium current as well as the control calcium current was irreversively suppressed by ω-conotoxin GVIA (ω-CgTX). However, 20-50 % of the control calcium current were resistant to 10 μM ω-CgTX. The ω-CgTX-resistant component was not sensitive to Bay K 8644.

These results suggest that in the chick ciliary calyx, there are at least 2 components of high-threshold calcium currents: the one component is sensitive to both Bay K 8644 and ω-CgTX, and the other is insensitive to both.

532.4

ALTERATION IN MICROFILAMENT ORGANIZATION OF BOVINE ADRENAL CHROMAFFIN CELLS DURING MASTOPARAN-EVOKED EXOCYTOSIS. K.Kumakura[1], T.Sakurai[2*], M.-O.Imaizumi[1*] and Y.Nonomura[2*]. [1]Life Science Inst., Sophia Univ. and [2]Dept. Pharmacol., Univ. of Tokyo, Japan

Mastoparan, a tetradecapeptide from wasp venom, is known as a nonspecific secretagogue for a variety of cells. Using a real-time monitoring technique, we found that pulsatile stimulation of bovine adrenal chromaffin cells with mastoparan evoked the transient secretory response with a biphasic dose-response curve. By persistent stimulation with 3-7 μM mastoparan, continuous release of catecholamines with a constant magnitude was evoked till withdrawal of mastoparan. These secretagogue action of mastoparan was completely independent of Ca^{2+}. By use of FITC-phalloidin and immunofruorescence method, we observed that subplasmalemmal microfilamental network was disrupted during mastoparan-evoked exocytosis either in the presence or absence of Ca^{2+}. Such disassembly of microfilamental network was also observed during nicotine-evoked exocytosis. These results suggest that mastoparan is a useful tool to study cellular mechanism for exocytosis.

532.5

INCREASE OF EXTRACELLULAR K^+ TO 10-12 mM ABOLISHES EXCITATORY SYNAPTIC TRANSMISSION BETWEEN CEREBELLAR NEURONS IN TISSUE CULTURE. W. Raabe. Neurology, VA Med. Ctr., Depts. Neurology, Physiology and Neuroscience Graduate Program, University of Minnesota, Minneapolis, MN 55417.

Increased extracellular K^+ (K_o^+) has been reported to affect axonal conduction and possibly synaptic transmission. To investigate the effects of increased K_o^+ in further detail, whole cell patch voltage clamp recordings were obtained from large (>20 μm φ) cerebellar neurons, presumably Purkinje cells, grown in primary dissociated tissue culture. An unpolished patch pipette was used to stimulate small nearby cells (<10 μm φ), presumably granule cells, to evoke monosynaptic EPSCs.

Normal extracellular solution contained 5 mM K^+. Increases of K_o^+ to 10 mM reversibly abolished the evoked EPSCs in most cells. In those cells, in which 10 mM K_o^+ did not abolish the EPSC, 12 mM K_o^+ abolished the EPSC. When increased K_o^+ abolished the evoked EPSC, neurons were still able to generate action currents and to produce spontaneous TTX-independent miniature EPSCs. The abolition of the evoked EPSC was always preceded by a transient increase in spontaneous synaptic activity.

The abolition of excitatory synaptic transmission between cerebellar neurons by K_o^+ 10-12 mM is consistent with a depolarizing conduction block for action potential invasion into presynaptic terminals. This suggests that for the study of transmitter release from neurons it may be best to increase K_o^+ to 8-9 mM to obtain increased spontaneous synaptic activity without blocking transmitter release from presynaptic terminals. In addition, it can be suggested that in those seizures which increase K_o^+ to 10-12 mM, increased K_o^+ may abolish neuronal excitatory synaptic transmission and may contribute to seizure termination.

532.6

INCREASE OF EXTRACELLULAR K^+ ENHANCES AMMONIUM TOXICITY. J. Mortimer and W. Raabe. Neurology, VA Med. Ctr., Depts. Neurology, Physiology and Neuroscience Graduate Program, Univ. of Minnesota, Minneapolis, MN 55417.

NH_4^+ (5 mM) produces a conduction block in presynaptic terminals and interrupts synaptic transmission (J. Neurophysiol. 62:1461, 1989; Soc. Neurosci. Abstr. 16:1015, 1990). This study examines the synergistic action of NH_4^+ and K^+ on synaptic transmission. Whole cell patch voltage clamp recordings were obtained from large (>20 μm φ) cerebellar neurons, presumably Purkinje cells, grown in primary dissociated tissue culture. An unpolished patch pipette was used to stimulated small nearby cells (<10 μm φ), presumably granule cells, to evoke monosynaptic EPSCs.

NH_4^+ 5-6 mM or K_o^+ 10-12 mM abolished evoked EPSCs. Mixtures of NH_4^+ plus K_o^+ abolished evoked EPSCs when the sum of $NH_4^+ + K_o^+$ exceeded 10-12 mM. NH_4^+, K_o^+ and $NH_4^+ + K_o^+$ abolished EPSCs without affecting action currents or TTX-independent spontaneous EPSCs. Before affecting EPSCs, NH_4^+, K_o^+ and $NH_4^+ + K_o^+$ always increased spontaneous synaptic activity.

Because NH_4^+ acts synergistically with the depolarizing effect of increased K_o^+ to abolish evoked EPSCs, it can be inferred that NH_4^+ has in cerebellar neurons (like in spinal neurons) the same effect as K^+ and produces a depolarization block for action potentials in presynaptic terminals. The synergistic action of NH_4^+ and K_o^+ on evoked EPSCs also indicates that increases of K_o^+ can make subthreshold concentrations of NH_4^+, e.g., 3.5 mM, toxic to neurons. Thus, increases of K_o^+ enhance the toxicity of NH_4^+ on the CNS.

532.7

FREQUENCY-MODULATION OF SYNAPTIC EFFICACY IN EMBRYONIC RAT SPINAL CORD SLICE CULTURE. Chr.Lüscher, J.Streit, H.P.Clamann and H.R.Lüscher, Department of Physiology, University of Berne, Switzerland

Frequency-dependent alterations of synaptic efficacy are believed to functionally determine neural networks during embryonic development. We looked for evidence for such a mechanism during the development of synaptic connections between DRG-cells and motoneurons in an organotypic co-culture of spinal cord, DRG and muscle. EPSPs and IPSPs were recorded from motoneurons during stimulation at different frequencies of DRG-cells, using conventional intracellular electrodes. A depression in amplitude by up to 100% of EPSPs and IPSPs and an increase in latency by roughly 1ms was found, when the frequency was increased by the factor 10 (range 0.1-10Hz). The decrease in amplitude showed the same quantal steps as were seen with amplitude fluctuations at constant frequency of stimulation. In addition, steps of multiple quanta were consistently found, suggesting a propagation failure at a higher order in the arborisation, leading to quantal events switched off in groups. Reducing extracellular Ca^{++} from 5mM to 1mM shifted the frequency-dependence of these effects towards higher frequencies. When action potentials invading DRG somata after stimulation of their central axons were investigated using the same frequency protocols, an increase in latency (17-35%) and its variance as well as the appearance of partial and complete failures were observed. These effects were again less pronounced with a reduced Ca^{++}-content of the perfusion-solution. From these findings it is concluded that synaptic depression is of presynaptic origin, and that there is strong evidence for a conduction failure in axonal arborisation as a underlying mechanism.

532.8

EFFECTS OF PYRETHROID INSECTICIDES ON SYNAPTIC FUNCTION J.T. Eells, J.M. Propp* and P.A. Holman* Dept. of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, WI 53226.

The neuroexcitatory actions of two toxicologically distinct classes of pyrethroid insecticides were characterized in rat brain synaptosomes using ^{3}H-tetraphenylphosphonium to measure membrane potential and measuring the release of ^{3}H-acetylcholine (^{3}H-ACh). The type II pyrethroids, deltamethrin (E_{max} 25 mV depol.; EC_{50} 10^{-8} M), cypermethrin (E_{max} 24 mV depol.; EC_{50} 10^{-7} M) and fenvalerate (E_{max} 17 mV depol.; EC_{50} 10^{-6} M) elicited concentration-dependent, tetrodotoxin (TTX)-sensitive plasma membrane depolarizations which were specific for the neurotoxic pyrethroid isomer. These pyrethroids also elicited TTX-sensitive release of ^{3}H-ACh. These data support a stereoselective interaction of type II pyrethroids with the voltage-sensitive sodium channel producing sodium influx, synaptic membrane depolarization and neurotransmitter release. In contrast, the type I pyrethroids, allethrin and tetramethrin, produced profound synaptosomal depolarizations (>100 mV) that were not blocked by TTX and were apparent when the plasma membrane was depolarized (135 mM K^+). These type I pyrethroids also evoked TTX-insensitive increases in ^{3}H-ACh release. These findings suggest that the neuroexcitatory actions of type I pyrethroids are not mediated by an action on the voltage-sensitive sodium channel and may involve a disruption of calcium homeostasis and mitochondrial depolarizaton. (Supported by NIH grants ES05006 and ES01985).

532.9

GABA VIA PRESYNAPTIC $GABA_B$ RECEPTORS INHIBITS EXCITATORY POSTSYNAPTIC POTENTIALS IN NEONATE RAT SYMPATHETIC PREGANGLIONIC NEURONS. S. Y. Wu*, S. L. Dun*, C. Ren*, N. J. Dun and A. G. Karczmar. Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43699 and Hines VA Hospital, Hines, IL 60153.

Nerve fibers and a few small diameter cells immunoreactive to GABA antisera were observed near the lateral horn of the rat spinal cord. The effects of GABA and baclofen on the synaptic activity of sympathetic preganglionic neurons (SPNs) in neonate rat thoracic spinal cord slices were evaluated with intracellular recording techniques. Superfusion of GABA (25-100 μM) and (-) baclofen (1-10 μM) consistently depressed the excitatory post-synaptic potentials (EPSPs) evoked by dorsal root stimulation, without causing a significant change of membrane potential or input resistance of the SPNs nor the depolarizations induced by pressure applications of glutamate. GABA and baclofen also eliminated spontaneously occurring EPSPs. The synaptic depressant effects of GABA and baclofen were antagonized by the $GABA_B$ receptor antagonist 2-OH-saclofen (20 μM) but not by the $GABA_A$ receptor antagonist bicuculline (10-50 μM). These results suggest that GABA may be involved in the regulation of transmitter release by acting on presynaptic $GABA_B$ receptors in the lateral horn. (Supported by NS18710)

532.10

THE FUSION PORE OPENS BEFORE MEMBRANE LIPIDS MIX WHEN A VIRAL FUSION PROTEIN MEDIATES CELL FUSION F.W. Tse, A. Iwata & W. Almers. Dept. Physiol. & Biophys., U. Washington, Seattle, WA 98195.

We patch-clamped 3T3 fibroblasts expressing HA (the fusion protein of the influenza virus envelope) while they fused to single human red blood cells (RBC). The electrical conductance of the cell-cell connection (fusion pore) was estimated from the membrane capacitance (C) measured with a lock-in amplifier under whole-cell clamp (30°C). The RBC membrane was loaded with dipalmitoyl-phosphatidylethanolamine tagged with Texas Red, so that the diffusion of lipid into the fibroblast could be observed with an image intensifier and later analyzed digitally. When HA was activated by acidification of the external medium, an abrupt increase in C was seen 40 - 350 s later, indicating the opening of the fusion pore. Over the next 40 - 120 s, the pore conductance increased from <1nS to >5nS. Only then could we see fluorescent lipid move into the fibroblast, with lipid flux continuing for >20 min. In one fusion where the pore conductance remained 1 - 3 nS for >2.5 min, <3% of the fluorescent lipid passed into the fibroblast within 6 min. Assuming that only the outer leaflet of the RBC was labeled, this represents a lipid flux of <10,000 phospholipid molecules/s. Clearly the fusion pore must open and dilate before significant lipid mixing can occur. This fits with the idea that fusion begins with the opening of a narrow pore that, initially proteinaceous, only later dilates by the incorporation of lipids (Almers & Tse, Neuron 4, p. 813). Supported by GM-39520.

532.11

A NOVEL EFFECT OF OPIOIDS ON SYNAPTIC INHIBITION. V.A. Doze, G.A. Cohen and D.V. Madison, *Department of Molecular & Cellular Physiology, Stanford University School of Medicine, Stanford, California 94305.*

Opioid receptor agonists, such as the stable enkephalin analog, D-Ala^2-Met^5-enkephalinamide (DALA), potently reduce synaptic inhibition of pyramidal neurons in the rat hippocampus as evidenced by a decrease in the evoked inhibitory postsynaptic potential (IPSP). Although DALA has been shown to directly hyperpolarize and to reduce the action potential discharge of interneurons, it is not known whether these are the mechanisms for its disinhibitory action.

Experiments were performed on 500 μm hippocampal slices from male, 150-250 g Sprague-Dawley rats. Using intracellular and whole-cell patch-clamp recordings and various pharmacological probes, we examined the effect of DALA on: 1) polysynaptic evoked IPSPs, 2) monosynaptic evoked IPSPs (recorded in the presence of the glutamate antagonists CNQX and APV to ensure monosynaptic isolation), 3) monosynaptic spontaneous IPSPs (recorded with KCl and QX-314 in the recording electrode, and CNQX/APV in the extracellular ACSF), and 4) miniature spontaneous IPSCs (recorded with KCl and QX-314 in the recording electrode, and CNQX/AFV and TTX in the ACSF to block action potential-dependent release) to study the synaptic location for the opioid disinhibition.

DALA (10 μM) potently reduced the amplitude of both the polysynaptic and monosynaptic evoked IPSPs by greater than 50%. DALA also reduced the frequency of monosynaptic spontaneous IPSPs by about 50%. However, most interestingly, DALA potently and reversibly blocked the frequency of miniature spontaneous IPSCs by more than 50% without causing any change in the shape of their amplitude distribution. These results show that opioids block the action potential-independent release of GABA from inhibitory interneurons. Thus, the locus of opioid disinhibition may reside in the presynaptic interneuron terminal.

V.A.D. is a NIMH Predoctoral Fellow. G.A.C. is a Howard Hughes Medical Institute Predoctoral Fellow. D.V.M. is a Lucille P. Markey Scholar and this work was supported by a grant from the Lucille P. Markey Charitable Trust.

532.12

THE ROLE OF CA^{2+} AND NA^+ IN POSTTETANIC POTENTIATION AT THE CRAYFISH NEUROMUSCULAR JUNCTION. R. M. Mulkey and R. S. Zucker, Dept. of Mol. & Cell Biology, Univ. of Calif., Berkeley, CA 94720.

Published evidence suggests posttetanic potentiation (PTP) in invertebrates may be due to intracellular sodium accumulation that occurs during tetanic stimulation. It has been proposed that elevation of intracellular sodium concentration ($[Na^+]_i$) raises intracellular calcium ($[Ca^{2+}]_i$) by release of calcium from internal stores or by calcium entry through Na/Ca exchange. We have elevated $[Na^+]_i$ in presynaptic boutons of crayfish opener muscle using pharmacological and physiological methods while monitoring $[Ca^{2+}]_i$ with the indicator fura-2. Elevation of $[Na^+]_i$ using ouabain (0.5 mM), veratridine (0.5-1.0 μM) or sodium injection through microelectrodes increased $[Ca^{2+}]_i$ at rest by 136 ± 4 nM (N=14), 396 ± 127 nM (N=7), and 430 ± 223 nM (N=7), respectively. This increase in $[Ca^{2+}]_i$ occurred by calcium influx through Na/Ca exchange, as $[Ca^{2+}]_i$ elevation was dependent on external calcium. Loading presynaptic terminals with Na^+ and Ca^{2+} using high K^+ solutions slowed the extrusion of elevated $[Ca^{2+}]_i$ as the decay time for $[Ca^{2+}]_i$ was 12.3 ± 5 min with Na^+ loading and 4.0 ± 1 min without Na^+ loading, as measured in 7 preparations. In 5 preparations elevation of $[Na^+]_i$ during a tetanus, by blocking the Na/K pump with ouabain, increased the posttetanic time constant for decay of transmitter release from 4.4 ± 2 min in normal to 17.3 ± 8.5 min in ouabain and prolonged the decay of $[Ca^{2+}]_i$ from 5.8 ± 8.8 min to 35 ± 18 min. Blocking the Na/Ca exchange with lithium following a tetanic train also prolonged the time constant for $[Ca^{2+}]_i$ decay from 6.5 ± 5.2 min in normal to 15.7 ± 14 min in lithium (N=7). These results suggest PTP is influenced by intraterminal sodium accumulation which slows the rate of calcium removal via Na/Ca exchange. Supported by grant NS-15114.

532.13

KINETICS OF Na/Ca EXCHANGE IN RAT BRAIN PRESYNAPTIC TERMINALS. G. Fontana* & M.P. Blaustein. Dept. of Physiol., Univ. of Maryland Med. Sch., Baltimore, MD 21201

^{45}Ca fluxes were measured in rat forebrain synaptosomes in the presence and absence of external Na^+, Na_o. To determine "initial rates" and some kinetic parameters of Na/Ca exchange, 1 to 3 sec incubations were used; uptake was linear for 3 s at $[Ca^{2+}]_o$ = 0.2 mM, but declined after 1 sec at $[Ca^{2+}]_o \geq 0.5$ mM. Virtually all the ^{45}Ca uptake activated by removal of Na_o ($\Delta J_{Ca/Na}$) was internal Na^+- (Na_i-) dependent. $\Delta J_{Ca/Na}$ was measured as a function of $[Ca^{2+}]_o$ at 1 sec, at 37°C: apparent K_{Ca} = 0.25-0.7 mM; $J_{Na/Ca(max)}$ ≈1200-1400 pmol/mg protein x sec (p/mgs); Hill coefficient (n_H) ~1. The Na_i-dependent ^{45}Ca uptake ($[Ca^{2+}]_o$ = 0.2 mM) was inhibited by Na_o with an apparent K_{Na} = 50 mM and n_H ~ -2.6 (1 sec uptake, 37°C). The Na_i-dependent ^{45}Ca uptake was observed in the apparent absence of external and internal K^+; however, raising $[K^+]_o$ from nominal "0" to 5 mM increased the Na_i-dependent uptake in a concentration-dependent manner. After ^{45}Ca loading (≈ 6 nmol total Ca/mg protein, almost all the ^{45}Ca efflux was Na_o-dependent (Na/Ca exchange): ~1100 p/mgs without FCCP; ~1600 p/mgs with FCCP (to release Ca^{2+} from mitochondria and raise $[Ca^{2+}]_i$). Thus, maximal exchanger-mediated Ca^{2+} flux rates in both directions were about 1200-1400 p/mgs at 37°C. With this very large capacity ($J_{Na/Ca(max)}$), the exchanger in nerve endings may play a major role in regulating $[Ca^{2+}]_i$, and may even participate in the termination of transmitter release by rapidly extruding Ca^{2+}.

532.14

LOCALIZATION OF Na/Ca EXCHANGERS IN NEUROMUSCULAR PREPARATIONS BY IMMUNOFLUORESCENCE MICROSCOPY. P.W. Luther*, R.K. Yip, R.J. Bloch, A. Ambesi†, G.E. Lindenmayer†† and M.P. Blaustein. Physiol. Dept., U. MD Med. Sch., Baltimore, MD 21201 & †Pharmacol. Dept., Med. U. SC, Charleston, SC 29425.

Ion flux measurements reveal a large-capacity (high maximal flux) Na/Ca exchanger in vertebrate nerve terminals. We used polyclonal antibodies raised against dog heart sarcolemmal Na/Ca exchanger to localize the exchanger in neuromuscular preparations by immunofluorescence microscopy. This antibody recognizes the major polypeptide of the Na/Ca exchanger (MW ≈ 160 kD) in immunoblots of dog heart and rat skeletal muscle and brain. In frozen sections through the neuromuscular junction (nmj) of rat skeletal muscle, the antibody, but not preimmune serum, labelled junctional regions more intensely than nearby extrajunctional areas. In fixed and detergent-permeabilized Xenopus nerve and muscle co-cultures, the antibody labelled the nerves in a punctate fashion, suggesting that the exchanger is distributed heterogeneously. Labelling was observed where the nerve traversed the muscle as well as in nearby regions of axons not in contact with muscle. At nmj's where the nerve had been torn away from the muscle, the labelling remained associated with the neuron. The nmj was not labelled with preimmune serum. Specific labelling of muscle could not be detected. We conclude that the Na/Ca exchanger is present at high concentrations in presynaptic regions of the nmj, where it likely contributes to Ca homeostasis.

532.15

REDUCTION OF SLOW CHOLINERGIC EPSPs IN THE HIPPOCAMPUS OF AGED RATS. L. Taylor and W.H. Griffith. Dept. Med. Pharmacol. & Toxicol., College of Medicine, Texas A&M University, College Station, TX 77843.

Central cholinergic transmission has been proposed to decline as a function of age. We have tested this directly using cholinergic synaptic transmission in the *in vitro* rat hippocampus. Intracellular recordings were made from CA1 pyramidal cells using electrodes containing 2 M K-methyl sulfate. Slow excitatory postsynaptic potentials (slow-EPSPs) were evoked by repetitive stimulation (20 Hz, 500 ms duration) of the stratum oriens in the presence of physostigmine (1μM). Reproducibility was defined as 4-6 identical trains yielding consistent slow-EPSPs. In order to enhance reproducibility, the slow-EPSPs were evoked at membrane potentials of -52 mV to -55 mV. Slow-EPSPs were cholinergic in nature since they were blocked by atropine (0.5 μM). Slices were obtained from male rats (Sprague-Dawley and Fisher 344) from 3 age groups (1-2 months, 8-10 mo, 18-23 mo). The amplitude of the slow EPSPs was reduced in 18-23 mo rats as compared to the other two groups ($p < 0.02$, ANOVA followed by Scheffe's post-hoc test). In 1-2 mo rats, the amplitude was 8.2 ± 1.3 mV, 6.8 ± 1.1 mV for 8-10 mo and 3.5 ± 0.6 mV for 18-23 mo (n=11 for each group). These results suggest a decline in hippocampal cholinergic synaptic transmission with age.

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532.16

PHARMACOLOGICAL PROFILE OF THE SELECTIVE D_2 DOPAMINE AGONIST, N-0923. D.J. Friedman, D.N. Krause and S.P. Duckles. Dept. of Pharmacology, College of Medicine, Univ. of California, Irvine, CA 92715.

We have previously shown in the rat tail artery that N-0923 activates prejunctional D_2 receptors to inhibit norepinephrine release. However, concentration response curves were complex, such that at concentrations of 10^{-7} M N-0923 no longer inhibited norepinephrine release. When α_2 adrenoceptors were blocked with yohimbine (10^{-6} M), concentration-response curves for N-0923 became monophasic up to a concentration of 10^{-7} M. N-0923 (10^{-7} M) inhibited contractile responses with an EC_{50} of 5.2 x 10^{-9} M and a maximum inhibition of 68 ± 2% and inhibited [^{3}H] efflux with an EC_{50} of 8.6 x 10^{-9} M and a maximum inhibition of 72 ± 3%. Inhibition of both contractile responses and [^{3}H]norepinephrine efflux was blocked by sulpiride confirming a D_2 receptor site of action. At a concentration of 10^{-6} M N-0923 increased baseline contractions as well as [^{3}H] efflux in tissues prelabeled with [^{3}H]norepinephrine, effects which were unchanged in the presence of yohimbine or sulpiride. N-0924, an enantiomer with less potency at D_2 receptors, was equipotent at increasing baseline [^{3}H] efflux indicating a lack of stereoselectivity. These findings suggest that N-0923, at concentrations of 10^{-7} M or higher, blocks α_2 adrenoceptors causing an increase in adrenergic transmitter release. Given the lipophilic nature of these compounds, the increase in baseline [^{3}H] efflux produced by 10^{-6} M N-0923 or N-0924 may reflect a tyramine-like action. *Supported by NIH fellowship MH09902.*

532.17

SENSITIVITY OF PREJUNCTIONAL α_2 ADRENOCEPTORS TO THE AGONIST UK14304 DECLINES WITH AGE IN THE RAT TAIL ARTERY. J. Buchholz and S.P. Duckles. Dept. of Pharmacology, College of Medicine, Univ. of California, Irvine, CA. 92717.

Our previous studies have shown that fractional norepinephrine release from adrenergic nerves in the F-344 rat tail artery significantly increases with age. Furthermore, the sensitivity of prejunctional α_2 adrenergic receptors to the antagonists yohimbine or idazoxan also declines with age. We have complemented these studies with the use of the selective α_2 agonist UK14304. This agonist inhibits fractional norepinephrine release as measured by HPLC and electrochemical detection. Our data (table 1) show that there is a significant age related difference in the inhibitory effect of UK14304 on fractional norepinephrine release at each concentration. These findings suggest that the function of inhibitory prejunctional α_2 adrenergic receptors is significantly altered with age in the rat tail artery. This may be one important variable in explaining the age-related increase in stimulation evoked norepinephrine release previously reported.

	% Inhibition of Fractional NE Release by UK14304		
Age in Months	UK 10^{-8} M	UK 10^{-6} M	UK 10^{-4} M
6	38.4 ± 5.5 *	50.6 ± 5.3 *	60.5 ± 3.5 *
20	7.2 ± 3.2	19.5 ± 5.9	33.6 ± 3.6

* = Significantly different from 20 months, P<0.01.

Supported by NIH AG06912 and AG05498.

532.18

MULTIPLE TARGETS OF ARACHADONIC ACID IN THE MODULATION OF SYNAPTIC TRANSMISSION. J. E. Richmond, F. Bahls, W. L. Smith* and P. G. Haydon. Dept of Zoology and Genetics, Iowa State University, Ames, IA, 50011.

FMRFamide causes a presynaptic inhibition of synaptic transmission by neuron B5 of *Helisoma trivolvis*. This modulation is due to a coordinate reduction in the calcium current and in the response of the secretory machinery to internal calcium. By voltage-clamping acutely dissociated spherical neurons of B5, we now show that FMRFamide (threshold concentration $3x10^{-8}$M) also increases an outward current. This current reverses around -85 to -90 mV, shows little rectification and shifts by 40-50 mV with a 10x increase in external K^+, consistent with it being a voltage-independent K^+ current.

We have investigated the role of the arachadonic acid (AA) cascade in mediating some of these modulatory actions. Bath application of AA (50μM) increases an outward current that has properties similar to the FMRFamide evoked K^+ current. Furthermore, addition of the phospholipase-A_2 inhibitor BPB (10μM) or the lipoxygenase and cycloxygenase blocker NDGA (10μM) reduces FMRFamides activation of the outward current.

Somata of neurons B5 and B19 form a giant chemical synapse under appropriate culture conditions. We have developed a technique for producing long-lasting asynchronous release of miniature inhibitory postsynaptic currents (MIPSCs) at this synapse by elevating calcium levels through the micro-injection of the calcium cage DM-nitrophen. Under these conditions both FMRFamide and AA (10 and 50μM) cause a reduction in the frequency of MIPSCs in the postsynaptic neuron B19. Addition of AA (but not FMRFamide) also reduces the magnitude of MIPSCs. The effects of BPB and NDGA on the inhibition of secretion by FMRFamide are currently in progress. Taken together these data suggest that the AA cascade mediates some elements of synaptic inhibition produced by FMRFamide.

532.19

SYNAPTIC TRANSMISSION IS ALTERED IN ADULT SHR RATS.
J.C. Magee and G.G. Schofield. Dept. Physiology, Tulane Medical School. New Orleans, LA 70112.

Exaggerated sympathetic nervous activity, due to an increased neuronal excitability, has been implicated in both the development and maintenance of hypertension in the spontaneously hypertensive rat (SHR). However, transmission of high frequency stimuli through the superior cervical ganglion (SCG) of adult SHR is diminished. This suggests that synaptic transmission has been altered during the development of hypertension in SHR. In order to test this various parameters of synaptic transmission were assessed using *in vitro* isolated SCG from SHR, WKY and Wistar rats. Preganglionic stimulation was achieved using a suction electrode on the cervical trunk and fast EPSPs were recorded using standard intracellular techniques. Use of the variance method determined that quantal content was increased, but quantal size was the same in SHR neurons when compared to those from WKY. Short term facilitation, determined from paired stimuli with interpulse intervals of 25-1000 ms, was found to be smaller in magnitude and shorter in duration in SHR neurons compared to WKY neurons. Trains of EPSPs were observed to facilitate less and depress more in SHR neurons when compared to WKY neurons. In low Ca/ high Mg solution these differences were reduced, but not removed. The small amount of depression observed in SHR neurons during paired stimulation in normal solution was eliminated by the low Ca/ high Mg solution. In addition, under these conditions trains of presynaptic stimuli gave rise to EPSPs in SHR and WKY neurons which increased in size throughout. These observations will be discussed in terms of the relationship of sympathetic nerve activity and hypertension. Supported by PHS grant HL 43656.

532.20

COMPARISON OF THE ACTIONS OF BACLOFEN AND ADENOSINE AT PRESYNAPTIC RECEPTORS IN HIPPOCAMPAL SLICE CULTURES.
Scott M. Thompson, Helmut Haas, and Beat H. Gähwiler.
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We have used intracellular recordings from CA3 cells to characterize the mechanism by which baclofen (BAC) and adenosine (ADO) block synaptic transmission in the hippocampus. BAC, but not ADO, reduced the amplitude of monosynaptic IPSPs (mIPSPs) elicited in the presence of CNQX and D-APV, suggesting that inhibitory terminals do not possess ADO receptors. Both substances reduced the amplitude of isolated EPSPs elicited with mossy fiber stimulation. BAC and ADO produced no postsynaptic hyperpolarization in pertussis toxin-treated cultures, but both substances could still block EPSPs. However, BAC had no effect on mIPSPs. Stimulation of protein kinase C with phorbol ester reduced all presynaptic actions of both BAC and ADO. The postsynaptic hyperpolarization elicited by BAC was also reduced, but the hyperpolarization elicited by ADO was not. Barium (1 mM) blocked the postsynaptic hyperpolarizations elicited by both substances, as well as the action of BAC on mIPSPs. However, both ADO and BAC were able to depress EPSPs in the presence of barium. These results suggest that the presynaptic action of baclofen at inhibitory synapses is the same as the postsynaptic action: an increase in a G-protein mediated, barium-sensitive K^+ conductance, which prevents GABA release by hyperpolarizing the terminals. The actions of baclofen and adenosine at excitatory axon endings must be mediated by some other mechanism.

532.21

INTRACELLULAR Ca^{2+} MEASUREMENTS WITH FURA-2 IN PRESYNAPTIC TERMINALS IN BULLFROG SYMPATHETIC GANGLIA.
Y.-Y. Peng and R. S. Zucker. Dept. of Molecular and Cell Biology, Univ. of California. Berkeley, CA 94720.

To investigate the Ca^{2+} dependence of neurotransmitter release in bullfrog sympathetic ganglia neurons, where the preganglionic C fibers release both ACh and LHRH, a method was developed to fill the presynaptic terminal boutons with Fura-2. Fura-2 appears to be transported to the terminal boutons via a fast axonal anterograde transport system, because: 1) the fura-2 signal moved at about 2 mm/hr.; 2) it can be stopped by cooling to 4°C. The filled boutons have diameters of 1-3 μm. The organization of the presynaptic axon and terminal boutons with respect to the ganglionic neurons is similar to that studied by HRP methods. Typically, at least 30 surface cells possessed filled boutons. For a given neuron, up to 50 individual boutons were observed. Fura-2 concentration in boutons was 100-500 μM. Ca^{2+} elevation in synaptic boutons due to a single stimulus to the presynaptic fiber can be detected by a photomultiplier.

Most boutons had a resting Ca^{2+} concentration of ~30 nM. Only when the resting Ca^{2+} was >70 nM, was there spontaneous release of ACh, which was measured as m.e.p.s.p.s. Exogenous LHRH (salmon, 10 μm) caused a decrease of the resting Ca^{2+} in these boutons. For boutons on C neurons, the LHRH receptor antagonist ((D-pGlu[1], D-Phe[2], D-Trp[3,6])-LH-RH, 20 μM) decreased the Ca^{2+} elevation evoked by stimulation to the preganglionic fibers. Supported by NIH grant 15114 to R.S.Z..

532.22

RELATION OF PRETERMINAL NODES AND TERMINAL MORPHOLOGY TO DEPOLARIZATION OF THE MAMMALIAN MOTOR NERVE TERMINAL.
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The mammalian motor nerve terminal (NT) is thought to be passively depolarized by inward Na^+ current from preterminal nodes of Ranvier. The spacing of nodes, morphology of the NT arbor, and distribution of ion channels in the nerve membrane may affect the depolarization of the terminal, and thus its function. Aged animals, in particular, frequently exhibit NT constrictions that might decrease the current to distal branches. Since direct measurement of the NT V_M is not currently feasible, these issues were approached by morphologic and computer-modelling studies. The shapes of mouse motor NT's and their preterminal axons were determined with vital fluorescent stains. Discrete cable models of these terminals were constructed from the morphologic data and from published work on ion channel density, distribution, and kinetics. Membrane voltages and currents were computed during simulations over a range of electrotonic parameters. The close (30-60 μm) spacing of the preterminal nodes resulted in a high safety factor for conduction, so that NT action potentials (AP's) occurred even after block of Na^+ channels in two preterminal nodes. Decreasing the internodal spacing did not significantly increase the current flow to the terminal, and the measured internodal distances were not correlated with the size or input impedance of the NT. Conduction through the NT was modelled as purely passive. Even for small values of leak resistance (10 Ω-cm²), small NT's were electrotonically compact. However, in large NT's, especially those with constrictions, AP amplitudes declined towards the distal ends. We conclude that preterminal internodal spacing provides safety of transmission, but is not fine tuned to match NT impedance. Also, in the passive case there are inhomogeneities in the depolarization of some NT's. Supported by NIA AG00105-07 and NIA AG08886.

532.23

CALCIUM-ACTIVATED POTASSIUM CHANNELS REGULATE SYNAPTIC TRANSMSSION AT THE FROG NEUROMUSCULAR JUNCTION.
R. Robitaille and M.P. Charlton, MRC Group, Physiol. Dept., Univ. of Toronto, Toronto, Canada M5S 1A8.

Transmitter release is steeply related to Ca^{++} concentration. The size of the Ca^{++} transient is modulated by the duration of the presynaptic action potential (AP). We tested the role of Ca^{++}-activated K^+ channels (IKca), in the regulation of transmitter release at the frog neuromuscular junction (NMJ). The motor nerve was stimulated and transmitter release was monitored using intracellular postsynaptic recordings and specific IKca channel blockers. Charybdotoxin (CTX, 2 nM), but not apamin (50 nM), increased release (163.6% ± 58.7%) without affecting MEPP frequency and amplitude. Similar results were obtained in low and normal Ca^{++} concentrations. CTX (2 nM) had no effect on release after a membrane permeant Ca^{++} buffer (DMBAPTA-AM) was loaded in the nerve terminal. This indicates that the toxin acts primarily on Ca^{++}-activated channels. Paradoxically, the intracellular Ca^{++} buffer first induced a transient increase of transmitter release (26.8% ± 6.3%) before the decrease occurred. This increase did not occur when the loading was performed in presence of IKca blockers (CTX 2 nM, Ba^{++} 2 mM) indicating that opening of IKca channels is reduced due to the attenuation of the Ca^{++} transient by the buffer . The early increase of transmitter release did not occur when EGTA-AM, a Ca^{++} buffer with similar affinity as BAPTA but slower forward rate constant, was used. We conclude that IKca channels are an important regulator of synaptic transmission at the frog NMJ, where they cause spike narrowing, and are probably clustered close to the Ca^{++} channels at release sites. Supported by MRC Canada.

532.24

NEOCORTICAL EPSP QUANTAL SIZE AND NUMBER ARE INFLUENCED BY POSTSYNAPTIC MEMBRANE POTENTIAL AND PRESYNAPTIC FIRING PATTERN
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Dept. Physiology, Royal Free Hospital School of Medicine, London NW3 2PF, UK.

In slices of adult rat neocortex, single axon epsps due to the firing of one pyramidal cell were recorded from one other, averaged and fluctuations in epsp amplitudes analyzed. These partially NMDA receptor mediated epsps increase in amplitude with postsynaptic membrane depolarization. Standard deviation time course matched average epsp shape at negative membrane potentials (-80mV), but exhibited an additional late component that increased further with depolarization from potentials less negative than -70mV. Altering postsynaptic membrane potential caused no significant change in the number of apparent failures but altered the position of peaks within the epsp amplitude distribution. In contrast, increasing presynaptic firing rate (1-5 spikes/s) greatly increased the proportion of apparent failures. These data support the view that fluctuation analysis of neocortical epsps can indicate whether a change in epsp amplitude is due to pre- or post-synaptic effects.

533.1

SIMULTANEOUS PATCH-CLAMP RECORDING AND CONFOCAL IMAGING OF CA^{2+} INFLUX INTO CA1 NEURONES OF THE RAT HIPPOCAMPAL SLICE. S. Alford,* B.G. Frenguelli* and G.L. Collingridge.* Dept. of Pharmacology, The Medical School, Birmingham University, Birmingham U.K. (Spon: European Neuroscience Association)

Long-term potentiation (LTP) is a long lasting increase in synaptic efficacy induced by tetanic stimulation. In CA1 pyramidal cells of the hippocampus, the induction of LTP is initiated by the activation of NMDA receptors and a postsynaptic Ca^{2+} influx (Collingridge et al 1983; J. Physiol 334, 33-46: Lynch et al 1983; Nature 305, 719-721). Ca^{2+} entry as a consequence of tetanic stimulation has been imaged using FURA-2 but the relative contributions of voltage-operated and ligand-gated Ca^{2+} entry has not been separated (Regehr and Tank 1990; Nature 345, 807-810). By combining whole-cell patch-clamp techniques in slices with confocal laser scanning microscopy of a Ca^{2+} fluorescent dye FLUO-3, introduced into cells through the patch pipette, we have attempted to differentiate, in various subcellular locations, between activation of voltage-operated and ligand-gated Ca^{2+} channels during the tetanus.Tetanic stimulation increases the fluorescence in voltage-clamped dendrites.This effect is blocked by the NMDA receptor antagonist AP5 and shows anomalous voltage-dependence consistent with NMDA receptor activation in the presence of extracellular Mg^{2+}. These data indicate tetanus-induced Ca^{2+} entry into dendrites directly through NMDA receptor channels. In contrast, Ca^{2+} enters the soma during the tetanus under current- but not voltage-clamp, indicating that the Ca^{2+} entry into the soma is through voltage-operated Ca^{2+} channels. In support of this, depolarising voltage steps from -70 to -10 mV caused a marked increase in somatic but not dendritic $[Ca^{2+}]$. The analysis of small areas of dendrite (approx 4 μm long) shows the decay in intracellular $[Ca^{2+}]$ is heterogeneous and oscillates as it returns to baseline levels. This may be due to Ca^{2+}-induced Ca^{2+} release.

533.2

Confocal Imaging of Calcium Transients and Whole-Cell Recording from Pyramidal Cells in Area CA1 of Rat Hippocampal Slices. R.J.Adams and T.J.Sejnowski. Computational Neurobiology Laboratory, The Salk Institute, La Jolla, Ca 92037.

Calcium transients are considered to be important mediators of synaptic plasticity in the hippocampus and elsewhere in the nervous system. We are using confocal microscopy to image changes in intracellular calcium ion concentration in pyramidal cells in area CA1 of 400μm thick slices of rat hippocampus. Cells about 100μm below the surface of the slice are recorded from using whole-cell techniques using electrodes of about 5MΩ resistance. The recording electrode filling solution includes fluo-3 as a fluorescent indicator of calcium ion concentration. Low noise electrical recording with simultaneous high temporal- and spatial-resolution imagining can be achieved with this technique.

Synaptic stimulation from axons of the Schaffer collateral/ commissural pathway produces calcium transients in the dendrites and soma of these cells. Changes in intracellular calcium in response to a single excitatory postsynaptic potential, causing the firing of a single action potential, can be detected in the proximal dendrites and soma.

533.3

CONFOCAL IMAGING OF CHANGES IN SYNAPTIC STRUCTURE IN LIVING HIPPOCAMPAL SLICES. A. Fine, T. Hosakawa* and T.V.P. Bliss. Dept. Physiol. & Biophys., Dalhousie Univ., Halifax, N.S., Canada B3H 4H7 and Div. Neurophysiol. & Neuropharm., NIMR, Mill Hill, London UK.

Laser-scanning confocal microscopy can be used to obtain submicron-resolution images of synaptic morphology in living hippocampal slices. We have investigated whether such morphologic changes occur in association with changes in physiological function. Synaptic structures are labelled by microinjection of a concentrated oil solution of DiI into the superfused slice; dye droplets are removed after 30 min. Anterograde diffusion of dye permits rapid, and often intense, labelling of dendritic spines and axon terminals. Viability of labelled neurons is assessed by ethidium bromide exclusion. Field potentials from labelled regions are normal, and LTP can be induced by electrical or chemical stimulation. Brief exposure to high concentrations of kainic acid can induce large changes in dendritic shaft and spine morphology; smaller and inconsistent changes can be seen in control slices and after induction of LTP by elevated extracellular calcium.

533.4

ANALYSIS OF THE VARIABILITY OF EVOKED SYNAPTIC CURRENTS DURING LONG-TERM POTENTIATION (LTP) IN HIPPOCAMPAL SLICES P. Renner*, T. Manabe*, D.M. Kullmann*, R.A. Zalutsky & R.A. Nicoll Depts. Pharmacol. and Physiol., UCSF, San Francisco CA 94143.

Quantal analysis of synaptic transmission was originally restricted to a small number of different synapses where the quantal amplitude could be easily resolved. The development of high resolution whole-cell recording techniques raises the possibility of extending this analysis to CNS synapses with small quantal size. These techniques have recently been used to analyze changes in the variability of evoked excitatory postsynaptic currents during LTP (Malinow and Tsien, Nature 346:177, 1990; Bekkers and Stevens, Nature 346:724, 1990). This analysis revealed a decrease in the coefficient of variation (CV), which was interpreted as an increase in transmitter release. We have repeated this analysis using different conditioning paradigms to elicit LTP. We first checked whether the CV analysis could detect some well known pre- and postsynaptic changes in transmission. Manipulations which affect the presynaptic site (applying baclofen, changing the Ca/Mg ratio, paired pulse facilitation), showed the expected change in CV, and the postsynaptically acting glutamate receptor antagonist CNQX had no effect. LTP was induced using low frequency stimulation (0.2 Hz) paired with postsynaptic depolarization (pairing). In 14 cells showing an average two-fold potentiation we saw no change in CV, whereas paired pulse facilitation, which was monitored in the same experiments gave the expected decrease in CV. When a tetanus was used instead of pairing to induce LTP, the results were more variable and, on average, a decrease in CV was observed (15 cells). Since the CV was unchanged, our results favor the hypothesis that LTP induced by low frequency pairing is expressed postsynaptically. This interpretation however depends on several assumptions which may well not apply for synapses onto CA1 pyramidal cells (Korn et al., Nature 350: 282).

533.5

THE EFFECT OF LONG-TERM POTENTIATION ON MINIATURE SYNAPTIC CURRENTS IN HIPPOCAMPAL SLICES. T.Manabe*, P.Renner*, D.Dixon and R.A.Nicoll. Depts. Pharmacol. and Physiol., UCSF, CA 94143.

Brief repetitive activation of excitatory synapses in the CA1 region results in long-term potentiation (LTP) of synaptic transmission in pyramidal cells. While there is general agreement that the induction of LTP occurs in the postsynaptic cell, the site of LTP expression remains controversial. We have used whole-cell recording from guinea pig slices to analyze spontaneous excitatory postsynaptic currents (sEPSCs) before and after LTP. Cells were voltage-clamped at -80 mV and EPSCs were evoked with electrical stimulation of afferent fibers at 0.2 Hz. LTP was induced by pairing 0.2 Hz stimulation with depolarization of the postsynaptic cell to 0 mV for 2.5 min. Presynaptic stimulation alone caused the frequency of sEPSCs to nearly double with little change in size. Following LTP induction, however, a significant increase in the size of sEPSCs was detected in 6 of 7 cells. In a second set of experiments we used a glass pipette, filled with 0.5 M sucrose in normal Ringer, to evoke miniature EPSCs (mEPSCs). This pipette was also used for focal electrical stimulation of afferents. Control mEPSCs and EPSCs were recorded in the absence of tetrodotoxin (TTX). Following LTP induction TTX was added to abolish spontaneous presynaptic action potentials and mEPSCs were again recorded. A significant increase in the size of mEPSCs was observed in 6 of 7 cells. Although we cannot exclude an increase in the amount of transmitter per quantum, a change in mEPSC size is usually interpreted as reflecting a change in postsynaptic sensitivity to the transmitter.

533.6

CHANGES IN NEURONAL EXCITABILITY DURING LONG-TERM POTENTIATION. W.D.A. Garner & R.F. Thompson. Neurosciences Program, University of Southern California, Los Angeles, CA 90089-2520.

High-frequency stimulation of the Schaffer axons, often inducing long-term potentiation, decreased the afterhyperpolarization (aAHP and mAHP) associated with the A current and M current. Intracellular recordings from CA1 neurons (Vm = -77 + 2 mV SD; n = 102 cells) showed that the decrease in the aAHP lasted about 30 s and was enhanced by application of 4-aminopyridine and 3,4-diaminopyridine, and by depolarization of the postsynaptic cell immediately prior to high-frequency stimulation. Conversely, when the postsynaptic cell was hyperpolarized immediately prior to stimulation, the aAHP increased in magnitude and the AP duration decreased slightly relative to controls. The decrease in the mAHP lasted significantly longer (at least 75 min). We postulate that suppression of the A current transiently increases $[Ca^{2+}]_{in}$ which, in turn, enhances activation of Ca^{2+}-activated K channels. Overall, CA1 cell excitability decreases during and immediately following high-frequency stimulation of Schaffer axons. Suppression of the M current can increase cell excitability by a mechanism yet to be determined.

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533.7

AVP-INDUCED POTENTIATION AND DEPRESSION OF EVOKED POTENTIALS IN THE DENTATE GYRUS IS REGULATED BY CALCIUM. C.Chen, R.E.Brinton, T.J.Shors and R.F.Thompson. Neurosci.Prog.,USC,Los Angeles,CA 90089-2520.

Vasopressin (AVP) has been shown to modulate memory process (de Wied, 1984) and the level of NE-induced cAMP formation in rat hippocampus in a Ca-dependent fashion (Brinton & McEwen, 1989). Rat hippocampal slices, maintained at the interface of perfusion solution and a humidified oxygen mixture, were used to assess the effect of AVP on synaptic transmission. Field potentials were evoked by a bipolar electrode in the perforant path and were recorded by an extracellular microelectrode in the granule cell layer of the dentate gyrus. AVP (500 nM) applied in the perfusion solution has been found to have dual effects on response of cells in the dentate depending on the external Ca. In 1.5 mM Ca, AVP potentiated the evoked response as measured by the change of population EPSP slope by 31.4±8.1% (t_7=5.72, p≤0.001). In 2.5 mM Ca, AVP depressed the evoked response by 20±5.2% (t_7=-3.76, p≤0.007). Both the potentiation and the depression occured within 5 min and lasted for more than 30 min after AVP application. Moreover, the changes in EPSP persisted following wash out of AVP. Thus, AVP-induced changes in hippocampal synaptic efficacy are long-lasting and are regulated by external calcium.

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533.8

INDUCTION OF HIPPOCAMPAL LONG-TERM POTENTIATION (LTP) BY PAIRING WEAK INPUT STIMULATION WITH IONTOPHORETIC K^+ APPLICATION. B.A. Ballyk and J.W. Goh. Department of Pharmacology & Toxicology, Queen's University, Kingston, Canada K7L 3N6.

While postsynaptic depolarization is required for LTP induction in the hippocampal CA1 region, the expression of LTP is at least partly presynaptic. Thus, a retrograde messenger must be involved, and our work suggests a role for K^+. We previously reported that elevating bath K^+ (10mM) during a weak tetanus facilitates the induction of LTP (IBRO abstracts, 1991). It was observed, however, that elevating K^+ in this manner potentiates the response during the K^+ application, raising the possibility that increasing bath K^+ acts at the stimulation site to recruit more fibres, thus converting a "weak" input to a "strong" input. This led to the present studies, in which we increased K^+ by iontophoresis at the synaptic zone, which does not potentiate responses during the exposure period, suggesting a synaptic mechanism for the co-operativity. Population EPSPs were recorded in CA1 region of guinea pig hippocampal slices by stimulation of stratum radiatum. Weak EPSPs (<300μV) failed to exhibit LTP following a 100Hz, 1s tetanus (98±9 % control, 30min post tetanus, n=6). K^+, iontophoretically applied at the site of recording for 10s, did not potentiate EPSPs (104±7 % control, 30min post K^+, n=6). However, delivery of weak tetanus with simultaneous iontophoretic (200nA) application of K^+ resulted in significant potentiation of EPSPs (168±5 % control, 30min post K^+ & tetanus, n=6). Preliminary experiments conducted in voltage clamped CA1 neurons (n=2) suggest that K^+ elevation at the synaptic zone in the absence of postsynaptic depolarization can facilitate LTP induction. We propose that the K^+ efflux from CA1 neurons critical for the induction of LTP is localized to the synaptic zone, possibly resulting from activation of NMDA receptors.

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533.9

MODERATE CONCENTRATIONS OF POTASSIUM CHANNEL BLOCKERS INTERFERE WITH HIPPOCAMPAL LONG-TERM POTENTIATION. T. Bonhoeffer[1] and C.M. Müller. Max-Planck-Institut für Entwicklungsbiologie and Max-Planck-Institut für biologische Kybernetik, Tübingen, FRG. [1]current address: Max-Planck-Institut für Hirnforschung, Frankfurt/M, FRG.

Several experiments suggest that maintenance of long-term potentiation (LTP) includes mechanisms located remote from the postsynaptic induction site. One possibility to convey signals over some distance is by means of ionic concentration changes. In order to test the hypothesis that shifts in potassium concentrations may be a crucial factor we investigated the effect of the potassium channel blockers teraethylammonium (TEA) and barium (Ba^{2+}) on the induction of LTP in the CA1 region of rat hippocampal slices. We observed that moderate concentrations of TEA (5-10mM) or Ba^{2+} (0.5-1mM) reliably and reversibly prevented the induction of long-term synaptic enhancement following tetanic stimulation. As bath application of these drugs acts on, both, neurons and glial cells, we intracellularly recorded responses of both cell types to afferent stimulation. We observed increased depolarization in pyramidal cells, which should indeed facilitate the induction of LTP. In contrast, the responses of identified astroglial cells were clearly diminished in the presence of TEA or Ba^{2+}. These data accord with the hypothesis that activation of glial cells is an intermediate step in long-term plasticity.

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533.10

BASAL- VS APICAL-DENDRITIC LTP IN HIPPOCAMPAL CA1 FOLLOWING COMMISSURAL STIMULATION IN THE ANESTHETIZED RAT. **T.Kaibara and L.S.Leung. Depts.of Physiol.and Clin. Neurol. Sci, Univ. of West. Ont., London, Canada.**

In urethane-anesthetized rats, basal- vs apical-dendritic long-term potentiation (LTP) in CA1 was studied following stimulation of the contralateral CA3 and different layers of contralateral CA1 (str.oriens, pyramidale or radiatum/moleculare). Average evoked potentials were recorded at 50 μm depth intervals and analyzed by a one-dimensional current-source-density (CSD) before and at 30-100 min. following a tetanus. Single test pulses evoked excitation(sinks) at both the basal- and apical-dendrites of CA1. Primed burst tetanization(1 pulse followed 190ms later by 10 pulses @ 100Hz) of the contralateral CA1 str. oriens yielded strong basal- (154.6% of baseline ±9.8,n=8) but little apical-dendritic sink LTP (106.9±6.4, n=8) while str. radiatum tetanization gave an unchanged basal-dendritic sink (103.2 ±19.6,n=6) and a depression of the apical-dendritic sink (83.3±7.5, n=6). Tetanization of str. pyramidale yielded a strong LTP of both basal (149.7±18.2, n=7) and apical-dendritic (143.1±15.8, n=7) sinks. Tetanization of CA3 showed a strong basal-dendritic (130.0±14.4) and weak apical-dendritic sink increase (109.0±10.3, n=11). Test responses evoked by CA1 str.radiatum stimulation after the CA3 tetanus revealed heterosynaptic depression of both basal- (75.9±10.5, n=7) and apical-dendritic (72.3±9.9,n=8) sinks. The CSDs indicated that both basal and apical dendrites are activated by hippocampal commissural stimulation, in some cases with more apical- than basal-dendritic excitation. However, the basal dendritic synapse seems to possess a greater propensity for LTP than the apical dendritic synapse, as in the behaving rat(Leung,Neursc.Abs.16:652).Support by Upjohn, NSERC.

533.11

HOMOSYNAPTIC LONG-TERM DEPRESSION IN CA1 OF RAT HIPPOCAMPUS *IN VITRO*. M.F. Bear, S.M. Dudek and J.T. Gold*. Center for Neural Science, Brown University, Providence, RI 02912.

A prominent form of synaptic plasticity in cerebral cortex, especially during development, is the weakening of ineffective synaptic inputs. For example, there is evidence in visual cortex that input activity which fails to correlate with postsynaptic depolarization beyond some threshold level leads to the down-regulation of the effectiveness of the active input. We have hypothesized that this "modification threshold" (θm) relates to the levels of postsynaptic response at which a significant NMDA receptor conductance is recruited during synaptic transmission. Further, we have proposed that the value of θm is not fixed, but rather is a function of the history of average postsynaptic activity.

The aim of the present study was to assess whether long-term synaptic depression (LTD) would result from persistent synaptic activation under conditions that are not favorable for recruiting NMDA receptors. The Schaffer collateral → CA1 pathway was investigated using conventional extracellular recording methods in transverse hippocampal slices prepared from adult rats. In attempts to induce LTD, low frequency stimulation (LFS; 0.5 Hz) was applied to the Schaffer collaterals for 15-30 minutes.

In naive slices, LFS produced a significant depression (as assessed using an F-test) in only 2 of 13 attempts. Even so, 9 of the 11 remaining cases showed a reduced response after LFS. The mean change (±SEM) after LFS for the group is -14 ±2%, which is significantly different from pre-LFS baseline values using a t-test. The depression of the CA1 response lasted at least 60 minutes and appeared to be stable. In striking contrast to the naive slices, LFS applied 60 minutes after the induction of LTP produced a significant depression in 6/6 attempts. The mean change in this group is -25 ±4%, this LTD is significantly greater than that observed in the naive slices. These findings suggest that this preparation can be used a model to study the mechanisms of use-dependent synaptic weakening in the cerebral cortex. (Supported by ONR Young Investigator Award N00014-88-K-0756)

533.12

LONG TERM POTENTIATION INDUCED BY PRIMED BURST STIMULATION IS REDUCED IN HIPPOCAMPAL AREA CA1 OF AGED, LEARNING IMPAIRED RATS. C.I. Moore*, G.M. Rose and M.D. Browning, Dept. of Pharmacology, UCHSC, and Medical Research, VAMC, Denver, CO 80262.

Long-term potentiation (LTP) is a form of synaptic plasticity that is widely thought to underlie certain forms of learning. Aged animals have been shown to exhibit marked deficits in a variety of learning tasks, and such animals also show reduced LTP in the hippocampal dentate gyrus (Barnes, JCPP, 93:74, 1979). In the present study we have focussed on area CA1 of the hippocampus. Specifically we have compared LTP induced by Primed Burst (PB) potentiation (a priming pulse followed 170 ms later by a 4-pulse burst at 200 Hz) in 3 and 24 month old male Fischer 344 rats which had been behaviorally tested in the Morris water maze. PB stimulation closely mimics endogenous patterns of hippocampal activity known to occur in behaving animals. All of the 3 month old animals rapidly learned the location of the hidden platform in the water maze. By contrast, none of the 24 month old rats learned over the 5 day training period. Following behavioral testing, field potentials were recorded from the CA1 pyramidal cell layer in *in vitro* hippocampal slices. Young and aged animals showed similar baseline response amplitudes at similar stimulus intensities. However, the potentiation observed in response to PB stimulation was significantly reduced in the 24 month old group. Subsequent analysis revealed that magnitude of both short-(1 minute post train) and long-(20 minutes post train) term potentiation were less in the old animals. Interestingly, there was no difference between age groups in LTP generated by conventional high frequency stimulation (a 1.0 sec/200 Hz train) delivered 20 minutes after PB stimulation. Supported by NIA grant AG 04418 to MDB and VAMRS to GMR

533.13

TIME-COURSE AND PLASTICITY OF RAT HIPPOCAMPAL CA3 PYRAMIDAL CELL RESPONSES TO INPUT FROM RECURRENT COLLATERALS EXAMINED BY WHOLE-CELL RECORDINGS. R. B. Langdon, J. Johnson, and G. Barrionuevo. Departments of Behavioral Neuroscience and Psychiatry, University of Pittsburgh, Pgh., PA.

Pyramidal cells in the CA3 field of hippocampus make direct excitatory synapses onto each other. We have studied these (1) as a mechanism whereby neuronal activity is synchronized, and (2) because studies of LTP of responses in CA3 depend on discrimination between EPSCs evoked via collaterals and via mossy fibers; the latencies and rise-times of the former have not been well-studied and may provide criteria for such discriminations. To evoke EPSCs via collaterals in CA3b pyamidal cells we applied stimuli to the alveus of region CA3c, with, interposed near the CA3b/CA3c border, a cut extending 125 to 200 μm radially from the alveus. In 5 out of 11 cells, the rising phase (10 to 90%) of the EPSC lasted between 1.4 to 3.0 msec. EPSCs with longer rising phases often exhibited multiple inflections, suggesting temporal dispersion of inputs. The mean latency to EPSC onset was 2.7 msec (S.D. = 0.4, range = 1.9 to 3.2) for CA3b cells which were between 570 and 780 μm from the stimulus site (n = 10). By comparison, direct conduction over this same distance by mossy fibers should require 1.4 to 2.0 msec (at 33° C), based upon the latencies of antidromic responses in *s. granulosum*, evoked by stimulation of the *s. lucidum*. To induce LTP, we applied 100 Hz stimulus trains at least 30 min after break-in, with cells clamped at -70 mV; presumably these conditions resulted only in LTP expressed polysynaptically and not on inputs to the cell recorded. Although late phases of the EPSC exhibited the greatest potentiation, nevertheless, the initial 3 msec of the EPSC was enhanced appreciably. In conclusion, the time-course of responses of CA3b pyramidal cells to inputs from collaterals can be rapid, and activity-induced potentiation of the collateral connections can affect early phases of the EPSC to collaterals. Supported by NINDS NS24288 and NIMH MH45156.

533.14

ISOLATED MOSSY-FIBER SYNAPTIC CURRENTS IN CA3 PYRAMIDAL NEURONS. Z. Xiang, A.C. Nobre, and T.H. Brown. Dept. of Psychology, Yale University, New Haven, CT 06520.

The mossy-fiber synapse is particularly suitable for neurophysiological and optical experimentation of plasticity due to its large size and electrotonic proximity to the soma. Because there are many synaptic systems present in the CA3 region of the hippocampus, it is difficult to excite the mossy-fiber synapse selectively when using extracellular microstimulation. For example, if the stimulating electrode is placed either in the stratum granulosum or in the stratum lucidum, it is possible to activate not only mossy fibers, but also fibers of the perforant path, commisural fibers, CA3 recurrent collaterals, and local inhibitory circuits.

We have used previous characterizations of mossy-fiber synapses (Brown & Johnston, 1983; Williams & Johnston, 1991) and our own observations to devise a set of minimal criteria for identifying isolated mossy-fiber excitatory postsynaptic currents (epscs). These include: fast rise time (under 3 ms), voltage independence of the decay time constant, small variability of the epsc latency, and ability to follow high-frequency stimulation. These criteria are not easy to satisfy. Slower currents of unkown origin as well as polysynaptic contamination are much more frequently observed. Using whole-cell recording methods, which has improved our signal-to-noise ratio by a decade, it is possible to dissect more components of evoked epscs. We have noted that small slow currents, which would be below the noise level using intracellular methods, are often evoked in conjunction with currents satisfying the mossy-fiber criteria. Criteria for isolating mossy-fiber epscs should be employed regularly, since failure to obtain pure mossy-fiber epscs may lead to significant interpretational errors. (Supported by ONR and NIH.)

533.15

ASSOCIATIVE LONG-TERM POTENTIATION IN THE HIPPOCAMPUS USING ANTIDROMIC STIMULATION AS THE CONDITIONING TETANUS. J. M. Jester and T. J. Sejnowski, Computational Neurobiology Laboratory, Salk Institute, La Jolla, CA 92037.

Associative long-term potentiation (LTP) is a long-lasting increase in synaptic response produced when a stimulus insufficient to cause LTP is paired with a conditioning tetanus. In area CA1 of the hippocampus, associative LTP is mediated by depolarization of the post-synaptic membrane which relieves the Mg^{++} block of the N-methyl-D-aspartate (NMDA) type of glutamate receptor. Can antidromically-elicited action potentials in the soma of CA1 pyramidal neurons depolarize the post-synaptic membrane in dendrites enough to prime the NMDA receptor?

Extracellular field potentials were recorded from 400 µm thick slices of rat hippocampus. The orthodromic test stimulus site (TEST) was in stratum radiatum of CA1 and the antidromic stimuls site (ANTI) was in the alveus. The ANTI site received a train of 50 bursts of 5 pulses at 100 hz with an inter-burst interval of 200 msec. The TEST site received 50 shocks at 5 Hz so that the shocks arrived simultaneous with the bursts to ANTI.

The conditioning stimulation to ANTI when given alone did not produce a change in either population spike or EPSP of the test site (0.0 ± 3.9%, n=6 PS, -5.4 ± 9.6%, n=5 EPSP), nor did the 5 hz stimulation to TEST (-2.5 ± 6.3%, n=11, PS and 0.6 ± 2.7%, n=11, EPSP); however, when the ortho- and antidromic stimulations were paired an increase in the population spike was observed (+24.0 ± 9.4%, n=5) which lasted at least 30 minutes. Application of 2-amino-5-phosphonovalerate (AP5) reversibly blocked this associative LTP. Thus, antidromically stimulated action potentials may invade the apical dendrites of CA1 hippocampal pyramidal cells and provide the depolarization necessary for associative LTP.

533.16

CURRENT-SOURCE DENSITY ANALYSIS OF LTP INDUCTION IN HIPPOCAMPAL SLICES. G. Capocchi and M. Zampolini
Clinical Neurology, Univ. Sch. of Med. Perugia. Italy

In a previous study we showed that the Long Term Potentiation (LTP) in the CA1 area of hippocampus was more easily obtained in the proximal portion of Apical Dendrites (pAD) compared to the Distal Portion (dAD). The slope increase was greater in pAD than in dAD. In order to investigate the origin of these differences we performed a current-source density (CSD) analysis before and after LTP induction. The experiments were carried out on rat hippocampal slices maintained in vitro. The stimulating electrode was placed in dAD. The recordings were obtained with two electrodes: one placed in CA1 somatic layer to test the constancy of spike population and the other used to explore, with 50 µm steps, the dendritic tree. The data were stored in the computer and one-dimensional CSD was calculated. The LTP was induced with short burst (4 pulses at 100 Hz), repeated 10 times with 200 ms of interval. In some experiments a 5 sec. of interburst interval was used. The input-output curves were also computed before and after LTP.

The results showed that in some experiments an early sink (ES) in pAD after induction of LTP appeared. Stimulation at 5 sec of interburst interval showed LTP and ES in pAD whereas no modification was observed in dAD. The modification in pAd was not influenced by DL-APV (50 µM) perfusion following LTP induction.

The data suggest that the patterned stimulation inducing LTP modifies the properties of pAD increasing depolarizing currents and support the hypotesis of a second (dendritic) component in the expression of LTP. This modification is not mantained by NMDA receptor.

533.17

LONG-TERM POTENTIATION CANNOT BE INDUCED IN THE CA1-FIELD OF THE HIPPOCAMPUS OF MICROENCEPHALIC RATS. Ramakers,G.M.J.[1*], Urban, I.J.A.[1], De Graan, P.N.E.[1], Cattabeni, F.[2], DiLuca, M.[2], and Gispen, W.H.[1]
[1] Rudolf Magnus Institute, University of Utrecht, The Netherlands. [2] Institute of Pharmacological Sciences, Faculty of Pharmacy, University of Milan, Italy.

Injection of methylazoxy methanol (MAM) to pregnant rats produces in offspring a.o. microencephaly, hypoplasia in the cerebral cortex and CA1 field of the hippocampus, disturbances in cognitive behavior and a reduction in the phosphorylation state of the B50/GAP43 protein (a specific substrate for proteinkinase C (PKC)) in the hypoplastic brain areas. Since long-term potentiation in the hippocampus involves a PKC-dependent phosphorylation we decided to examine the hippocampal LTP in slices from the rats with MAM-induced microencephaly (25 mg/kg of MAM injected on day 15 of gestation (G15)) and from control (saline on G15) rats. Field potentials (FPs) were evoked in the pyramidal and radiate layers of the CA1 field or in the granular layer of the dentate gyrus (DG) by stimulating (at 0.1 Hz and 1/2 maximum intensity) either Schaffer collaterals or the perforant path. Glass-electrodes (2 MOhm; filled with perfusing medium) were used for the recording. After 20 min of base-line FPs, a tetanic stimulation (1 sec, 100 Hz) of the same intensity was given. The recording of FPs was continued for the 60 min following the tetanus. The magnitude of the population spike (PS) and the slope of the EPSPs of averaged FPs (every 5 min; n=30) were computed. None of the MAM-treated slices (n=7) exhibited LTP in the CA1 field, whereas control slices (n=7) exhibited after the tetanus a LTP-like increase in FPs (increase of PS by >50% and the slope of EPSPs by 45-50%). In the DG slices from MAM-treated and control rats (n=7) showed a long-lasting (45-50%) increase in the slope of EPSPs. Wether the inability of tetanic stimulation to induce LTP in the CA1 field of the microencephalic rats is due to the profound neuronal disorganisation found in this field of the MAM-treated rats or to abnormal intrinsic properties of the CA1 neurons is currently investigated.

533.18

HETEROSYNAPTIC LONG-TERM POTENTIATION IN THE LATERAL ENTORHINAL CORTEX; AND EXTRA AND INTRACELLULAR STUDY ON THE ISOLATED IN VITRO BRAIN PREPARATION. Marco de Curtis(*) and Rodolfo R. Llinas, Dept. of Physiology and Biophysics, New York University, New York and (*) Dept. Neurophysiology, Ist. Neurologico, Milano, Italy.

A study of the properties of LTP in the entorhinal cortex (EC) (Alonso, de Curtis and Llinas; PNAS; 87, 9280-84, 1990) demonstrated that a non-Hebbian potentiation can be evoked in the layer II neurons by direct manipulating the postsynaptic membrane potential. This potentiation does not require simultaneous activation of the presynaptic fibers, suggesting that postsynaptic mechanisms are operant in this form of LTP. The ability to induce heterosynaptic LTP to a non-tetanized input following a high frequency stimulation of a separate input converging on the same cell population has been described (Bradler and Barrionuevo; Synapse, 4, 132, 1989). Utilizing the isolated brain preparation maintained *in vitro* by arterial perfusion (Llinas, Muhlethaler and Walton; J. Physiol. 414, 16, 1989), we tested both extra and intracellularly the possibility of heterosynaptic LTP induction in the spiny stellate and pyramidal neurons of layer II in the lateral EC. Two separate inputs converging on the apical dendrites of these cells were studied. The EPSPs evoked by both stimulations were characterized pharmacologically. LTP was induced either by a theta-frequency tetanic stimulation of the one input or by intracellular postsynaptic manipulations. In both cases heterosynaptic and non-Hebbian potentiation of the responses evoked by both stimuli were obtained. In contrast, short-term post-tetanic potentiation (or depression) was observed only in the response to the tetanized input. Supported by N.I.H. grant NS13742.

533.19

LONG-TERM POTENTIATION AND DEPRESSION OF THE EXCITABILITY OF THE CORTICOSTRIATAL TERMINAL FIELD. M. Garcia-Munoz, S.J. Young and P.M Groves
Department of Psychiatry, University California San Diego, La Jolla, CA 92093-0603.
We have found that long-lasting changes in the excitability of glutamatergic corticostriatal terminals can be induced by tetanic stimulation . Antidromic responses, recorded from medial-prefrontal cortex, were elicited by stimulation of the contralateral corticostriatal terminal field in urethane anesthetized rats. Excitability was assessed by determining the threshold current for antidromic activation of the terminal field before and after tetanization. Tetanizing stimuli (100Hz/250ms, every 6 sec, 5 times) were applied either through the striatal stimulating electrode or near the cortical recording site with a current sufficient to consistently elicit action potentials. Cortical tetanic stimulation typically produced a long-lasting (62 ± 13 min) decrease in excitability (-22 ± 7 %, 6/7 cells). In most cases, the excitability change persisted as long as the recording could be maintained. In contrast, long-term increases in excitability were induced in animals in which dopamine and GABAergic transmission were both disrupted either by 1) pretreatment with alpha-methyl-paratyrosine (AMPT, 250 mg/Kg, i.p) and bicuculline (BIC, 0.5 mg/Kg, i.v.) (26 ± 4%, 56 ± 16 min, n=7), or 2) kainic acid (KA) lesions combined with haloperidol (HAL, 0.1mg/Kg, i.v.) pretreatment (11 ± 2 %, 88 ± 38 min, n=4) or 3) AMPT combined with KA (11 ± 1%, 69 ± 22 min, n= 5). However administration of HAL with BIC or disruption of either neurotransmitter system alone produced variable effects. These results suggest that long-term decreases in excitability occur with marked activation of cortical glutamatergic afferents when GABA and dopamine transmission are intact. If both inhibitory inputs are reduced, long-term increases in excitability may be induced presynaptically. Interestingly, direct application of tetanizing pulses to the striatal terminals typically increased excitability (5/7 cells, 12 ± 2%, 48 ± 6 min) in untreated rats. Striatal administration of AP-7 (10μM) blocked this effect (3/4 cells) suggesting the involvement of NMDA receptors. Similar to other systems, corticostriatal afferents appear to express long-term decreases and increases in excitability. The relative levels of activity in inhibitory and excitatory striatal afferents and postsynaptic elements may determine the occurrence and direction of the long-lasting excitability change. This work was partially supported by grants from NIDA and the Office of Naval Research.

533.20

LONG-LASTING MODIFICATION IN SYNAPTIC EFFICACY AT PRIMARY AFFERENT SYNAPSES WITH NEURONS IN RAT SUPERFICIAL SPINAL DORSAL HORN. R. Cerne, M.C. Jiang* and M. Randic. Dept. of Vet. Physiology and Pharmacology, Iowa State University, Ames, IA 50011.

Although a great deal is known about long-term potentiation (LTP) in the brain, the existence of a similar synaptic plasticity at primary afferent synapses has not been demonstrated, as yet. We have investigated the excitatory synapse between small primary afferent fibers (Aδ and C) and neurons in the superficial laminae (LI-III) of spinal dorsal horn (DH) using intracellular recording from dorsal horn neurons in a transverse slice preparation of rat (25-39-days-old) spinal cord. Single electrical stimuli applied to the dorsal root evoked monosynaptic and/or polysynaptic excitatory postsynaptic potentials (EPSPs) in all DH neurons. Both types of EPSPs were blocked (n=13) by a new non-NMDA receptor antagonist 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo (F) quinoxaline (NBQX, 0.5-1.0μM for 2-4 min) indicating that a non-NMDA subtype of glutamate receptor mediates most of the fast EPSPs. However, the participation of a slower NMDA receptor-mediated component has also been shown. The depolarizations evoked by L-glutamate and L-aspartate were depressed by only 15-60% in the presence of NBQX (1-10μM). Brief, high frequency electrical stimulation of primary afferent fibers (100 Hz, 1s) produced a long-lasting potentiation of primary afferent-evoked EPSPs that appeared to be monosynaptic. In addition, in several cells a long-term depression of monosynaptic excitatory transmission was elicited. The results demonstrate that distinct and long-lasting changes in synaptic efficacy can be induced by high-frequency stimulation of primary afferents and that these may be physiologically relevant for transmission and integration of sensory information, including pain. (Supported by NS 26352 and BNS 841 8042).

ION CHANNELS: LIGAND-GATED

534.1

IDENTIFICATION OF A REGION WITHIN GLUTAMATE RECEPTOR SUBUNITS THAT CONTROLS CALCIUM PERMEABILITY. R. Dingledine, R.I Hume and S.F. Heinemann. Molecular Neurobiology Lab., The Salk Institute, La Jolla, CA 92037.

The glutamate receptor clones, GluR1 and GluR3, when transcribed and expressed individually or together in Xenopus oocytes, assemble into receptors that exhibit marked inward rectification and calcium permeability in response to kainate. Co-assembly of either subunit with GluR2, however, results in a receptor with a linear kainate i-v curve and greatly reduced calcium permeation (Hollmann et al., Science 1991). We have constructed subunit chimeras and point mutations to locate sites controlling the permeation characteristics of these glutamate receptors. A key residue was identified, R607 in GluR2 and its equivalent, Q612, in GluR3 (numbered from the initiator Met). Interconversion of amino acids at this position in GluR2 and GluR3 switched their phenotypes. Thus, GluR2(R607Q) expressed alone was active in contrast to the parent GluR2, which showed little activity, and GluR2(R607Q) behaved functionally like GluR3 in that its i-v curve displayed marked inward rectification in normal (high Na) solutions. In solutions containing low Na and high Ca or Ba, this construct responded to kainate with an inwardly rectifying current. Conversely, GluR3(Q612R) had a GluR2 phenotype since it was inactive when expressed alone but when co-expressed with GluR1 or GluR3 assembled into a receptor with linear i-v curve in normal solutions and outward Goldman rectification in low Na, high Ba solutions. Other mutants of GluR3 around position 612 produced similar but not identical effects. These findings suggest that the region identified is in or near the ion permeation path, and is an important determinant of ion permeability through non-NMDA receptor channels.

534.2

GLUTAMATE RECEPTOR CHANNEL KINETICS IN EMBRYONIC *DROSOPHILA* MYOTUBES
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Glutamate receptor channel kinetics were studied in embryonic *Drosophila* myotubes formed in culture. There were many brief events together with prolonged events. Long openings were interrupted by brief closures, thus showing bursting behavior. The burst histogram was fitted with three exponentials, the brief, intermediate and long component. The mean duration of long bursts increased with the glutamate concentration. The closed time histograms within and between long bursts were fitted with two exponentials at low concentrations and three at high concentrations.

The concentration dependent change in the burst duration, the mean duration and the relative amplitude of the long closed time component were fitted with a set of rate constants in the four state cooperative model in which two glutamate molecules bind to the receptor and open the channel. Generally, the scheme well predicted these changes but there were systematic deviations from the basic scheme.

534.3

THE DIVERSITY OF NON-NMDA CHANNELS REFLECTS THE DIVERSITY OF DIFFERING SUBSETS OF HIPPOCAMPAL NEURONS. C-M. Tang and Q-Y. Shi*. Dept. of Neurology, Univ. of Penn., Phila. PA.

Electrophysiological recordings from single non-NMDA channels suggest that there is a diverse group of channels. The diversity is exemplified by the widely varying single channel conductances, open times, Kd, and desensitization kinetics. In an attempt to categorize these channels into distinct subsets we have also found that cell morphology to be one more useful parameter. Channels obtained repeatedly from a single neuron show characteristics that are strongly conserved when compared to channels obtained from a neighboring but morphologically dissimilar neuron. This association is less rigid but still present when channels from different but morphologically similar neurons are compared. The diversity of non-NMDA channels and their association with specific subsets of neurons may reflect different roles that these neurons serve within the hippocampus.

534.4

THREE CLASSES OF KAINATE ACTIVATED CHANNELS ARE PRESENT ON CULTURED MOUSE FOREBRAIN NEURONS.
C. Poulopoulou and L.M. Nowak. Department of Pharmacology, Cornell University, Ithaca, NY 14853.

Excitatory amino acid (EAA) receptors are broadly classified as NMDA and nonNMDA types. Functional separation of the numerous nonNMDA receptors coupled to ion channels has proved difficult because of poor pharmacolological tools. Recent molecular biology studies indicate a large diversity nonNMDA EAA receptor channels which is likely to underly the diversity seen in electrophysiology. Thus, we are continuing our efforts to characterize nonNMDA receptor-channels by measuring biophysical properties of kainate (5-200 μM) activated channels in outside-out patches excised from mouse forebrain neurons grown in dissociated cell culture for 10-35 days. Recording solutions contained (in mM): 150 NaCl, 2.5 KCl, 1.0 $CaCl_2$, 10 Hepes - Na (pH 7.2) and 300 nM tetrodotoxin. Pipettes (3-5 MΩ) contained (in mM): 145 CsCl, 4 ATP-Mg, 10 EGTA/1$CaCl_2$ and 10 Hepes-K(pH 7.2). Three different channels populations were identified. Most patches contained more than one channel type, but some patches, showing mainly one class were obtained for all 3 populations. The brief ($\tau \sim 0.8$ ms) 30-40 pS channel appeared to desensitize rapidly. The 12-14 pS channel openings were interrupted by brief closures (τ_1 = 20-30 ms; τ_2 = 0.5 - 0.8 ms from noise). The 1 pS conductance (estimated from noise variance) had a main τ of 20-40 ms (90% to 98% of the power density). 1 pS and 12-14 pS channels were activated by kainate, domoic acid, and Br-willardiine. The 12-14pS channel was more sensitive to inhibition by CNQX than the 1pS channel. Supported by NS 24467 to LMN.

534.5

NON-STATIONARY CURRENT-VARIANCE ANALYSIS OF GLUTAMATE RECEPTOR-MEDIATED EPSCs AT THE MOSSY FIBER-GRANULE CELL SYNAPSE. S.F.Traynelis, R.A.Silver*, and S.G.Cull-Candy*. Department of Pharmacology, University College London, Gower Street London WC1E 6BT.

We have exploited the excellent temporal resolution of electrical recordings from cerebellar granule cells to examine the mean unitary conductance underlying both non-NMDA and NMDA receptor-mediated components of EPSCs recorded in response to stimulation of presumed mossy fiber axons. A classically slow decaying (τ = 57ms) NMDA component could be recorded in 10μM bicuculline/3μM glycine/0mM Mg^{++}; rapidly decaying non-NMDA EPSCs (τ = 1.4ms) were studied in isolation using 10μM bicuculline/15μM APV/10μM 7-Cl-kynurenate. An exponential function with τ constrained to that of the average EPSC timecourse was fitted to each individual EPSC, and the fitted decay subtracted. The resulting record was divided into sections, the variance for each section summed over all EPSCs within a given cell, and baseline variance subtracted. The unitary current (i) and number of channels (N) were calculated by fitting the current-variance (I,σ^2) relationship with $\sigma^2 = iI - I^2/N$. Mean unitary conductances of 11.4 ± 1.3 pS (n = 4 cells) and 41.8 ± 6.0 pS (n = 5) were found for non-NMDA and NMDA receptor-mediated EPSCs (respectively), which represent the weighted means for all channels participating. Confidence intervals for measurements of unitary conductance underlying fast EPSCs were estimated from unfiltered EPSCs simulated by summing simultaneous conductance steps of random, exponentially distributed durations. Analysis of the current-variance plots from these simulated currents for different numbers of summed channels (10-150), numbers of EPSCs (30-150), and decay times (τ = 0.25-2.0ms) resulted in 95% confidence intervals ranging between ± 5.6 and ± 10.5%.

534.6

NMDA-MEDIATED SINGLE CHANNEL CURRENTS FROM MAMMALIAN BRAIN RECONSTITUTED IN ARTIFICIAL LIPID BILAYERS. F. Wirtz-Brugger*, S. Auerbach, L. Wheeler* and C. Wilmot. Dept. Biol. Sci. Rutgers Univ. Piscataway, NJ 08855 and Hoechst-Roussel Pharm. Inc., Somerville, NJ 08876.

Ligand-gated single channel activity was recorded from rat brain membranes reconstituted into phospholipid bilayers formed on the tips of patch electrodes. Using cesium as the charge carrying ion, the reconstituted receptor exhibited properties of the NMDA receptor complex. Addition of NMDA (20 μM to 100 μM) to the pipette solution produced channel openings. With glycine (10 μM to 100 μM) and strychnine (1 μM) present along with NMDA, channel conductance was 55 pS. The gating of this channel was independent of voltage in the absence of Mg^{++}. Addition of 100 μM Mg^{++} resulted in increased channel bursting and voltage dependency of opening. Addition of the NMDA antagonist MK801 (100 μM) completely blocked channel opening. This method provides an opportunity to study the effects of the NMDA receptor-channel complex under tightly controlled experimental conditions. (Supported by PHS RR 007085-23).

534.7

LOCAL ANESTHETIC AND PROTON BLOCK OF ACH-EVOKED CURRENTS IN RAT PARASYMPATHETIC NEURONS. T.J.Nutter*, J.Cuevas* and D.J. Adams, Dept. of Pharmacol., Univ. of Miami Sch. Med., Miami, FL 33101

The dose- and voltage-dependence of local anesthetic (procaine, QX222) and proton (H^+) block of ACh-evoked currents was investigated in cultured parasympathetic neurons dissociated from rat intracardiac ganglia. Whole-cell currents evoked by 100 ms pulses of ACh applied from a pressure ejection pipette were recorded with a patch pipette containing (mM): 140 NaCl or CsCl, 2 MgATP, 2 Na_4BAPTA, 10 HEPES-NaOH, pH 7.2. The external pH was varied in some experiments using histidine buffer. Bath application of procaine or QX222 reduced ACh-evoked current amplitude in a voltage-dependent and reversible manner. An e-fold reduction in current amplitude occurred with ~120 mV hyperpolarization in the presence of 100 μM QX222. The effect of the local anesthetics on the time course of ACh-evoked currents was most apparent as a prolongation (~50%) of the decay phase at -80 mV. The dose-reponse relationships obtained for procaine and QX222 at -80 mV gave K_d's of 1 μM and 30 μM, respectively. The K_d's obtained for local anesthetic block of the neuronal ACh receptor-channel are an order of magnitude lower than found at the frog motor endplate (J. Physiol. **268**,291; **277**,153). Changes in external pH alter both ACh-evoked current amplitude and time course of decay. Both the current amplitude and half-time of decay were maximal at pH 8.0 and declined at acid and basic pH. The reduction in ACh-evoked current amplitude at acid pH suggests a titratable ionic group with a pKa of ~6.7. At pH 6.5, a 30% increase in the rate of decay of ACh-evoked currents was observed independent of membrane potential. Changes in external pH did not alter the reversal potential for ACh-evoked currents, indicating no measurable proton permeability. Taken together, the higher affinity of local anesthetics and H^+ for neuronal ACh-activated channels may be related to differences in the primary structure between the neuronal and muscle nicotinic receptor-channels.

534.8

METHYLLYCACONITINE (MLA) IS A POTENT ANTAGONIST OF NICOTINIC ACETYLCHOLINE RECEPTORS (nAChR) ON RAT HIPPOCAMPAL NEURONS: WHOLE-CELL PATCH-CLAMP STUDIES. M. Alkondon[1], S. Wonnacott[2] and E.X. Albuquerque[1]. [1]Dept. Pharmacol. & Exp. Ther., Univ. of Maryland School of Medicine, Baltimore, MD 21201; [2]Dept. Biochem., Univ. Bath, Bath, England.

MLA, an alkaloid isolated from the seeds of *Delphinium brownii* has been shown to antagonize the nAChR of insect, nematode and mammalian tissues. Recent studies showed that MLA is able to inhibit the binding of [^{125}I]α-BGT from the rat brain membranes with high affinity (*FEBS Lett.*, **270**: 45, 1990). We have earlier demonstrated nicotinic responses in tissue cultured neurons of embryonic rat hippocampus (*FEBS Lett.*, **222**:63, 1987), which were sensitive to α-cobratoxin (*Eur. J. Pharmacol.*, **191**: 505, 1990). In the present study using the whole-cell patch-clamp technique, we tested the ability of MLA to inhibit nAChR responses of fetal rat (17-18 day gestation) hippocampal neurons grown in culture for 2-5 weeks. MLA (50-1000 pM) elicited a concentration-dependent inhibition of currents evoked by either ACh or (+)anatoxin-a with an IC_{50} of ~150 pM. However, NMDA-evoked currents were unaffected by MLA up to 100 nM. The block of nicotinic currents was observed within 2-10 min of perfusion of the neurons with MLA, and was completely reversed by washing within 10-20 min. Current-voltage plots indicated a voltage-independent effect of MLA from -100 mV to +80 mV. The ability of MLA to antagonize the nicotinic currents in hippocampal neurons and the ability of the same to inhibit α-BGT-binding in several preparations are in accordance with the concept of the existence of an α-BGT-sensitive nAChR in these neurons. Support: U.S. Army Med. Res. & Devel. Comm. Contr. DAMD-17-88-C-8119 and USPHS NS25296.

534.9

POINT PROCESS ANALYSIS OF FROG ENDPLATE SINGLE CHANNEL CURRENTS. N.G. Castro*, E.F. Nobre*, and Y. Aracava, Lab. Mol. Pharmacol. II, IBCCF, UFRJ, RJ, Brazil 21941; Dept. Pharmacol. Exp. Ther., Sch. of Medicine, Baltimore, MD 21201.

Methods based on the theory of stochastic point processes were applied to the study of the temporal pattern of activation of endplate channels. Single channel currents activated by acetylcholine (ACh, 0.4-2.0 μM) were recorded from enzymatically dissociated muscle fibers with cell-attached patch clamping. Recordings started within 2 min of the initial exposure of the patch to ACh, allowing us to observed the relaxation to steady-state of slow desensitization. The digitized records were searched for opening transitions, which were taken for point events in time. The series of inter-openings intervals (IOIs) were statistically analyzed in order to characterize the structure of the underlying point process. This analytical approach depicted two main features of the receptor-channel desensitization process: i) a decreasing trend in the frequency of openings, the rate of which increased with ACh concentration, and ii) a tendency for openings to occur in clusters of bursts, also directly concentration-dependent. When several channels contributed to the currents, the trend could be fit by maximum likelihood, assuming a non-homogeneous Poisson model. In addition, expectation density function estimates showed evidences of tong term ($\approx$1 min) dependency between openings suggestive of a coupled regulatory process. Support: FINEP/UMAB Mol. Pharmacol. Training Program, CAPES & CNPq; U.S. Army Med. Res. & Devel. Comm. Contr. DAMD17-88-C-8119.

534.10

ION CHANNEL BLOCKADE BY OXIMES AND RECOVERY OF SOMAN-POISONED DIAPHRAGM MUSCLE IN VITRO. J.E.H. Tattersall. Biology Division, Chemical Defence Establishment, Porton Down, Salisbury, Wilts. SP4 OJQ, UK.

Oximes, which reactivate inhibited acetylcholinesterase (AChE), are used to treat poisoning by organophosphorus anticholinesterases. Some oximes, however, can produce neuromuscular recovery even when the inhibited AChE has aged and is no longer available for reactivation. I have tested the possibility that this "direct" recovery may be due to ion channel blockade.

The recovery produced by oximes and non-oxime analogues was measured in soman-poisoned guinea pig diaphragms in vitro. This was compared with their blocking activities on nicotinic receptor ion channels at the endplates of dissociated mouse muscle fibres.

There was a strong correlation between several parameters of ion channel blockade and the degree of direct recovery in diaphragms over the range of 12 compounds tested. Those which caused the greatest recovery produced a very rapid, flickering type of open channel blockade. Furthermore, the threshold concentrations for direct recovery and channel blockade were very similar.

The results are consistent with oxime-induced neuromuscular recovery from soman poisoning in vitro being due to ion channel blockade.

534.11

MAPPING OF THE LIGAND BINDING SITE OF NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS USING CHIMERIC SUBUNITS.

C. W. Luetje, M. Piattoni and J. W. Patrick. Division of Neuroscience, Baylor College of Medicine, Houston TX 77030.

A family of genes has been identified which encodes subunits of neuronal nicotinic acetylcholine receptors (nAChR). The α2, α3 and α4 subunits can each form functional nAChR when expressed in *Xenopus* oocytes in pairwise combination with either the β2 or β4 subunits. Each αβ subunit combination has unique pharmacological properties. α2β2 is insensitive to blockade by neuronal bungarotoxin (NBT) (IC50>1μM), and is more sensitive to nicotine than to acetylcholine. In contrast, α3β2 is highly sensitive to NBT blockade (IC50<10nM), and is less sensitive to nicotine than to acetylcholine. Amino acid residues involved in NBT and nicotine sensitivity were mapped by generating a series of chimeric subunits composed of portions of α2 and α3, and co-expressing them with β2 for analysis. NBT sensitivity was localized to a region of α3 between lys84 and ile215. Both chimeric subunits constructed at glu195 of α3 (α2-glu195-α3 and α3-glu195-α2) formed receptors insensitive to NBT, suggesting that amino acid residues involved in NBT sensitivity flank glu195. Chimeric subunit α2-ile215-α3 formed a receptor more sensitive to nicotine than to acetylcholine while chimeric subunit α3-ile215-α2 formed a receptor less sensitive to nicotine than to acetylcholine. Neither chimeric subunit constructed at glu195 (α2-glu195-α3 and α3-glu195-α2) formed a receptor more sensitive to nicotine than to acetylcholine. These results demonstrate that amino acid residues involved in nicotine sensitivity are located between the amino-terminus and ile215, and may flank glu195.

534.12

THE EXTRACELLULAR DOMAIN OF THE NEURONAL NICOTINIC SUBUNIT β4 DETERMINES THE PHARMACOLOGY OF RECEPTORS FORMED WITH α3 Roger L. Papke, Robert Duvoisin*, & Stephen F. Heinemann Molecular Neurobiology Laboratory, Salk Institute, La Jolla CA. 92037

The neuronal nicotinic acetylcholine receptor alpha subunit, α3, forms functional receptors when co-expressed in *Xenopus* oocytes with either β2 or β4 subunits. While the alpha subunits of nicotinic acetylcholine receptors are believed to contain the principle agonist binding sites for activation, in the case of neuronal nicotinic receptors beta subunits also influence receptor pharmacology, as well as the kinetics of agonist binding and channel activation. For example, it has been shown that α3β4 receptors are resistant to neuronal bungarotoxin (n-btx), while α3β2 receptors are blocked by this toxin (Duvoisin *etal.*, 1989). Also, α3β4 receptors are effectively activated by cytisine, while α3β2 receptors have been reported to be 100 fold less sensitive to cytisine than to ACh (Luetje & Patrick, 1991). In order to identify that portion of the beta subunits which may influence agonist and antagonist effects, we have created chimeric beta subunits which contain reciprocal exchanges between the β4 and β2 subunits in the first transmembrane domain. The chimeric subunits were constructed using PCRs to create half subunit pieces that shared a common terminal sequence. A final PCR then used the region of overlap to prime the synthesis of full length chimeric subunits. When β4/2 (β4 extracellular sequence and the remainder β2 sequence) is co-expressed with α3, receptors are formed which are resistant to neuronal-bungarotoxin and are activated equally well by cytisine and ACh. The macroscopic concentration-response relationships of receptors formed with β4/2 also show the higher Hill slope that is typical for β4-containing receptors. Conversely, α3β2/4 receptors are blocked by n-btx, are relatively insensitive to cytisine, and have a lower Hill slope.

534.13

DIFFERENTIAL MODULATIONS OF GABA RECEPTOR CHANNEL BY POLYVALENT CATIONS. M.Yan and T.Narahashi. Dept.of Pharmacol.,Northwestern Univ.Med.Sch.,Chicago,IL 60611.

$GABA_A$ receptor channel complex is a multimeric receptor protein with an integral chloride ion channel. We report here that polyvalent cations modulate the GABA-induced chloride current in a differential manner. The currents were recorded from the rat dorsal root ganglion neurons in primary culture by the whole cell patch clamp technique. The mechanisms underlying the modulations were also studied. Among all the cations tested at a concentration of 1 mM, Cu^{2+}, Zn^{2+}, Co^{2+}, Ni^{2+}, Cd^{2+} and Pb^{2+} suppressed the GABA-induced chloride current to different degrees whereas Mn^{2+} and Al^{3+} had no effect. Zn^{2+} and Cu^{2+} were most potent and abolished the GABA response almost completely. La^{3+}, however, increased the response 2-3 fold. The modulations by polyvalent cations were reversible after washing with normal solutions. Suppression by Cu^{2+} and Zn^{2+} and augmentation by La^{3+} occurred in a dose-dependent manner at a holding potential of -60 mV. La^{3+}, Cu^{2+} and Zn^{2+} all exerted their effects in a voltage-independent manner at holding potentials ranging from -80 mV to +60 mV. Competition studies showed that Cu^{2+} and Zn^{2+} did not compete with GABA for a binding site. These data suggest the existence of a cationic regulatory site distinct from the GABA site at or near the external orifice of the chloride channel.

534.14

INHIBITORY NEUROTRANSMITTER RECEPTORS PRESENT IN CULTURED RAT MEDULLARY NEURONS. C. A. Lewis and D. Faber, Neurobiology, SUNY at Buffalo, Buffalo, NY 14214.

Whole-cell current responses to bath application of glycine, ß-alanine, taurine, and GABA were studied in medullary neurons cultured from embryonic rats. Two-component responses were evoked by bath application of agonist, one component which desensitized and another which did not. The two current components have different dose-response characteristics with the nondesensitizing component being activated more effectively at lower concentrations than the desensitizing one and also reaching its peak at lower concentrations. For all agonists, the two current components have different strychnine sensitivities, with the desensitizing component being more sensitive. The complete occlusion between the responses to glycine and ß-alanine or glycine and taurine suggest that these agonists activate the same receptors. Taurine and ß-alanine are less potent agonists than glycine with relative potencies of 1: 0.4 :0.1 for glycine: ß-alanine: taurine. On the other hand, there was incomplete occlusion between the responses to glycine and GABA, suggesting that these agonists activate both similar and different populations of receptors. In confirmation, the receptor population activated by either glycine or GABA was blocked by both strychnine and picrotoxin. In conclusion, there appear to be at least 3 populations of inhibitory neurotransmitter receptors--GlyR, GABAR, and Gly/GABAR.

534.15

SINGLE-CHANNEL PROPERTIES OF NEWBORN RAT SPINAL CORD GLYCINE RECEPTORS EXPRESSED IN XENOPUS OOCYTES

Quoc Thang NGUYEN*, Andres MORALES* and Ricardo MILEDI, Laboratory of Cellular and Molecular Neurobiology, Dpt. of Psychobiology, University of California, Irvine CA92715 USA

Using the noise-analysis technique, we measured the single-channel characteristics of glycine receptors (expressed in Xenopus oocytes) by heavy (H) and light (L) mRNA isolated from the newborn rat spinal cord (cf.ref.1 & 2). Oocytes were injected with H or L fractions and recorded four to six days after injection. Cells were clamped at -60mV and perfused with 1-2x10-4M glycine in Ringer. Power spectra of newborn L receptors were well fitted by a Lorentzian. The unitary current at -60mV was -0.94+/-0.46pA (mean+/-S.D, n=17), the mean open time tau being 50.9+/-6.3ms, a near three-fold increase over the value found in the adult (ref.2). In oocytes injected with H fractions, some spectra could be fitted by one Lorentzian; in those cases the unitary current was -1.23 +/-0.45ms and tau was 7.7+/-2.4ms (n=9), values identical to the adult.

1.Akagi & Miledi (1988) Science 242, 270-273
2.Morales & Miledi(1991)J.Physiol.Proc.434:25P

534.16

MOLECULAR CLONING AND FUNCTIONAL EXPRESSION OF A SEROTONIN-GATED ION CHANNEL. A.V. Maricq*, A.S. Peterson*, A.J. Brake*, L. Tecott, R.M. Myers*, and D. Julius*. Depts. of Pharmacology and Physiology, University of California, San Francisco, CA 94143-0450.

Serotonin is a ubiquitous neurotransmitter whose diverse functions are mediated by activation of pharmacologically distinct receptor subtypes. Although most serotonin receptors appear to transduce extracellular signals by interacting with G-proteins, the 5HT3 subtype of serotonin receptor (5HT3R) was recently shown to incorporate a ligand-gated cation-selective ion conductance. We have isolated a functional clone encoding a 5HT3 receptor (5HT3R-A) by screening a neuroblastoma cDNA expression library for serotonin-gated currents in *Xenopus* oocytes. The isolated clone encodes a single protein that has many of the predicted secondary structural features shared by other ligand-gated ion channels (e.g. the nicotinic acetylcholine, GABA-A, and glycine receptors). When the cloned receptor is expressed in oocytes, the pharmacological and electrophysiological characteristics of the serotonin-gated current are largely consistent with the known properties of the 5HT3R. Potent and selective 5HT3R antagonists such as ICS 205-930 block the serotonin-gated current at nanomolar concentrations. In agreement with previous reports, the current passes through a non-specific cation conductance that is modulated by the external concentration of divalent cations. However, we also find that I-V plots of the serotonin-gated current display a region of negative slope conductance that is sensitive to the external divalent cation concentration and that resembles the voltage and divalent cation mediated conductance changes observed in the NMDA subtype of excitatory glutamate receptor.

534.17

NORADRENERGIC α_1 RECEPTOR-MEDIATED DEPOLARIZATION OF NEURONS OF THE MEDIAL PONTINE RETICULAR FORMATION OF THE RAT, *IN VITRO*. D.R. Stevens, R.W. McCarley, and R.W. Greene. Neuroscience Lab., Dept of Psychiatry, Harvard Med. School and VAMC, Brockton, MA 02401

The medial pontine reticular formation (mPRF) plays an important role in REM sleep and in the startle reflex in waking. Neurons of the locus coeruleus display firing activity inversely correlated to the expression of REM sleep. Noradrenergic neurotransmission is important in behavioral state control and influences the appearence of REM sleep. The action of norepinephrine (NE) on neurons of the mPRF was examined in rat brain slices *in vitro* using intracellular electrophysiological methods.

Application of NE to mPRF neurons results in a depolarization of 74% of the neurons tested (n=28). NE is mimicked by the α_1 agonist phenylephrine (PE, n=19). NE and PE inward currents were accompanied by a decrease in membrane conductance. The reversal potential of the inward current elicited by PE corresponded to that of K^+ and was sensitive to alterations of K^+_o in a Nernstian manner. The current-voltage relation recorded during voltage ramp commands indicates a strong voltage-dependence of the PE sensitive current. The conductance change elicited by PE is greater at depolarized membrane potentials. The mechanism of the PE induced conductance change is under study.

These results support the conclusion that NE acting at α_1 receptors causes a decrease in potassium conductance. The potassium conductance(s) involved in the action of NE has not yet been identified.

534.18

REGULATION OF Ca^{2+} RESPONSES TO ENDOTHELIN-1 BY VOLTAGE- AND RECEPTOR-GATED Ca^{2+} CHANNEL ANTAGONISTS AND DRUGS ACTING ON PHOSPHOLIPASE C AND PROTEIN KINASE C. J. Chan and D.A. Greenberg. Department of Neurology, University of California, San Francisco, CA 94110.

Endothelin-1 (ET-1, 5 nM) produced biphasic (transient peak and sustained plateau) elevations of free intracellular Ca^{2+} (Ca^{2+}_i) detected by fura-2 fluorescence in NG108-15 neuroblastoma x glioma cells. Both peak and plateau responses were attenuated by depletion of extracellular Ca^{2+}. Peak responses were also reduced by the putative receptor-gated Ca^{2+} channel antagonist SK&F 96365 (30 μM), while plateau responses were abolished by 100 nM nimodipine. The phospholipase C (PLC) inhibitor U-73122 (1 μM) and the protein kinase C (PKC) activator phorbol 12-myristate 13-acetate (PMA, 1 μM) reduced peak responses by 80% and 35%, respectively, compared to responses in the presence of the inactive analogs U-73343 and 4α-PMA. In Ca^{2+}-depleted buffer, both U-73122 and PMA abolished peak Ca^{2+}_i responses, while the PKC inhibitor staurosporine (100 nM) increased peak responses by 250%. These findings suggest that peak Ca^{2+}_i responses to ET-1 result from influx of Ca^{2+} through receptor-gated channels and PLC-activated mobilization of intracellular Ca^{2+}, and are inhibited by PKC, while plateau responses involve influx of extracellular Ca^{2+} through dihydropyridine-sensitive, voltage-gated channels.

534.19

DOMAIN STRUCTURE OF THE INOSITOL-1,4,5-TRISPHOSPHATE RECEPTOR. G. A. Mignery*, C. L. Newton* and T.C. Südhof Howard Hughes Medical Institute, Department of Molecular Genetics, UT Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75235

The inositol-1,4,5-trisphosphate ($InsP_3$) receptor is composed of a homotetramer of highly conserved 313Kd subunits which contain eight membrane spanning regions in the COO-terminal fourth of the protein. The structural features of the receptor were examined by mutagenesis and transient expression in COS cells. Deletion of the eight transmembrane regions resulted in the expression of a soluble, monomeric protein which binds $InsP_3$ with an affinity and specificity similar to the wild type receptor. This data indicates that the membrane spanning regions are essential for subunit tetramerization but do not contribute to the ligand binding site and that each receptor subunit contains an independent ligand binding domain. To identify the sequences composing the ligand binding site COO-terminal deletion mutants were constructed. $InsP_3$ binding analysis on the truncated receptor proteins showed that the ligand binding region resides in the NH_2-terminal fourth of the receptor subunit which is separated from the putative Ca^{2+}-channel by over 1400 amino acids. Gel filtration experiments demonstrate a large conformational change of the receptor as a function of ligand binding suggesting a mechanism by which ligand binding might cause channel opening. Taken together, these results allow each receptor subunit to be divided into a NH_2-terminal ligand binding domain, a central coupling/regulatory domain and a COO-terminal cluster of membrane spanning regions which interact to form the receptor homotetramer and the Ca^{2+}-channel.

ION CHANNELS: CELL FUNCTION

535.1

EXPRESSION OF CONNEXIN26 GAP JUNCTION CHANNELS IN CELLS WITH NEURONAL PHENOTYPE. B. Eghbali*, J.A. Kessler, M. Dougherty*, H. Hsu*, M. Chanson*, and D.C. Spray. Einstein Coll. Med., Bronx, NY 10461

Connexin26 (Cx26) is an abundant gap junction protein in the developing nervous sytem; in the adult brain it is confined to specific cell types (e.g., pinealocytes and leptomeningeal cells) and is usually coexpressed with other connexins. To define the properties of Cx26 we stably transfected pc12 cells (a well-characterized, communication-deficient neuronal system) with Cx26 cDNA, using a plasmid containing a dexamethasone (dex)-inducible promoter and the selectable marker *neo*. G418-resistant colonies were injected with Lucifer Yellow and four dye coupled colonies were separated for further analysis. Whereas mRNA encoding connexins 32, 43 and 26 were not detectable in the parental cell line using Northern blot analysis, two bands (presumably elongated transcripts including portions of the vector) were found to hybridize to the Cx26 probe in the transfected colonies. Immunofluorescence using connexin-specific antibodies failed to reveal connexins 26, 32 or 43 in the parental line, but in the single transfected clone thus far analyzed, punctate Cx26 immunoreactivity was prominent. Junctional conductance was barely detectable in the parental line (0.03±0.03 pS; n=19) but was moderate in dex-treated transfectants (4.0±0.8 nS; n=41). Two populations of unitary junctional events were detected in Cx26 transfectants after dex treatment, <100 and >175 pS; because the latter event was seen in an untransfected dex-treated pc12 cell pair and in a pair of untreated pc12 transfectants, we attribute the smaller event to channels formed by the introduced Cx26. The stable expression of Cx26 in a well-coupled cell line in which neuronal phenotype can be induced should allow biophysical analysis of Cx26 and illuminate the role of gap junctions in neuronal differentiation and function.

535.2

EFFECTS OF METABOLIC INHIBITORS ON INSECT MUSCLE: A COMPARATIVE STUDY OF DIPTERA AND LEPIDOPTERA. E.M. Fitzgerald and M.B.A. Djamgoz.* Dept. Biol., Imperial College, London, SW7 2BB, U.K.

Work on insect muscle has shown that Diptera have a ouabain-sensitive Na^+/K^+-ATPase, whilst Lepidoptera have a ouabain-insensitive K^+-pump. Using K^+-selective microelectrodes, we have quantified the effects of various metabolic inhibitors on membrane potential (E_m) and intracellular K^+ activity (aK_i) in skeletal muscle of Phormia terranovae (Diptera) and Spodoptera exigua (Lepidoptera). The following inhibitors were used: dinitrophenol, cyanide, azide, rotenone, dicyclohexylcarbodiimide, salicylhydroxamic acid, ouabain, ethacrynic acid, vanadate, amiloride. Each inhibitor (conc. 1mM) was applied for 60 mins. at R.T. (20°C) and E_m and aK_i recorded continuously from different cells (20-50 cells; 5-7 insects).

Most inhibitors caused significant reduction of aK_i ($p < 0.001$) apart from ouabain and amiloride, which were ineffective in Spodoptera and Phormia, respectively. Strong inhibition by vanadate was common to both insects, suggesting that both have P-type ATPases. However, differences in ouabain and amiloride sensitivities suggest the molecular structures of the two pumps are different. All inhibitors except ouabain, caused significant depolarisation of E_m ($p < 0.001$). For a given species, the inhibitor profiles of aK_i and E_m were largely similar, implying that changes in E_m and aK_i are closely linked.

535.3

THE PERINEURAL WAVEFORMS AFFECTED BY DINOFLAGELLATE *PTYCHODISCUS BREVIS* TOXIN. M.C.Tsai, M.L.Chen*. Department of Pharmacology, College of Medicine, National Taiwan University, Taipei, Taiwan, R.O.C.

The dinoflagellate *Ptychodiscus brevis* induced numerous fish kills and human toxicity. The toxins in the dinoflagellate initially facilitated the transmitter releasing process followed by complete block of the neuromuscular transmission. Brevetoxin-B was one of the toxic component in the dinoflagellate. It increased the MEPP frequency and quantal content of EPP of mouse diaphragm. The effect of the toxin on the ionic currents of the nerve terminal was studied by the perineural waveform recordings of adult I.C.R. mouse (*Mus musculus* from Institute of Cancer Research). Waveforms were evoked by stimulating the motor nerve bia a suction electrode every 2-30 s with supramaximal pulses of 0.05 ms duration. At lower concentration (0.11 μM), brevetoxin-B increased the sodium and potassium currents of nerve terminal while it decreased the calcium activated potassium current and slow calcium currents of the nerve terminal. At this concentration, the toxin had no effect on the fast calcium current and slow potassium currents. At higher concentration (1.11 μM), the toxin decreased all of the perineural waveforms on the nerve terminal. The effect may contribute to the human toxicity of the dinoflagellate. (Supported by a grant, NSC080-0412-B002-13Z, NSC, ROC).

535.4

REGIONAL DISTRIBUTION OF ION CHANNELS IN A CULTURED CRUSTACEAN PEPTIDERGIC NEURON. D. E. R. Meyers and I. M. Cooke. Békésy Lab. of Neurobiology and Dept. of Zoology, University of Hawaii, Honolulu, HI 96822.

A subpopulation of neurons isolated from the X-organ of the tropical land crab (*Cardisoma carnifex*) and grown in primary culture in defined medium are reactive against crustacean hyperglycemic hormone (CHH) antisera. When plated with a neurite, a large lamellipodium forms (PNAS 86:402, 1989). Regular firing and bursts of action potentials can be recorded. Under whole-cell voltage clamp, Ca, Na and two types of K current can be isolated (Graf & Meyers, Meyers & Graf, FASEB J. 4:1200; 1201, 1990). The conductances resident in isolated somata and lamellipodia after 24 h in culture have now been examined. Conventional "whole-cell" patch-clamp recordings were made from somata and lamellipodia which had been separated by severing the neurite (internal and external solutions as previously).

Somatic and lamellipodial I_{Ca} were similar. From a V_h of -40 mV, the I(V) curve is without notches and shifting V_h from -40 to -80 mV has little effect on maximal I_{Ca}. Decay was slow and incomplete. In paired-pulse experiments in somata, the relation between test-pulse current amplitude and prepulse potential was U shaped. In contrast, lamellipodial test current did not recover with large depolarizing prepulses. I_{Na} was best developed in lamellipodia. Comparison of I_{Na} in somata with their lamellipodia showed that peak somatic I_{Na} was less than 30% of lamellipodial I_{Na} (at 0 mV, V_h = -40 mV). K current similar to I_A and I_K was observed in both regions. I_A was half-inactivated at -46 mV and inactivation was removed with a τ of 30 ms. The I(V) relations for I_K, and V_R measured in either region were similar. I_A was sensitive to 4-AP (6 mM) and I_K to TEA-Br (100 mM). As in intact cells, 4-AP was sometimes associated with an increase in I_K (FASEB J. 4:1200). The data suggest that voltage-dependent K channels with similar properties are distributed throughout the CHH neuron. Ca channels are also similar except that lamellipodial I_{Ca} is not enhanced by large prepulses. Na channels appear to be preferentially located in the distal neurite and/or lamellipodium. Supported by grants from the Whitehall Foundation, NSF BNS89-10432 and NIH G12RR03061.

535.5

GRADED AND REGENERATIVE POTENTIALS IN NORMALLY NONSPIKING STRETCH RECEPTOR NEURONS IN A SAND CRAB. D.H.Paul and J.Bruner. Biology Department, University of Victoria, Victoria BC V8W 2Y2

Electrical responses in the four dendrites forming the telson-uropod stretch receptor of *Emerita analoga* were triggered by stretch of the elastic receptor strand or by intracellular current injection. Investigations of three types of isolated receptor-ganglion preparation [intact neuron, isolated distal segment (DS), similar segment isolated from the receptor strand (DSWS)], using conventional two-microelectrode current clamp, revealed that the four neurons are not identical in all of their electrical properties. 1) Active responses to stretch do not normally occur, but in saline with 50mM TEACl (substituted for NACl) stretch triggers a regenerative potential out-lasting the stimulus; this potential is abolished in Cd^{2+} (1mM) and is not observed in DS. 2) Both DS and DSWS of all four dendrites show linear I/V relations between -120 and -30 mV (MP = -60 to -65 mV) and delayed rectification (abolished by 50 mM TEA^+) above -30 mV. 3) In dendrites III and IV depolarization triggers a fast, graded, TTX-sensitive potential which is not made regenerative by potassium channel blockers (50 mM TEA, 5 mM 4-AP). This transient is very small or absent from dendrites I and II. 4) In dendrite III, but apparantly not in the other dendrites, the transient is followed by a slowly increasing depolarization which develops over several hundreds of milliseconds.

Supported by NSERC, Canada

535.6

MEMBRANE PROPERTIES AND GABA RESPONSES OF THE CULTURED TRIGEMINAL GANGLION CELLS OF THE MARINE CATFISH, PLOTOSUS. T. OGURA AND S. OBARA*, Dept. Physiol., Teikyo Univ. Sch. of Med., Tokyo 173, Japan

The trigeminal ganglion cells, isolated and cultured for 1-4 days, were examined with the whole-cell recording technique. The action potential showed a hump in the falling phase, with a half-duration of 1.8 ms. TEA enhanced the hump when the membrane potential had been shifted to more positive levels. Hence, the TEA-sensitive K current seemed to operate in repolarizing the spike. The spike duration was prolonged with CsCl-filled electrodes, which was reduced by Co2+, both suggesting an involvement of the Ca current. Two types of the Ca current, LVA and HVA, were confirmed. These currents seemed to govern the encoding pattern, with the maximum firing rate reaching only about 70 HZ. Perfusion with GABA of 0.1-1 mM induced inward, mostly Cl, current while glycine had no effect. In some cells, GABA also reduced the Ca current moderately. Thus, GABA might exert presynaptic inhibition directly by hyperpolarization, and also probably by modulation of an intracellular signal transduction, at the proximal endings of the trigeminal nerve.

535.7

INTRACELLULAR RECORDING AND STAINING OF TERMINAL NERVE-GNRH CELLS IN A FISH BRAIN IN VITRO. **Y.Oka. Zool. Inst., Fac. Sci., Univ. of Tokyo, Tokyo 113**

We have previously shown in the dwarf gourami that terminal nerve (TN) cells are a major component of the GnRH system and that the TN-GnRH system is structurally independent from the preoptic/hypophysial-GnRH system which facilitates gonadotropin release from the pituitary. Thus, we have hypothesized that GnRH may function as an important neuromodulator (Oka and Ichikawa, '90). A whole-brain in vitro preparation has been developed to facilitate studies on the ionic and biochemical mechanisms of the TN-GnRH neuromodulatory system. In the present study, I examined the spontaneous electrical activity of TN cells using both extra- and intracellular recording techniques and determined the morphology of individual cells by intracellular injections of either Lucifer Yellow or biocytin. TN cells showed endogenous activities of either slow regular beating (1-6 Hz) or bursting patterns according to the physiological conditions (e.g., sexual maturity) of the fish. Anatomical observation of intracellularly-labeled cells revealed multiple axonal branches that projected to those areas where we had previously demonstrated dense GnRH-immunoreactive fibers. The electrophysiological and morphological characteristics of TN-GnRH cells seem to be advantageous for their possible role as a neuromodulator; the intrinsic membrane properties of modulator neurons change according to the animal's physiological conditions, and these modulator neurons in turn modulate neural activities in a wide variety of brain regions simultaneously via multiple axon branches.

535.8

THALAMIC FIRING PATTERNS ARE HIGHLY SENSITIVE TO VARIATION IN LOW THRESHOLD CALCIUM CHANNEL: COMPUTER SIMULATIONS OF VOLTAGE AND CURRENT CLAMP EXPERIMENTS. W.W. Lytton and T.J. Sejnowski, Salk Institute, La Jolla, CA, 92037

The low-threshold calcium channel (T channel) is believed to be critical in producing the anode break behavior characteristic of thalamic relay cells. We used computer simulations of Hodgkin-Huxley like models to explore the influence of different models of this channel on responses to simulated current clamp. We first reproduced characteristic current/voltage curves under simulated voltage-clamp using a model of the high threshold calcium channel (L channel) combined with a model of the T channel obtained from either of two sources (Coulter et al., *J Physiol* 1989, 414:587-604; Crunelli et al., *J Physiol* 1989, 413:543-561).

Thalamic cells show a complex variety of responses under current clamp. Characteristic firing patterns include: 1) a single spike on a plateau in response to depolarization from rest 2) repetitive spiking in response to increased depolarization from a depolarized potential 3) a brief burst of spikes as an anode break response following hyperpolarization (Jahnsen and Llinas, *J Physiol* 1984, 349: 227-247). We were able to reproduce these 3 firing patterns using a model that included 8 channels found in this cell type. Firing behavior was highly dependent on the details of the T channel model used and varied widely between the two sets of parameters from the two different voltage clamp studies. These differences may reflect artifactual variation due to the different voltage-clamp techniques or actual differences due to species and developmental stage. Relatively minor channel variations could be exploited in control mechanisms that alter the firing pattern of the cell.

535.9

ESTIMATION OF PASSIVE ELECTROTONIC PARAMETERS USING THE INVERSE FOURIER TRANSFORM TECHNIQUE TO STUDY ETHANOL EFFECTS ON GRANULE CELLS. M. Patil, D. Durand, G.L. Yuen and R. Chintalacharuvu*, Applied Neural Control Lab., Dept. of Biomedical Engineering, Case Western Reserve University, Cleveland, OH-44106.

Accurate estimation of the passive electrotonic parameters of a neuron is essential for understanding the neuronal integration and information processing. The shunt cable model (Durand et al., 1982), which allows for different somatic and dendritic time constants was used to model the dentate granule cells and was modified to include an electrode artifact. The input impedance of the model and its voltage response to a current stimulus were calculated in the frequency domain, converted to the time domain using the inverse Fourier transform, and the parameters were estimated by nonlinear, least-square fitting of the model output to the voltage response to a current stimulus. Optimization of the objective function was obtained using the gradient method (NL2SOL) algorithm. In order to quantify the consequences of acute ethanol treatment on the neurons, parameters such as the input resistance, dendritic and somatic time constants, resistances, and capacitances were obtained before and after exposure to low doses (50 mM) of ethanol and compared.

Intracellular responses to short hyperpolarizing current stimuli (0.5 msec, 2.5/5/7.5 nA) were recorded in 12 cells from hippocampal slices prepared from adult rats. The membrane time constant (τ_m) was found to decrease in ethanol by 24 %, while the somatic time constant (τ_s) increased by 27 %. The electrotonic length also increased in ethanol from (mean ± SD) $L_c = 1.026 \pm .12$ to $L_e = 1.202 \pm .17$, ($p<.05$). Although the mechanism(s) for these changes is unclear at this time, they may influence the neuronal integration in ethanol.

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535.10

CHOLINERGIC MODULATION OF ENTORHINAL CORTEX LAYER II NEURONS' INTRINSIC EXCITABILITY. A. Alonso and R. Klink. M.N.I., McGill University, Canada. H3A-2B4.

The entorhinal cortex (EC) layer II is the major source of afferents to the hippocampus via the perforant path and receives inputs from the entire cortical mantle. It is also innervated by multiple subcortical structures including notably the cholinergic neurons of the basal forebrain (Alonso and Kohler, J. Comp. Neurol., 1984). By using intracellular recordings in "in vitro" rat brain slices, we have investigated the effects of bath application of the muscarinic cholinergic agonist carbachol (15-50uM) on the two previously described groups of EC layer II neurons: the stellate cells (SCs) and the pyramidal-like cells (PLs) (Alonso and Llinas, Soc. Neurosc., 1990). On the SCs, carbachol induced a modest depolarization, decreased the fast afterhyperpolarization and suppressed cell firing accommodation. It also reduced a subthreshold Na-dependant component of the rebound potential at the break of hyperpolarizing current pulses suggesting a cholinergic modulation of a subthreshold Na-conductance. The Na-dependant subthreshold oscillatory activity characteristic of the SCs also showed a small reduction in frequency and rhythmicity with carbachol. On the PLs, among other effects, carbachol drastically affected the cells firing behavior by inducing a slow rhythmic bursting pattern which was associated with a substitution of the post-train afterhyperpolarizations by long-lasting plateau depolarizations. The results demonstrate a potentially strong influence of basal forebrain cholinergic inputs to the modulation of EC layer neurons' intrinsic excitability by which the processing of incoming neocortical information to the hippocampus will be strongly affected. (Supported by Canadian MRC).

535.11

INWARD AND OUTWARD RECTIFICATION AND SUBTHRESHOLD OSCILLATIONS IN ENTORHINAL CORTEX LAYER II STELLATE CELLS. R. Klink and A. Alonso. MNI, McGill University, Canada H3A 2B4.

The stellate cells from entorhinal cortex layer II display Na-dependent (not Ca) rhythmic subthreshold oscillatory activity that probably underlies the generation of the theta rhythm in this cortical area (Alonso and Llinas, Nature, 1990). By using intracellular recordings in "in vitro" rat brain slices, we have investigated some of the non-linear membrane properties that may contribute to generation of the oscillatory behavior. Following blockage of Na conductances with TTX, analysis of the current-voltage relationships revealed a robust time-dependent inward rectification as well as a fast (non-transient) outward rectification which developed strongly at the oscillatory voltage level. Time-dependent inward rectification could be abolished by bath application of 1-2mM Cs which had little effect on the outward rectification but had no effect on the time-dependent inward rectification. Carbachol (30-60μM), 4-AP (2mM) and Ca-channel blockers Co or CD, had no effect on the non-linearity of the membrane in the oscillatory voltage level. In control ringer solution both Cs and Ba ions almost completely abolished the subthreshold oscillatory activity. The results suggest that in addition to a persistent inward Na-current and a Q-like current, a voltage-dependent K outward current plays a major role in the generation of the rhythmic subthreshold oscillatory activity in entorhinal cortex layer II stellate cells. (Supported by MRC Canada).

535.12

SEROTONIN DEPOLARIZATION OF MOUSE PITUITARY CORTICOTROPE CELLS (AtT-20/D16V). L. M. Konopka, K.M. Braas, J.F. Fiekers and V. May. Dept. Anatomy & Neurobiology., Univ. Vermont College of Medicine, Burlington, VT 05405.

Serotonin (5-HT) is localized to a subset of gonadotrophs in the mammalian anterior pituitary gland (Payette et al., 1985) and elicits ACTH release from dissociated anterior pituitary cells (Spinedi and Negro-Vilar, 1983). In the present experiments the effects of 5-HT were examined on a homogeneous cell line of corticotrophs (AtT-20/D16V cells). 5-HT was applied by pressure ejection onto single AtT-20 cells plated on glass coverslips and maintained in HEPES-buffered solution. 5-HT activated these cells by producing a transient depolarization which could be superimposed upon spontaneous membrane oscillations. The amplitude and duration of the response to 5-HT depended on the pulse duration and did not desensitize. The depolarization was attributed to an increased conductance and exhibited a complex voltage dependence. These results demonstrate a direct excitant effect of 5-HT on AtT-20 cells which may initiate or regulate the release of ACTH. (Supported by NIH grants NS25973, NSF9010044)

535.13

SEROTONIN PRODUCES TRANSIENT AND SUSTAINED ELEVATIONS OF CYTOSOLIC CALCIUM IN FURA-2 LOADED CULTURES OF MOUSE PITUITARY CORTICOTROPE CELLS (AtT-20/D16v) J. F. Fiekers, L. M. Konopka and V. May. Dept. Anatomy & Neurobiol., University of Vermont College of Medicine, Burl., VT 05405.

The actions of serotonin (5-HT) were examined on AtT-20 mouse corticotrope cells. Individual cells were loaded with fura-2/AM and examined for changes in intracellular calcium concentration using ratio fluorescence of fura-2. 5-HT produced a concentration-dependent increase in $\{Ca\}_i$ in more than 80% of the cells studied. The response was repeatable, lasted for minutes, and was characterized by a transient elevation either with or without a plateau. At the peak of the transient calcium elevation, some cells exhibited additional, rapid, repetitive calcium transient responses which were also observed in the absence of extracellular calcium. Twenty percent of the cells in culture exhibited rhythmic spontaneous oscillations of $\{Ca\}_i$ and exposure to 5-HT induced rythmic oscillations of $\{Ca\}_i$ in quiescent cells. 5-HT responses could be superimposed on these oscillations. These results demonstrate that 5-HT increases $\{Ca\}_i$ and may modulate the release of ACTH from corticotropes. (supported by NIH grants NS27319 and NS25973)

535.14

VISCERAL C-FIBER NEURONS ARE HETEROGENEOUS WITH RESPECT TO THE K+ CONDUCTANCES CONTRIBUTING TO ACTION POTENTIAL REPOLARIZATION . E.P. Christian and K.E. Naper. Department of Pharmacology, ICI Americas, Inc., Wilmington, DE 19878.

Visceral C-fiber neurons in the nodose ganglion synthesize and release several tachykinin peptides that may be involved in hyperreactive conditions, such as asthma. Because action potential repolarization is an important locus for the control of transmitter release and repetitive firing, we examined the contribution of different K^+ currents to the action potential. Single microelectrode current- and voltage-clamp techniques were applied to study C-fiber neurons in intact guinea pig nodose ganglia maintained *in vitro*. C-fiber neurons that could not be otherwise distinguished by standard electrophysiological parameters differed with regard to the K^+ conductances contributing to action potential repolarization. Three components were identified: 1) in ~50% of the neurons charybdotoxin prolonged action potential duration to a maximal 160% of control in a concentration-dependent, and Ca^{2+}-sensitive manner (EC_{50} = 22.0 nM; n=10); 2) in ~70% of the neurons a Ca^{2+}-insensitive "A-like" current shortened the duration of the action potential by up to 25%, when it was de-inactivated by hyperpolarizing prepulses negative to -55 mV; and 3) in *all* neurons studied, tetraethylammonium (5-30 mM) increased the action potential duration (50-500%), independent of the presence of Ca^{2+}, or prepulse potential. Thus a Ca^{2+}-dependent, and at least two other voltage-sensitive K^+ conductances appear to be differentially available for action potential repolarization in subpopulations of C-fiber neurons.

535.15

LIMITED CORRELATION BETWEEN SYMPATHETIC NEURONE SUBTYPE AND PEPTIDE CONTENT IN GUINEA PIG PREVERTEBRAL GANGLIA. E.M. McLachlan, J.R. Keast and R.L. Meckler, Department of Physiology and Pharmacology, University of Queensland, Qld 4072, AUSTRALIA.

Guinea pig sympathetic neurones can be classified as phasic, tonic or long afterhyperpolarizing (LAH), by their discharge pattern following a depolarizing current step. These patterns result from the expression of different populations of voltage- and calcium-dependent K+ channels. Each neurone type receives a characteristic pattern of synaptic input. The proportions of each type in prevertebral ganglia of young guinea pigs varied rostrocaudally from the coeliac (CG) to the distal inferior mesenteric ganglion (IMG), with tonic cells increasing in the caudal direction (from 15 to 80%) and LAH cells being virtually absent in the IMG. The proportion of phasic neurones was 5-20% in all ganglia.

Colchicine-treated ganglia were stained immunohisto-chemically using antibodies to somatostatin (SOM) and neuropeptide Y (NPY). The proportions of NPY+ (20-40%) and SOM-/NPY- (30-40%) cells varied little between ganglia, although the proportion of SOM+ cells increased caudally (from 20% [CG] to 45% [IMG]). Physiologically-characterized neurones were filled with biocytin and subsequently immunostained. All SOM+ cells in CG were tonic (n=5). However SOM- cells were tonic (n=4), phasic (n=3) or LAH (n=4). There was no correlation between peptide content and neuron type in the IMG (n=14).

535.16

INTRACELLULAR ANALYSIS OF BIOPHYSICAL MEMBRANE PROPERTIES THAT MAY INFLUENCE TRANSMISSION IN THE CALYX-TYPE SYNAPSE OF THE CHICK CILIARY GANGLION. G.H. Fletcher *and V.A. Chiappinelli, Dept. of Pharmacological and Physiological Science, Saint Louis University Medical Center, St. Louis, MO 63104.

In order to determine those ion channels which contribute to the normal functioning of synaptic mechanisms in the chick ciliary ganglion, intracellular recordings were obtained from presynaptic calyx nerve terminals (n=69) and postsynaptic ciliary neurons (n=48). Under current-clamp conditions, depolarizing pulses (0.6-2.0 nA, 65 ms duration) applied to presynaptic calyces more usually elicited a single action potential (amplitude=84.9±1.2 mV, duration = 1.00±0.03 ms), whilst ciliary neurons fired either single or multiple spikes (amplitude=89.0±3.1 mV, duration=1.32±0.06 ms) in response to current steps of lower intensity (0.2-0.8 nA). 1μM TTX totally abolished spike generation in calyces and ciliary cells, suggesting that a fast inward Na^+ current is responsible for the upstroke of the action potential. Bath application of Ba^{2+} (5mM), TEA (10mM) and 4-AP (1mM), which are known to block M-current, the delayed rectifier potassium current and A-current respectively, prolonged spike generation and increased neuronal excitability by decreasing the threshold for action potential generation, when compared to similar current pulses delivered to these cells in normal recording medium. The results are a first step towards identifying those ion channels which are involved in transmission in this parasympathetic ganglion. Supported by NIH grant EY06564 to VAC.

535.17

EFFECTS OF AXOTOMY OR TARGET TISSUE ATROPHY ON THE ELECTRICAL PROPERTIES OF SYMPATHETIC NEURONS M. Sánchez-Vives and R. Gallego*. Instituto de Neurociencias, Universidad de Alicante, Spain.

We have studied the electrical properties of rat sympathetic neurons after axotomy or atrophy of their target tissue in an in vitro preparation of the superior cervical ganglion. Cells innervating the submandibular gland were identified by antidromic stimulation in preparations from control animals and from animals in which the gland was atrophied by ligature of the salivary duct three months before. The effects of axotomy were studied 7-10 days after sectioning the postganglionic branches.

The action potential was smaller in axotomized cells (75±1.3 mV, mean±s.e., n=61) and in cells from the atrophy group (76±1.3 mV, n=46) than in control cells (82±0.8 mV, n=157). Axotomy also produced a marked decrease in afterhyperpolarization duration, 89±8 ms against 208±6 ms for the controls. These changes are difficult to interpret because passive properties were also affected: axotomized cells showed a decreased membrane potential and input resistance, whereas this latter property was increased in the atrophy group. (Supported by DGICYT grant PM-89/0021, Spain)

535.18

MODELING THE PROPAGATION POTENTIAL, AN EXTRACELLULAR VOLTAGE PLATEAU PRODUCED BY ACTION POTENTIALS. S.E.Fox and A.P.Rudell. Dept. Physiol., SUNY Health Sci. Ctr., Brooklyn, NY 11203.

A propagation potential is recorded between two widely-spaced points outside an axon when an action potential is propagating in it. Between the peak recorded as the action potential passes the first point and the trough as it passes the second, the voltage does not return to zero, but maintains a constant value of the same sign as the initial peak and about one-third its magnitude. This propagation potential may be a general property of axons, since it is recorded from both frog sciatic nerves and earthworm giant axons.

The propagation potential cannot be explained on the basis of K^+ accumulation because TEA causes an increase, not a decrease, in its magnitude. The afterpotentials of earthworm giant axons are too steeply sloped to account for the flat plateau. It is not clear how such a flat plateau could be produced by volume conductor effects, especially since it could be recorded from nerves in air. Simple core-conductor models of axons do not generate the propagation potential. Even with the inclusion of the filtering properties of axon sheaths they do not produce flat plateaus.

Addition of longitudinal resistance gradients *does* produce flat plateaus. Integrated local circuit currents, equal and opposite on the rising and falling phases of the action potential, produce different extracellular voltage drops through the asymmetric longitudinal resistances. If the gradients are fixed in space, the sign of the plateau is reversed relative to the first peak when direction of propagation is reversed. This is not the case in real axons. The only way we have reproduced a propagation potential with a core-conductor model is by incorporating a transient change in longitudinal resistance associated with the passage of the action potential. Such longitudinal resistance changes might be caused by transient changes in the composition or size of the extracellular environment of the axon associated with the passage of the action potential. (Supported by NS17095.)

EXCITATORY AMINO ACIDS: PHARMACOLOGY VI

536.1

SUB-TYPES OF SODIUM-DEPENDENT HIGH AFFINITY (SDHA) GLUTAMATE TRANSPORT: I. LOCALIZATION. L.A. Dowd, J.D. Sinor, and M.B. Robinson, Children's Seashore House; Depts. Ped. and Pharm., U. of PA; Philadelphia, PA, 19104.

Evidence for a neuronal localization of SDHA glutamate (Glu) transport includes enrichment in "synaptosomal membrane fractions" and the decreases in uptake observed after neuronal lesions. Autoradiographic studies in primary cultures support a glial localization. Two subtypes of SDHA L-[^{3}H]-Glu transport can be differentiated by the selective inhibitors L-α-aminoadipate (AAD) and dihydrokainate (DHK). Uptake in cerebellum is inhibited by AAD and insensitive to DHK. The opposite pattern is observed in forebrain regions (Brain Res., 544(1991)196). In these studies, the localization of the subtypes of transporters was investigated. Subcellular fractionation of these tissues using discontinuous sucrose density gradients demonstrated that both subtypes were at least 4-fold enriched in the synaptosomal membranes fraction. Little or no transport activity was observed in myelin or mitochondrial fractions. Previous studies have demonstrated that both crude synaptosomal membranes (P2) and synaptosomes can contain significant amounts of glial protein (Brain Res. 101(1976)341). As ß-alanine is a more potent inhibitor of GABA uptake into glia than of GABA uptake into neurons (Biochem. Pharmacol. 24(1975)933), the inhibition of [^{3}H]-GABA uptake by ß-alanine was examined. In P2 preparations and fractionated synaptosomal membranes made from both brain regions, the inhibition of GABA uptake by ß-alanine was consistent with high and low affinity components (30-40% high affinity $p< 0.005$ 2-site fit). These data suggest that the uptake of Glu measured in "synaptosomal membrane fractions" may be glial and/or neuronal.

536.2

SUB-TYPES OF SODIUM-DEPENDENT HIGH AFFINITY (SDHA) GLUTAMATE TRANSPORT: II. ION-DEPENDENCE, PHARMACOLOGY AND REGULATION. J.D. Sinor, L.A. Dowd, and M.B. Robinson, Children's Seashore House; Depts. Ped. and Pharm., U. of PA; Philadelphia. PA, 19104.

Two subtypes of SDHA glutamate (Glu) transport can be differentiated by competitive inhibitors including dihydrokainate (DHK) (see accompanying abs. by Dowd et al.). These subtypes of L-[^{3}H]-Glu transport were characterized. The K_ms for sodium, measured at 50 and 100 μM L-[^{3}H]-Glu, were 5.3 mM in cerebellum and 12 mM in cortex. Of the over fifty EAA analogues tested, only 22 inhibited transport more than 65% at 1 mM. These compounds included EAA analogs that were not previously thought to interact with transport including (numbers in parentheses are IC_{50}'s in μM for cortex then cerebellum) kainate (160; 243), N-methyl-D-aspartate (428; 345), quisqualate (662; 95), D-glutamate (236; 57), and trans (dicarboxyl)-2,4-methanoglutamate (25; 5). Compounds that inhibited less than 65% at 1 mM included: ibotenate, quinolinate and AMPA. Cerebellar and hippocampal slices were transiently depolarized with 30 mM K^+. Following depolarization, the uptake of L-[^{3}H]-Glu was measured in crude synaptosomal membranes. Compared to control (incubation in normal K^+), the V_{max} for uptake was increased 2.1-fold by depolarization in cerebellar slices (DHK-insensitive). There was no change in hippocampus (DHK-sensitive). These data add further support for the differentiation of subtypes of SDHA Glu transport and suggest that these subtypes may be independently regulated. The effect of K^+ may have implications for the vulnerability of different brain regions to excitotoxicity during ischemic insults.

536.3

INHIBITORS OF EXCITATORY AMINO ACID (EAA) RECEPTORS COUPLED TO PHOSPHATIDYLINOSITOL (PI) TURNOVER INHIBIT L-[^{3}H]-GLUTAMATE (GLU) UPTAKE. L. Littman, J.D. Sinor, and M.B. Robinson. Children's Seashore House; Depts. Ped. and Pharm., U. of PA; Phila., PA 19104.

EAA receptors are coupled to increased PI turnover. The elucidation of the functional and developmental roles for these receptors is hindered by the lack of high-affinity antagonists. In the current studies, a possible relationship between inhibitors of these receptors and inhibitors of sodium-dependent high affinity Glu transport was investigated. Rat hippocampal slices prepared from neonatal animals were used to measure PI hydrolysis induced by agonists. The uptake inhibitor L-aspartate-ß-hydroxamate (AßHA) was a weak partial agonist with stimulation at 300 µM to 16 ± 4 % (n=5) of the maximal quisqualate response. PI hydrolysis stimulated by 100 µM quisqualate was inhibited up to 64 ± 4 % (n=3) by AßHA with an IC_{50} of 25 ± 6 µM. S-2-Amino-3-phosphonopropionic acid (S-AP3), previously identified as a non-competitive antagonist, has an IC_{50} of 370 µM (Mol. Pharm 38:222-228). Four phosphonocyclohexyl and cyclopentyl analogs of Glu did not inhibit PI hydrolysis (< 25 % at 1 mM). The transport of L-[^{3}H]-Glu was measured in crude synaptosomal membranes prepared from the cortex of adult animals. AßHA and S-AP3 inhibited transport with IC_{50}'s of 11 µM and 110 µM, respectively. As observed for inhibition of PI hydrolysis, the R-isomer was much less active (> 30-fold difference in potency). The cyclic analogs of Glu did not inhibit transport (less than 5 % at 1 mM). These studies support a correlation between potency for inhibition of PI hydrolysis and potency for inhibition of transport.

536.4

PHARMACOLOGICAL CHARACTERIZATION OF TYPE I AND TYPE II SODIUM-DEPENDENT EXCITATORY AMINO ACID TRANSPORT SITES. K.J. Anderson and T.W. Vickroy. Departments of Physiological Sciences and Neuroscience, University of Florida, Gainesville, FL 32610.

Sodium-dependent transport of excitatory amino acids (EAA) represents the primary means by which these neurotransmitters are inactivated, and may play a role in the prevention of excitotoxicity. D-[^{3}H]Aspartate has been used extensively as a tool to characterize this transport process due to its specificity and high affinity. We have recently described two separate sodium-dependent transporter binding sites based on different sensitivities to L- or D-threo-β-hydroxyaspartate. These two sites are anatomically distinct, being found primarily in the forebrain (Type I) or in the cerebellar molecular layer (Type II). In an attempt to further pharmacologically characterize these two binding sites, we have screened a series of compounds that are thought to interact with EAA systems. Briefly, assays were conducted using 6µm-thick, horizontal sections of rat brain. D-[^{3}H]Aspartate binding (100 nM in 50 mM Tris-acetate with 300 mM NaCl, pH 7.4) was carried out in delipidated sections at 0-2°C. Following rinsing in ice-cold buffer, sections were air dried and either exposed to [^{3}H]-sensitive film or cerebellum and forebrain were individually scraped from the slides and total radioactivity was determined by liquid scintillation counting. Drugs that showed at least 10-fold higher affinity for Type I sites included D-threo-β-hydroxyaspartate, D-aspartate-α-benzyl ester and L-2,4-*trans*-pyrrolidine dicarboxylate (supplied by R. Bridges, Univ. Calif., Irvine). At present no compounds have been identified that show more that 5-fold higher affinity for Type II sites. The discovery of specific drugs which interact at these transporters may provide a valuable means to affect specific EAA-using pathways. Supported by AG-08843 (KJA) and NS-28268 (TWV).

536.5

NMDA ANTAGONISTS BLOCK C-FOS EXPRESSION DURING OPIOID WITHDRAWAL. C.E. Inturrisi, M. Brodsky* and K. Rasmussen, Dept. of Pharmacology, Cornell U. Med. College, New York, NY and Lilly Research Labs, Indianapolis, IN.

Naltrexone (NTX)-precipitated withdrawal in morphine dependent rats results in the induction of the proto-oncogene, c-fos (Hayward et al., Brain Res. 525, 256, 1990) and NMDA antagonists (MK-801 (MK) and LY-274614 (LY)) block the behavioral signs of withdrawal (Rasmussen et al., Eur. J. Pharmacol., in press). In morphine dependent rats c-fos mRNA levels, as measured by a quantitative solution hybridization assay, are increased 2-4 fold at 1 hour after NTX administration in locus coeruleus (LC), amygdala (AD), nucleus accumbens (NA), frontal cortex (FC) and hippocampus (HC) (but not in striatum or spinal cord). Pretreatment of dependent rats with MK (1 mg/kg sc) or LY (100 mg/kg ip) prior to NTX reduced by 60 to 90% the NTX withdrawal-induced increase in c-fos mRNA in AD, NA, HC and LC but not in FC. Differential drug-CNS region effects were noted: LY was a more effective blocker of c-fos induction in NA, while MK was more effective in LC and HC. These results demonstrate that competitive (LY) and noncompetitive (MK) NMDA antagonists can block both the behavioral manifestations of withdrawal and c-fos induction in those brain regions associated with opioid effects and withdrawal. Supported in part by NIDA Grant DA-01457 (CEI).

536.6

THE ACTIONS OF GLUTAMATE RECEPTOR AGONISTS AND ANTAGONISTS ON THE ACTIVITY OF PHOSPHATE-ACTIVATED GLUTAMINASE (PAG). R. Dawson, Jr. and D.R. Wallace. Dept. of Pharmacodynamics, Univ. of Florida, Gainesville, FL 32610.

The conversion of glutamine (GLN) to glutamate (GLU) is catalyzed by PAG. PAG is under physiological regulation by end product inhibition due to GLU and ammonia and is activated by both phosphate and calcium. The pharmacological specificity of the binding site on PAG for GLU-induced allosteric inhibition of PAG activity was probed. In addition, the noncompetitive N-methyl-D-aspartate (NMDA) receptor blocker (+)MK-801 was also examined.

PAG activity was measured in crude synaptosomal (P_2) preparations by determining GLU formation after a 15 minute incubation in the presence of GLN. The assay buffer contained (in mM) sodium phosphate 10, Na_2EDTA 0.2, HEPES 20 and GLN 0.5. The GLU formed was measured by HPLC. PAG activity was measured in the cortex from 6 month old F344 rats (n=8) and 100 day old Sprague-Dawley (SD) rats (n=5).

PAG activity (expressed as percent of control) was significantly inhibited by 5mM of the following drugs: kainic acid (44±2%), NMDA (60±3%), quinolinic acid (44±2%), kynurenic acid (58±4%), AP4 (55±2%) and cis-piperdine dicarboxylic acid (79±3%). Interestingly, (+)MK-801 (5mM) stimulated PAG activity (209±10%) in F344 rats. (+)MK-801 (5mM) was tested in SD rats and was also found to stimulate PAG activity (183±4%). Ketamine (5mM) stimulated PAG activity (137±1%) whereas (-)MK-801 (5mM) had no effect on PAG activity. (+)MK-801 (5mM) was additive with the stimulating effects of calcium (1mM) on PAG activity and synergistically potentiated the effects of phosphate and substrate (GLN) on GLU synthesis by PAG. The results show that GLU analogs can inhibit PAG activity and demonstrate that high concentrations of (+)MK-801 can activate PAG activity in a stereoselective manner.

536.7

PRE-RELEASED ADENOSINE MODULATES NMDA-EVOKED NORADRENALINE (NA) RELEASE IN THE CORTEX. C.G. Craig and T.D. White. Dept. of Pharmacology, Dalhousie University, Halifax, N.S., Canada, B3H 4H7.

Adenosine, an important inhibitory neuromodulator, is released during NMDA receptor activation in the cortex. Maximal adenosine release occurs at levels of NMDA receptor activation which release minimal NA. We examined the potential modulatory role of adenosine released during NMDA receptor activation on NA release from slices of rat parietal cortex. The P_1 agonist R-PIA (10µM) decreased 100µM NMDA-evoked [^{3}H]NA release by 27%. Although the P_1 antagonist 8-phenyltheophylline (10µM, 8-PT) reversed R-PIA's inhibition of NMDA-evoked [^{3}H]NA release, alone it had no effect on 100µM NMDA-evoked [^{3}H]NA release. This suggests that adenosine released during NMDA receptor activation does not modulate [^{3}H]NA release. Indeed, adenosine release evoked by 100µM NMDA occurs later than [^{3}H]NA release, so that the released adenosine might not be temporally available to modulate [^{3}H]NA release. Pretreatment with an NMDA concentration (10µM) which releases substantial endogenous adenosine but very little [^{3}H]NA decreased subsequent 100µM NMDA-evoked [^{3}H]NA release by 63%. 8-PT partially reversed this inhibition, indicating that pre-released adenosine, acting at P_1-purinoceptors, modulated subsequent NMDA-evoked [^{3}H]NA release. These results suggest that adenosine released during submaximal NMDA receptor activation could provide an inhibitory threshold which must be overcome in order for other NMDA-mediated processes to proceed maximally. (Supported by the MRC of Canada).

536.8

DIVERSITY OF K^+ CHANNEL MODULATION BY SIGMA LIGANDS IN CULTURED CEREBELLAR NEURONS. P.G. Lysko, M.M. Gleason*, C.L. Webb*, K.A. Lysko*, and G. Feuerstein. Dept. of Pharmacology, SmithKline Beecham, King of Prussia, PA 19406.

Sigma ligands have been suggested to be modulators of K^+ channel activity, as determined by ^{86}Rubidium (Rb) efflux from preloaded rat cortical synaptosomes, where KCl-induced Rb efflux was reportedly blocked by σ-ligands but not by the NMDA antagonist MK-801. Here we studied Rb efflux from primary cultures of 7-9 da old rat cerebellar granule cells preloaded in buffer for 2 hr with ^{86}RbCl (2µCi/ml/35 mm dish). Rates of Rb efflux were measured at 37C by sequential removal, replacement, and counting of buffer solution every 2 min. Basal efflux was determined for 10 min, and stimulated efflux for an additional 12 min, at which time cells were solubilized with SDS and counted. Inhibitors were added 10 min prior to stimulation and included throughout. The basal rate constant of 0.133±0.005 min^{-1} (n=18) was increased 227% by 80 mM KCl and resolved by 10 min in spite of the continual presence of KCl. KCl-stimulated Rb efflux was differentially inhibited up to 60% by 100µM MK-801, DTG, SKF 10,047, and haloperidol, but not by (+)-3-PPP. When cells were incubated in the absence of glucose (in vitro ischemia),the basal rate constant increased to 0.153±0.005 min^{-1} (n=18); 100µM glutamate (excitotoxic) increased Rb efflux by 40%. This Rb efflux was inhibited up to 40% by MK-801, DTG, and SKF 10,047, but not by haloperidol or (+)-3-PPP. Kainate (100µM, excitotoxic) stimulated Rb efflux to the same extent as KCl, was not inhibited by any of the above compounds, but was inhibited by 100µM of the quinoxalinediones NBQX, DCQX, and MNQX. Data suggest selective inhibition of K^+ channels by σ-ligands, which may be neuroprotective by blocking NMDA channels.

536.9

CHANGES IN CONCENTRATIONS OF DOPAMINE, SEROTONIN AND THEIR METABOLITES INDUCED BY CARBON MONOXIDE (CO) IN THE RAT STRIATUM AS DETERMINED BY IN VIVO MICRODIALYSIS. M. Hiramatsu[1], S. Yokoyama[1]*, T. Nabeshima[2] and T. Kameyama[1]. [1]Dept. of Chem. Pharmacol., Meijo Univ., Nagoya 468, Japan and [2]Dept. of Hospital Pharmacy, Nagoya Univ. Sch. of Med., Nagoya 466, Japan.

Ischemic conditions can induce massive increases of neurotransmitters in the extracellular fluid of the brain. The increase in extracellular neurotransmitter concentrations has been implicated in the pathogenesis of hypoxic cell damage. In this study, striatal microdialysis was performed in rats subjected to hypoxia produced by CO-exposure. Extracellular changes of dopamine, serotonin and their metabolites were monitored before and after the CO-exposure at 15-min intervals by HPLC-EC analysis. Immediately after CO-exposure, extracellular dopamine increased, whereas 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) decreased. Dopamine was cleared from the extracellular fluid within 45 min and reached a baseline level after CO-exposure. Extracellular 5-hydroxyindoleacetic acid (5-HIAA) did not change after CO-exposure. The non-competitive N-methyl-D-aspartate (NMDA) antagonist, MK-801 (1 mg/kg, s.c.) tended to antagonize the increment of dopamine and the decrement of DOPAC. This protective effect might be due to the blockade of NMDA receptors since hypoxia induces glutamate release and glutaminergic system regulates the striatal dopaminergic system.

536.10

DEVELOPMENTAL PATTERNS OF GLUTAMATE-INDUCED INTRANEURONAL CALCIUM ACCUMULATION. E.F. May*, M.A. DeCoster and F.C. Tortella. Neuropharm. Branch, Walter Reed Army Inst. Res., Washington, DC 20307-5100.

The Adherent Cell Analysis and Sorting System (ACAS) has been used to measure glutamate (Glu)-induced intracellular calcium (Ca_i) changes in primary rat cortical neurons. (DeCoster et al., Trans. Am. Soc. Neurochem., 22: 1991). In this study, neurons of varying ages (7-29d) were treated with Glu (5-80 μM) and Ca_i measured using the ACAS. In neurons of all ages, 40-80 μM Glu caused an immediate sustained rise (ISR) in Ca_i. The amplitude of this response was greatest in 22d neurons. At 20 and 10 μM Glu, 14d neurons showed a greater amplitude of Ca_i increases than 7d neurons; however, the patterns of responses were heterogeneous, and included biphasic and ISR types. The responses to 5 μM Glu were also heterogeneous at different ages, but the ISR response was most often observed in older neurons, with the highest amplitude at 19-21d. In summary, ISRs were more frequent and greater in amplitude in older neurons. The heterogeneity of responses to lower doses of Glu at different neuronal ages may reflect the development of multiple Glu receptor types.

536.11

L-α-AMINOADIPIC ACID REDUCES KYNURENIC ACID PRODUCTION IN THE RAT HIPPOCAMPUS: A MICRODIALYSIS STUDY IN FREELY MOVING RATS. R. Schwarcz and H.-Q. Wu. Maryland Psychiatric Research Center, Baltimore, MD 21228.

Kynurenic acid (KYNA) is a broad-spectrum antagonist of excitatory amino acid (EAA) receptors in mammalian brain. Neuronal activity can influence KYNA synthesis in vitro and in vivo, and certain EAAs have been shown to regulate KYNA function in brain slices (Soc. Neurosci. Abstr. 15: 328.10, 1989). The effect of the most potent endogenous EAA in vitro, L-α-aminoadipic acid (αAA), was now studied by microdialysis in the rat hippocampus in vivo. αAA, administered through the dialysis probe, dose-dependently decreased extracellular KYNA concentration in the presence of kynurenine (500 μM), reaching 50% of control levels with 500 μM αAA. KYNA levels returned to basal level within 2 h after discontinuation of the αAA perfusion. 500 μM αAA also lowered endogenous extracellular KYNA levels by ~30%. Neuronal depletion by quinolinic acid lesions did not interfere with the αAA effect. Co-perfusion of 50 μM veratridine (which by itself potently decreases extracellular KYNA) with 500 μM αAA did not result in an additive KYNA reduction. Moreover, the αAA-induced effect was not blocked by tetrodotoxin (5 μM). These data suggest that αAA exerts its inhibitory effect through direct interaction with KYNA-producing glial cells. αAA may be an endogenous modulator of KYNA synthesis in the brain and may thus play a role in the function or dysfunction of cerebral EAA receptors. Supported by USPHS grant NS 16102.

536.12

MODULATION OF D-[^{3}H]ASPARTATE RELEASE BY KAINIC ACID IS NOT MEDIATED BY AUTORECEPTOR ACTIVATION. A.M. Palmer and C.T. Reiter. *Departments of Psychiatry and Pharmacology, University of Pittsburgh Medical Center, 3811 O'Hara Street, Pittsburgh, PA 15213.*

This study addresses the hypothesis that kainic acid receptors are present on excitatory amino acid (EAA) nerve terminals. Slices of rat cerebral cortex were preincubated in physiological media containing D-[^{3}H]aspartate, superfused (1.6 ml/min) and collection of twelve 0.5 min fractions begun at t = 30 min. Kainic acid (KA, 0.01 - 1 mM at t = 15-36 min) increased baseline counts in a dose-dependant fashion in both the presence and absence of Ca^{2+} (190 and 170% of control, respectively for a 1mM dose) but had no effect in media with Na^+ concentration reduced (with equimolar substitution of choline chloride) to 10mM. This suggests that the observed elevation is not mediated via interaction with the process of release but instead by inhibition of EAA uptake; D,L-threo-ß-hydroxy aspartate (100μM) increased baseline counts to 355% of control. In parallel studies, the Na^+-dependant binding of D-[^{3}H]aspartate was inhibited by both D,L-threo-ß-hydroxyaspartate and KA (IC_{50} = 0.5 and 78.3μM, respectively; n = 4-5). Elevated concentrations of K^+ (25-75 mM) at t = 31.5-32.5 min evoked a dose-dependent increase in the release D-[^{3}H]aspartate. Compared to baseline, 50mM K^+ evoked an elevation of 30.1 ± 3.3 (mean fold increase ± SEM, n = 6) that was higher than that occurring in the absence of Ca^{2+} (5.3 ± 0.2). KA inhibited this release (to 54 ± 5% of control for a 1mM dose) in a dose-dependant fashion and was insensitive to blockade of voltage-sensitive Na^+-channels (1μM tetrodotoxin at t = 15-36 min), application of an antagonist (30μM DNQX at t = 15-36min) and removal of Ca^{2+} ions; an alternative agonist (100μM domoic acid at t = 25-36 min) had no effect on release. These data are not consistent with the notion that KA receptors are present on EAA nerve terminals, but instead suggest that the effect of KA on EAA release are mediated via EAA uptake.

536.13

CHARACTERIZATION OF GLUTAMATE AND NMDA-EVOKED CALCIUM CHANGES IN SINGLE CULTURED CORTICAL NEURONS FROM FETAL RAT BRAIN. E.N. Fluhler[1], K.G. Fernandes*[1], S.L. Yates*[2], P.M. Lippiello[1]. [1]RJR-NABISCO, Winston-Salem, NC 27102 and [2]Duke University Medical Center, Integrated Toxicology Program, Durham, NC 27710

Excitatory amino acid (EAA) receptors, which are thought to play a role in learning and memory, have been shown to be significantly altered in the pathology associated with dementia of the Alzheimer's type (DAT). While a large amount of work has been done to characterize the functional properties of EAA receptors in the hippocampus and striatum, considerably less is known about EAA receptors found on mammalian cerebral cortical neurons. Our laboratory has previously developed a culture method for cortical neurons. These cultures have been shown to be essentially glia-free, and are ideally suited for functional studies based on fluorescence microscopic imaging techniques. Here we describe the results of experiments aimed at elucidating the pharmacological and functional properties of EAA receptors found on single cultured cortical neurons. The ion-selective fluorescent probe fura-2 was used to measure EAA-evoked changes in neuronal calcium under a variety of conditions. Application of the agonists glutamate (GLU) and N-methyl-D-aspartate (NMDA) caused increases in neuronal intracellular calcium in a dose dependent manner (ED_{50} ca. 0.4 and 8.4 μM respectively). The time course for GLU and NMDA-evoked responses were similar and approached a maximum within approximately 90 seconds. Magnesium (2.0 mM), MK-801 (1uM), and 2-amino-5-phosphonovaleric acid (AP5) all blocked NMDA-evoked responses. Interestingly, glycine did not significantly modulate NMDA-evoked calcium changes. These results indicate that cultured fetal rat cortical neurons possess EAA receptors which respond similarly, but not identically, to those found on cultured hippocampal and striatal neurons.

536.14

DOPAMINE RELEASE EVOKED BY SUCCESSIVE APPLICATIONS OF NMDA AND KAINATE: EVIDENCE FOR DESENSITIZATION OF NMDA RECEPTORS IN VIVO. D.P. Carrozza, T.N. Ferraro, G.T. Golden, P.F. Reyes, T.A. Hare. Thomas Jefferson University, Philadelphia, PA 19107 and DVAMC, Coatesville, PA 19320.

Intrastriatal (i.s.) application of NMDA or kainate (KA) via microdialysis causes dose-dependent release of striatal dopamine (DA). To further characterize the role of striatal excitatory amino acid (EAA) receptors in DA release, the effect of successive applications of NMDA and KA were compared. Male Sprague-Dawley rats underwent surgery for placement of guide cannulae at coordinates chosen to allow striatal microperfusion. After a 48 h recovery period, microdialysis probes were inserted in freely-moving, unanesthetized rats. Microdialysis was carried out at a rate of 3.0 μl/min with sample collection at 15 min intervals. Levels of DA, DOPAC, and HVA were measured using HPLC with coulometric detection. About 1 hour following probe insertion, a 5 min i.s. application of NMDA or KA (each 12.5 mM in Ringer's solution) was given followed by a 45 min perfusion with normal Ringer's and then a second 5 min application of NMDA or KA. Results showed that DA release evoked by the first and second applications of KA was similar (110.2 ± 39.9% of the first application, n=6). However, a second application of NMDA resulted in a significant reduction in DA release compared to the first (29.3 ± 11.5%, $p<0.004$, n=9). When aminophosphonovalerate (APV, 0.36 mM, 5 min, i.s.) was administered prior to the second NMDA treatment, the reduction of DA release associated with the second NMDA application was significantly inhibited (49.9 ± 9.1% of the first treatment, $p<0.015$ vs non-APV treated, n=7). Administration of dextromethorphan (DXT, 1.0 mM, 5 min, i.s.) also partially prevented the reduction in DA release associated with the second NMDA treatment (48.2 ± 11.6%, $p<0.03$ vs non-DXT treated, n=5). Results suggest that striatal EAA receptors which mediate NMDA-induced DA release in vivo are desensitized following successive applications of NMDA. This desensitization is attenuated by competitive and non-competitive antagonists. KA-induced DA release is mediated by EAA receptors which are not similarly desensitized.

536.15

COMPARISON OF CARBACHOL- AND GLUTAMATE-STIMULATED PHOSPHORYLATION OF RAT HIPPOCAMPAL MEMBRANE PROTEINS. L.M. Shaffer, M.A.N. Edgar* and L.A. Dokas*. Departments of Neurology*, and Biochemistry and Molecular Biology, Medical College of Ohio, Toledo, OH 43699.

Both cholinergic and glutaminergic agonists stimulate the hydrolysis of phosphatidylinositol 4,5 bisphosphate. These effects are differentially inhibited by active phorbol esters. Since phorbol esters stimulate the activity of protein kinase C, it was of interest to examine changes in the phosphorylation of membrane proteins correlated with manipulation of the cholinergic and glutaminergic systems in the rat hippocampus. Following incubation of adult rat hippocampal slices with 2.0 mM carbachol, no differences in the [γ-^{32}P]-ATP phosphorylation of membrane proteins were observed. However, incubation with glutamate, ibotenic acid and NMDA (10^{-3}M) resulted in altered phosphorylation of membrane proteins. The most prominent of these phosphoproteins has been identified by immunoblot analysis to be B-50/GAP-43. In contrast, increased phosphorylation in response to carbachol, glutamate and ibotenic acid was observed in hippocampal slices from 4 day old pups. Supported by grants from the Ohio Department of Aging and NIH (NS 23598).

536.16

THE MODULATION BY SIGMA LIGANDS OF NMDA-EVOKED [^{3}H]NORADRENALINE RELEASE INVOLVES $G_{i/o}$ PROTEINS. F.P. Monnet, P. Blier, G. Debonnel and C. de Montigny. Neurobiological Psychiatry Unit, McGill University, Montreal, and Institut de Recherche Jouveinal, Fresnes, France.

We have previously documented, using *in vivo* extracellular unitary recordings, a selective potentiation of NMDA-induced activation of CA_3 dorsal hippocampus pyramidal neurons by DTG, (+)pentazocine and JO-1784 and the suppression of this effect by haloperidol and (+)3-PPP, which all share a high affinity for sigma (σ) binding sites (*Soc. Neurosci. Abstr.*, 16: 396.11, 1990). High affinity σ binding sites have been proposed to be linked to G proteins (*Eur. J. Pharmacol.*, 149: 399, 1988; *J. Neurochem.*, 53: 779, 1989). The present studies were undertaken to investigate the effect of σ ligands on NMDA-evoked [^{3}H]NE release in preloaded hippocampus slices of Sprague-Dawley rats and to verify the possible involvement of $G_{i/o}$ proteins in mediating this effect of σ ligands. To this end, $G_{i/o}$ proteins were inactivated with pertussis toxin (PTX, 1 µg, *in situ* 4 to 11 days prior the experiments) or *in vitro* preincubation with *N*-ethylmaleimide (NEM, 30 µmol/l, 30 min prior the experiments). The slices were incubated with 0.1 µM [^{3}H]NE for 30 min and then superfused with Mg^{++} free Krebs' containing DTG, JO-1784 or (+)3-PPP and, 40 min later, the calcium-dependant tritium overflow was evoked by 100 µM NMDA. JO-1784 (0.01-0.3 µM) (+)3-PPP (0.1-0.3 µM) increased, whereas DTG (0.03-3 µM) decreased, in a concentration-dependent manner, [^{3}H]NE release evoked by NMDA. Haloperidol (1 µM), which did not modify the NMDA-evoked [^{3}H]NE release, prevented the effects of JO-1784 and DTG. The PTX and NEM pretreatments abolished the effects of JO-1784 and (+)3-PPP, but only shifted to the right the concentration-effect curve of DTG. The present results indicate that σ sites can modulate the NMDA-evoked [^{3}H]NE release and that these are coupled to $G_{i/o}$ proteins.

536.17

GABAERGIC MEDIATION OF PHENCYCLIDINE-INDUCED CHANGES IN STRIATAL NEUROPEPTIDE Y LEVELS IN THE RAT BRAIN. L.P. Midgley, L.G. Bush*, J.W. Gibb and Glen R. Hanson. Dept. of Pharmacology and Toxicology, University of Utah, Salt Lake City, UT 84112.

Alterations in neuropeptide Y (NPY) tissue content in CNS or extracellular fluid have been found in several psychiatric disturbances and neurodegenerative diseases. Phencyclidine-HCL (PCP), a psychotropic drug of abuse, interacts with a number of neurotransmitter systems and has been identified as an antagonist at the NMDA receptor. We previously reported that administration of PCP or the non-competitive NMDA antagonist, MK-801, significantly reduces (by 30-50%) striatal NPY levels in the rat brain. In the present study, we observed that administration of the GABA-T inhibitors, amino-oxyacetic acid (AOAA) and MDL 71,754 (gamma-vinyl-GABA, GVG), had no effect on striatal NPY levels. Conversely, the GABA antagonist, bicuculline, significantly reduced striatal NPY levels by approximately 20%. Pretreatment of rats with AOAA or MDL 71,754 completely blocked the effects of PCP and MK-801 on striatal NPY levels. These data suggest that GABAergic receptors mediate PCP- and MK-801-induced changes in striatal NPY systems and contribute to interactions between NMDA and NPY systems. (MDL 71,754 was (graciously) provided by Marion Merrill Dow Inc., and the research was supported by USPHS grants DA 00869 and DA 04222).

536.18

EXTRACELLULAR FLUID GLUTAMATE CONCENTRATION IN THE PARAVENTRICULAR NUCLEUS: THE EFFECT OF POTASSIUM CHLORIDE DEPOLARIZATION AND GABAERGIC STIMULATION. M. Tristan-Morales*, W.W. Morgan and J.R. Haywood. Departments of Pharmacology and Cellular and Structural Biology, University of Texas Health Science Center, San Antonio, TX 78284.

The goal of these studies was to measure neuronal glutamate concentrations in the paraventricular nucleus (PVN) as an estimate of glutamate release using microdialysis. In chloral hydrate anesthetized rats, a 0.5 mm concentric design dialysis probe was inserted in the PVN through a previously placed guide cannula. Artificial cerebrospinal fluid was infused at a rate of 0.5 µl/min, and samples were taken at 30 min intervals. Glutamate was measured in the effluent using HPLC with electrochemical detection. Recovery of glutamate was determined at 3.8%. Potassium chloride depolarization increased extracelluiar glutamate concentration from 26.3±6.3 pg/µl to 92±20.9 pg/µl during the first collection period and 131.9±15.4 pg/µl during the second period. Administration of the GABA uptake inhibitor, nipecotic acid (100µM), reduced glutamate concentration by 44% (31.6±4.2 pg/µl to 17.6±2.4 pg/µl). These findings suggest that glutamate is neuronally released in the PVN. Further, the release of glutamate can be inhibited by increases in extracellular concentrations of GABA. (Supported by HL 36080 and HL 32977)

536.19

NEUROPROTECTIVE PHARMACOLOGY AND ONTOGENY OF QUINOLINATE INDUCED INJURY IN DEVELOPING BRAIN. W.H. Trescher, J.W. McDonald, and M.V. Johnston, The Kennedy Research Institute, Johns Hopkins University School of Medicine, Department of Neurology, Baltimore, MD 21205.

We evaluated the pharmacology and ontogeny of quinolinate induced injury in the immature rat brain. The dose response of quinolinate mediated injury was determined in the PND 7 rat pup by unilateral microinjection (0.5 µl) of 4 doses of quinolinate (50-400 nmol, n = 6-7/dose) directed into the anterior striatum. The severity of brain injury was quantified on PND 12 by comparison of the wet weights of the hemisphere ipsilateral (I) and contralateral (C) to the injection, and expressed as % Damage = 100*(C-I)/C. The extent of damage produced by quinolinate, which was maximal with 400 nmol, 28.9 ± 2.9%, was linearly related to the dose injected (r^2 = 0.99). The pharmacology was tested against injury produced by intrastriatal injection of 150 nmol of quinolinate. 15 min. after injection of quinolinate, animals received an intraperitoneal injection of PBS (control), dextromethorphan (DM, 20 mg/kg), dextrorphan (DX, 20 mg/kg), MK-801 (1 mg/kg), and CGS 19755 (10 mg/kg); n = 9-10/treatment. CNQX (20 nmol, n = 9) and 7-Cl-kynurenate (40 nmol, n = 5) were coinjected with quinolinate. Partial protection against injury was afforded by DX, DM ($p < 0.05$) and CNQX (n.s). Complete protection was provided by MK-801, CGS 19755 ($p < 0.001$) and 7-Cl-kynurenate ($p < 0.005$). To evaluate the ontogeny of quinolinate induced injury, we injected 150 nmol/0.5 µl into the anterior striatum at PND 7, 14, 21, and 90. The severity of injury was evaluated 5 days later by comparing the regional cross-sectional areas of the striatum on Nissl stained sections. Reduction in the cross-sectional area of the striatum was maximal at PND 7 (74.4 ± 6.4%) and minimal at PND 90 (38.2 ± 14.5%). The data demonstrate an attenuation of quinolinate neurotoxicity by NMDA receptor antagonists in the immature rat brain and suggest an enhancement of that toxicity in the developing brain. Supported by NIH-NINDS grants KO8 NS01482 (WHT) and RO1 NS28208 (MVJ)

536.20

EFFECT OF IBOTENIC ACID LESIONS ON NMDA-, DOPAMINE D1- AND D2-MEDIATED CHANGES IN STRIATAL NEUROTENSIN SYSTEMS. N. Singh, L. Midgley, J Wagstaff*, L. Bush*, J. Gibb and G. Hanson. Dept. Pharmacol. and Toxicol., Univ. of Utah, Salt Lake City, UT 84112.

N-methyl-D-aspartate (NMDA) receptors mediate dopamine D1-, but not D2-induced changes in striatal neurotensin (NT) systems. To elucidate further the role of NMDA receptors in regulating NT systems, rats were lesioned with the NMDA selective toxin, ibotenic acid (IBO) and NT levels were assessed by RIA after treatment with NMDA, SKF38393 (D1 agonist) and sulpiride (D2 antagonist). Substance P (SP) levels were used to determine the extent of the lesions. 6-8 days after rats were lesioned in the striatum with IBO (15 ug/2ul at 2 sites), they were treated with 3 doses of NMDA (100 mg/kg/dose), SKF38393 (20 mg/kg/dose) and sulpiride (80 mg/kg/dose) 6 h apart and sacrificed 18 h later. In animals with > 60 % striatal SP depletion, NT levels were unaltered by IBO lesions on the ipsilateral side, but were increased on the contralateral side when compared to unoperated rats. Ipsilateral IBO lesions blocked NMDA-induced increases in striatal NT but did not block sulpiride-induced increases in striatal NT when compared to unoperated controls. In preliminary studies, IBO lesions also appeared to block SFK38393-induced increases in NT. In contrast, basal increases in contralateral NT content after IBO lesions were additive with sulpiride- and SKF38393-induced NT changes but not with NMDA-induced changes when compared to unoperated controls. These findings confirm a role for NMDA receptors in controlling NT responses to D1 receptors and suggest that a compensatory NT change occurs in the contralateral side in response to IBO lesions. (Supported by USPHS Grants DA 00869 and 04222.)

537.1

STUDIES ON THE MECHANISM OF INTERACTION OF THE VOLATILE ANESTHETIC HALOTHANE WITH GABA-A RECEPTORS. B. Longoni and R.W. Olsen. Dept. of Pharmacology, School of Medicine, University of California, Los Angeles, CA 90024.

Volatile anesthetics enhance GABA-A receptor-chloride channel function in some neurons. The effects of halothane on GABA-A receptor-regulated chloride flux were evaluated in brain slices. Rat cortical slices were prepared according to the method of Yang and Olsen (1987). $^{36}Cl^-$ efflux was measured every minute for 24 consecutive minutes. GABA agonists such as muscimol (10 μM) added at the 14th minute of the assay significantly increased $^{36}Cl^-$ efflux via GABA-A receptor chloride channels. Simultaneous addition of halothane at clinically relevant concentrations of 0.8-1.7 mM (1-4 MAC) augmented and prolonged the muscimol-dependent $^{36}Cl^-$ efflux without affecting the basal flux. [^{3}H]Muscimol binding was augmented by halothane at similar concentrations. Halothane enhancement of muscimol-stimulated $^{36}Cl^-$ efflux appears to be dependent upon physiological concentrations of extracellular Ca^{2+}. In fact, in a Ca^{2+} free Ringer's solution, no halothane effect could be observed, while barbiturate enhancement of muscimol function was not impaired. GABA-mediated inhibition by halothane may be more complicated than direct modulation of GABA-A receptors by other CNS depressant drugs, and may contribute to our understanding of the role of GABA neurotransmission in general anesthesia.

537.2

PREFERENTIAL ANTAGONISM OF PENTYLENETETRAZOLE (PTZ)-INDUCED SEIZURES DIFFERENTIATES BENZODIAZEPINE (BZD) LIGANDS ACTING AS PARTIAL ALLOSTERIC MODULATORS AT $GABA_A$ RECEPTORS.

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PTZ and bicuculline (BIC) were infused i.v. at a constant rate into rats in order to progressively down regulate GABA action on $GABA_A$ receptors until seizures were elicited. The anti-PTZ and anti-BIC ED_{50} of several BZD and congeners were determined. Abecarnil (anti-PTZ EC_{50} = 1 μmol/kg iv) alprazolam (1.5), diazepam (3), chlordiazepoxide (20), behaved as full positive modulators of GABA action and have a PTZ/BIC antiseizure ratio close to 1, whereas the nonsedative BDZs (clonazepam, EC_{50} .6 and bretazenil EC_{50} .06) behaved as partial positive modulators (approximately half the maximal efficacy of diazepam) and had a PTZ/BIC antiseizure ratio 5 to 10 fold higher. Moreover when bretazenil was injected together with diazepam before BIC infusion, only the partial modulation of bretazenil was observed. These data suggest that bretazenil acts as partial positive modulator of $GABA_A$ receptors by blocking the full positive modulatory activity of diazepam. This indicates that BZD receptors can be occupied by compounds that have partial modulatory activity and prevent the action of the full positive modulators.

537.3

2-ARYL-3-INDOLEACETAMIDES (AIAM): A NEW CLASS OF POTENT AND SPECIFIC LIGANDS FOR THE MITOCHONDRIAL BENZODIAZEPINE RECEPTOR (MBR). E. Romeo, A.P. Kozikowski*, D. Ma*, V. Papadopoulos, J. Auta, A. Guidotti and E. Costa. F.G.I.N., Washington, D.C. 20007 and *Mayo Clinic, Jacksonville, FL 32224.

We have synthesized a series of AIAM that displace competitively ($n_H \approx 1$) and with high affinity (Ki 2-20 nM) [^{3}H] 4'Cl-diazepam and [^{3}H] PK11195 bound to rat MBRs prepared from cultured cerebellar astrocytes, brain homogenates and adrenal cortical cells. AIAM failed to displace the binding of [^{3}H] 4'Cl-diazepam to rat cerebellar granular neurons in primary culture or the binding of [^{3}H] flumazenil, [^{3}H] spiroperidol, [^{3}H] MK-801, [^{3}H] ketanserin, [^{3}H] AMPA, [^{3}H] 3-PPP, [^{3}H] glycine, and [^{3}H] naloxone to crude rat brain synaptic membranes. In C6-2B glioma cells the affinity of AIAM for the MBRs parallels their ability to increase mitochondrial pregnenolone synthesis. It is known that glial cells synthesize steroids that can modulate positively and negatively $GABA_A$ receptor function. Rats receiving small i.v. doses of AIAM show an increased behavioral output when placed on the elevated X-maze. When AIAMs are injected in doses twenty to fifty fold larger they produce sedation, ataxia, and respiratory depression. These effects are blocked by a pretreatment with subconvulsant doses of pentylenetetrazole but not of strychnine. A possible role of glia cell steroidogenesis in the pharmacological actions of AIAMs will be discussed.

537.4

PICROTOXIN BLOCK OF GABA CURRENT BY TWO DIFFERENT MECHANISMS. Kong-Woo Yoon, D. F. Covey, and Steven M. Rothman. Department of Anatomy and Neurobiology, Washington Univ. Sch. of Med., St. Louis, MO 63110.

We examined the effect of the picrotoxin (PTXN) receptor antagonist, α-isopropyl-methyl-γ-butyrolactone (αIMGBL) and PTXN on GABA induced chloride currents in dissociated rat hippocampal neurons. When applied by U-tube, PTXN (40 uM) decreased the peak GABA (10 uM) current by 36.6 ± 3.9%. The amount of PTXN block increased to 73.6 ± 2.8% with continued application with a time constant of 2.1 ± 0.2 sec. The amount of early and late depression by picrotoxin was not dependent on GABA concentration in the range of 1 to 30 uM but it was dependent on picrotoxin concentration (10, 40uM). The time constant for the delayed block decreased with increasing GABA concentration (20 sec at 1 uM, 1 sec at 30 uM) suggesting further that this PTXN effect is use dependent. In a separate set of neurons the picrotoxin receptor antagonist αIMGBL (5mM) almost completely abolished the delayed block (74.4 ± 4.2% to 38.5 ± 10.1%, n=7) while it had no effect on the early current depression. With brief application of 1 uM GABA, we saw no use dependent PTXN block and no reduction of block by αIMGBL. Our observations suggests that the PTXN block of GABA currents is mediated at two different sites producing a fast and slow (use dependent) block. αIMGBL appear to selectively antagonize the use dependent block.
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537.5

THE MUSCLE RELAXANT EFFECT OF FLUPIRTINE - INDICATIONS FROM ELECTROPHYSIOLOGICAL STUDIES. M. Wienrich and I. Szelenyi*. Dept. Neurobiology, Battelle Europe and ASTA-Pharma AG*, D-6000 Frankfurt am Main, Germany

Flupirtine (F) is a centrally acting, non-opioid analgesic. Analgesia caused by F is accompanied by a muscle relaxant activity. In order to elucidate the mechanism of this effect, we studied the interaction of F with GABA. We used primary dissociated cultures derived from whole embryonic rat brain and performed whole-cell recordings in the current-clamp mode. Due to our recording conditions, GABA induced reversible, concentration-dependent membrane depolarizations (EC_{50} $1.5x10^{-6}$M) which were antagonized by bicuculline, indicating the presence of a $GABA_A$ receptor. F also induced reversible, concentration-dependent membrane depolarizations (EC_{50} $1.5x10^{-4}$M) which were also antagonized by bicuculline. At $1x10^{-5}$M GABA induced a maximal effect. At the same concentration F, however, had no effect. Moreover, F at a threshold concentration of $3x10^{-5}$M potentiated the GABA effect, causing the concentration response curve for GABA to shift to the left (EC_{50} $5x10^{-7}$M). This indicates a possible over-additive interaction between the two compounds. We conclude that F has $GABA_A$-agonistic properties.

537.6

EXPRESSION OF POLYPEPTIDE DIAZEPAM BINDING INHIBITOR (DBI) AND PERIPHERAL BENZODIAZEPINE RECEPTORS (PBR) IN CULTURED PRIMARY ASTROCYTES. H. Alho[1], V. Varga[1*] and K. Krueger[2]. [1]Dept. of Biomed. Sci., Univ. of Tampere, BOX 607, 33101 Tampere, Finland; [2]Fidia-Georgetown Institute for the Neurosci., Washington DC 20007, USA.

The polypeptide diazepam binding inhibitor (DBI) modulates the $GABA_A$ receptor ionophore complex. It inhibits benzodiazepine (BZ) ligand binding to both central and peripheral benzodiazepine receptor sites (CBR, PBR). It has been suggested that the PBR is involved in neurosteroid synthesis in oligodendrocytes and glioblastoma cell lines. However, the exact role and localization of DBI and PBR in neuronal tissues is not completely understood. Therefore we studied the expression and distribution of DBI and PBR, and measured steroid hormone synthesis in rat primary astrocyte cultures.

Most of the astrocytes exhibited DBI-LI and DBI mRNA. DBI-LI was localized throughout the cytoplasm of glial cells. However, PBR-LI was found only in some of the astrocytes. These cultures produced measurable amounts of steroids. This steroid production was affected by the PBR ligands PK 11195 and Ro5-4864. These results demonstrate a wide distribution of DBI and co-localization with PBR in the cultured astrocytes. It was concluded that DBI may modulate central neuronal activity by acting indirectly via neurosteroids beside its direct interaction with CBR.

537.7

ABECARNIL: A β-CARBOLINE ANXIOLYTIC SHOWING A SELECTIVE ACTION AT CENTRAL BENZODIAZEPINE RECEPTORS.

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The β-carboline abecarnil shows anxiolytic and anticonvulsant effects in animal models at lower levels of benzodiazepine receptor occupation than diazepam, but does not exert muscle relaxant or ataxic effects. (Stephens et al, JPET 253: 334, 1990; Turski et al, JPET 253: 344, 1990). These observations suggest some form of selective activity at subpopulations of benzodiazepine receptors. Consistent with this hypothesis, abecarnil in vitro showed a 15 fold lower affinity for ^{3}H-lormetazepam binding sites in spinal cord than in cortex (IC_{50} 250 vs 15 nM), whereas diazepam showed a similar affinity for the two sites (250 vs 80 nM). In cerebellum, abecarnil was able to displace binding of ^{3}H-Ro 15-4513 from both its binding sites (IC_{50} 2 and 560 nM), whereas diazepam and other benzodiazepines displaced maximally 60 % of Ro 15-4513 binding (IC_{50} 20 and >10000 nM). Electrophysiological studies in frog oocytes expressing defined combinations of benzodiazepine receptor subunits confirmed a differentiation between abecarnil and benzodiazepines. These findings are consistent with abecarnil interacting with an overlapping but different subpopulation of receptors from benzodiazepines.

537.8

DISULFIRAM AND DIETHYLDITHIOCARBAMATE ARE COMPETITIVE INHIBITORS AT THE PERIPHERAL BENZODIAZEPINE RECEPTOR.

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In the present study, in vitro interactions of disulfiram (an agent used to induce ethanol intolerance in alcoholics), its metabolite diethyldithiocarbamate (DDC), and metronidazole (a disulfiram congener), with central benzodiazepine (BZ) receptors (CBR) and peripheral BZ receptors (PBR) were investigated in rat tissues. Disulfiram displaced specific binding of [^{3}H]PK 11195 from PBR in the cerebral cortex with IC_{50} value of 5 x 10^{-7} M. The binding of [^{3}H]PK 11195 and [^{3}H]Ro 5-4864 to PBR in the kidney was displaced by disulfiram with IC_{50} values of 7 x 10^{-7} M and 2 x 10^{-7} M, respectively. DDC displaced [^{3}H]PK 11195 binding to kidney membranes with IC_{50} value of 5 x 10^{-5} M. Binding of [^{3}H]flunitrazepam (FNZ) to CBR in the cerebral cortex was not affected by either disulfiram or DDC. Metronidazole (up to 10^{-4} M) did not affect [^{3}H]FNZ and [^{3}H]PK 11195 binding to CBR and PBR, respectively. Scatchard analysis of [^{3}H]PK 11195 saturation curve to kidney membranes, performed in the absence or presence of 7 x 10^{-7} M disulfiram revealed a decrease in ligand affinity without influencing the maximal number of binding sites, suggesting a competitive inhibition.

537.9

QUANTITATIVE AUTORADIOGRAPHIC CHARACTERIZATION OF THE BINDING OF ^{3}H-NO 328 (TIAGABINE) TO THE GABA UPTAKE CARRIER.

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Gaba (Gamma aminobutyric acid) is a major inhibitory neurotransmitter in the CNS. A decrease in Gaba-ergic neurotransmission is believed to be involved in a number of neurological conditions, including epilepsy. NO 328 (Tiagabine) is a potent and selective inhibitor of both Gaba uptake in vitro (IC_{50} = 67 nM) and DMCM and PTZ-induced convulsions in mice and rats. The present report examines the autoradiographic localization of the Gaba uptake carrier in rat brain using ^{3}H-NO 328. ^{3}H-NO 328 binding was regionally distributed in the rat brain, with the highest level of binding present in the prefrontal cortex, somatosensory cortex, superior colleculus, substantia nigra pars compacta, dorsal raphe, amygdaloid nucleus, CA$_3$ and CA$_4$ of the hippocampus, and molecular layer of the cerebellum. Scatchard analysis serial sections were linear with dissociation constant values ranging from 10 nM (frontoparietal cortex, motor area) to 165 nM (globus pallidus) and Bmax ranging from 456 Fmol/mg protein (substantia nigra pars riticulta) to 28 Fmol/mg protein (corpus collosum).

537.10

BENZODIAZEPINE INHIBITION OF TRIIODOTHYRONINE UPTAKE INTO HUMAN LIVER CELL LINE HAS A UNIQUE STRUCTURE ACTIVITY RELATIONSHIP

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We have reported that high affinity temperature-dependent cellular uptake of triiodothyronine (T_3) into HepG2 cells is inhibited by uM diazepam and peripheral ligand RO5 4864 (Soc for NS 1990, abstr #36.19). This inhibition is is not due to toxicity and is reversible with washing . We assayed the dose response of 25 benzodiazepines (BZ's) and determined apparent IC_{50}'s to illustrate structure for inhibition. ASSAY Confluent HepG2 cells in multiwell plates were incubated 3 hr prior to assay with serum-free media. Wells +/- drug and 40 pM ^{125}I-T_3 incubated x 1 hr at 22 or 4 C, then cells were washed on ice. The NaOH hydrolysate was gamma counted. T_3 uptake at 22 C - 4 C uptake = ~80% of total 22 C uptake. RESULTS At R_2', Cl> F> Br; at R_7, Cl> NO_2; at R_4', Cl > H; at R_3, OH> H; at R_1, alkyl groups > H; on imidazole of 1,2 annelated BZ's, CH_3 > H; the nonfused phenyl ring and the carboxyl at R_2 in the 1,4 BZ's are essential for potency. Most potent drugs were lormetazepam, triazolam, and prazepam and least were flumazenil and RO5 3663. Lormetazepam and triazolam showed no effect on T_3 binding to isolated rat liver nuclei, indicating that the interaction is not at the nuclear receptor. T_3 did not affect RO5 4864 binding to rat liver homogenate, indicating that it does not share binding sites with this peripheral BZ ligand. Study supported by NIH grant K11 DK 01456 to Dr. Kragie. P. Sorter of Hoffman LaRoche generously supplied benzodiazepines.

537.11

EFFECTS OF SEDATIVES ON GABA-MEDIATED CHLORIDE FLUX MEASURED IN THE MILLISECOND TIME SCALE.

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GABA-mediated chloride influx into rat cerebral cortical microsacs, and its potentiation by pentobarbital (PB), diazepam (DZ) and ethanol (EtOH), was studied in incubations lasting from 24 to 3000 msec. The 3000 msec assays were carried out manually, while all other assays were performed in a quench flow machine. The EC_{50} and Hill coefficient of each GABA concentration-response curve were found to depend on the incubation time; both were lower at longer incubation times. Pentobarbital enhanced the effect of GABA (at EC_{30} concentration) with greater efficacy in the 3000 msec than in the 50 msec incubations; the threshold PB concentration required for potentiation was also lower in the longer assay. Preincubation of microsacs with PB (40 - 80 μM) for 1 or 5 sec before a 50 msec incubation with GABA, did not increase the effect of PB beyond that obtained by adding PB only into the incubation. However, a 1 sec preincubation with DZ (1 μM) was necessary for showing its effect in the 50 msec assay. EtOH (50 mM) had no effect, whether or not it was added in the preincubation. When microsacs were preincubated with 32 μM GABA for 1 sec, far greater potentiation of the GABA effect was seen when PB was also present during the preincubation (1.47 ± 0.18 nmol Cl/mg protein) than when it was only added in the incubation (0.6 ± 0.12 nmol Cl/mg protein). This suggests that only PB binding which occurs after GABA binding potentiates the effect of GABA. Preincubation with both DZ and GABA produced greater influx (0.49 ± 0.12 nmol Cl/mg protein) than preincubation with GABA alone, but merely adding DZ into the incubation, after the preincubation with GABA had no effect (-0.16 ± 0.13 nmol Cl/mg protein). Ethanol also had no effect when added either during or after the 1 sec preincubation with GABA. The different effects of these sedatives on GABA-mediated chloride flux suggests that they do not have a common site of action.

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537.12

DEHYDROEPIANDROSTERONE SULFATE: A NEUROSTEROID THAT BLOCKS THE $GABA_A$ RECEPTOR NONCOMPETITIVELY.

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Dehydroepiandrosterone sulfate (DHEAS) was tested at $GABA_A$ receptors on neurons cultured from the ventral mesencephalon of fetal rats. The currents were measured under whole cell voltage clamp. DHEAS blocked peak currents induced by GABA noncompetitively with a dissociation constant of 4.5 μM and a stoichiometry of 1:1. In addition, DHEAS caused a pronounced, double exponential decay in the conductance. Analysis showed that this decay was not due to any of the following: (1) accelerated desensitization of the receptor, which showed single exponential decay, (2) voltage dependent occlusion of the ion channel by DHEAS, or (3) binding of DHEAS to the activated state of the receptor.

DHEAS also decreased the time constant for the decay of large, but not small, inhibitory postsynaptic currents. This observation is attributed to either diminished release of GABA or accelerated reuptake.

537.13

THDOC DECREASES PLASMA CORTICOSTERONE CONCENTRATIONS VIA A NON-GLUCOCORTICOID MECHANISM. M.J. Owens, J.C. Ritchie and C.B. Nemeroff. Depts. of Psychiatry and Pharmacology, Duke Univ. Med. Ctr., Durham, NC 27710.

Certain naturally occuring steroids that exist as trace metabolites of steroid hormones have been found to enhance GABAergic receptor function and binding. One of the most potent of these in *in vitro* studies is tetrahydrodeoxycorticosterone (THDOC; 5α-pregnane-3α,21-diol-20-one). THDOC, presumably acting via GABAergic mechanisms, possesses anxiolytic activity in the Vogel conflict test in the rat. Because enhanced CNS GABAergic activity appears to inhibit HPA activity and because the benzodiazepine, alprazolam, alters corticotropin-releasing factor (CRF) neurons in the locus ceruleus, we have examined the acute effects of THDOC on CRF neurons in several brain regions and on plasma corticosterone concentrations. In addition, we concomitantly examined the actions of the synthetic glucocorticoid, dexamethasone, and its purportedly active metabolite, 6β-OH-dexamethasone, on these same measures of CRF activity.

Following a single acute s.c injection, THDOC (2.5 mg/kg) and alprazolam (1 mg/kg) attenuated mild stress-induced increases in plasma corticosterone concentrations. In contrast, stress-induced corticosterone increases were completely abolished by dexamethasone and 6β-OH-dexamethasone. Attenuation of plasma corticosterone responses by THDOC were not due to any inherent glucocorticoid activity as THDOC had little affinity for the cytosolic glucocorticoid receptor. Unlike alprazolam, THDOC failed to decrease CRF concentrations in the locus ceruleus. While THDOC may plausibly act endogenously to reduce stress-induced endocrine and behavioral responses that are likely mediated in part by CRF neurons, these preliminary findings suggest that THDOC does not identically mimic the actions of alprazolam, another drug which potentiates GABAergic activity.

537.14

EFFECTS OF CHRONIC ANABOLIC/ANDROGENIC STEROID EXPOSURE ON INDICES OF FUNCTION OF THE GABA/BENZODIAZEPINE RECEPTOR COMPLEX. Daniel Bitran, Robert J. Hilvers*, and Carol K. Kellogg. University of Rochester, Department of Psychology, River Campus, Rochester, NY 14627.

The growing use of anabolic/androgenic steroids by athletes is of great concern to the medical and scientific communities. Although much research has addressed the mechanisms underlying steroid-mediated anabolic effects, little is known about the neural mechanisms that mediate the adverse effects of steroids on mental health. In addition to the classic genomic mechanism of steroid action, the modulation of neural excitability by 3α-hydroxylated pregnane steroids via effects on GABA/BDZ receptors has been demonstrated. Reduced metabolites of testosterone potentiate GABA-mediated function in a manner similar to that observed for reduced pregnane steroids. Since anabolic-androgenic steroid metabolites are potent agonists at the GABA/BDZ receptor complex, it was hypothesized that behavioral and neurochemical indices of the GABA/BDZ receptor system would be affected by chronic treatment, and subsequent withdrawal from testosterone-proprionate (TP) exposure.

In one experiment, 4 weeks following the implantation of a TP-filled silastic capsule (30 mm), male rats displayed increased anxiety as shown by an increase in the duration of burying behavior in the defensive burying paradigm. The androgenic efficacy of the steroid treatment was evidenced in a doubling of the weight of the ventral prostate gland in TP-treated males. The effector response of the GABA/BDZ receptor system was investigated using $^{36}Cl^-$ uptake in cortical synaptoneurosomes. Whereas TP treatment did not affect the maximal response to GABA, a significant decrease in the EC_{50} for GABA-stimulated Cl^- influx was observed. This pattern of results has been previously demonstrated following discontinuation from chronic treatment with BDZs, and is consistent with an upregulation in the function of the GABA/BDZ receptor complex. In our studies, silastic capsules were found empty upon sacrifice, suggesting that the rats were in a withdrawal phase. Current experiments will more precisely characterize changes in behavior and neural function during TP exposure and following steroid withdrawal.

537.15

ANXIOLYTIC ACTIVITY OF THE PROGESTERONE METABOLITE 5α-PREGNAN-3α-OL-20-ONE. S. Wieland, N.C. Lan, S. Mirasedeghi* and K.W. Gee. Dept. of Molecular Pharmacology and Toxicology, Sch. of Pharmacy, Univ. of Southern California, Los Angeles, CA 90033

3α-hydroxylated pregnane steroids have been shown to possess anesthetic, hypnotic, anticonvulsant and anxiolytic properties. In this study, metabolites of progesterone and deoxycorticosterone, 5α-pregnan-3α-ol-20-one (3α-OH-DHP) and 5α-pregnan-3α,21-diol-20-one (5α-THDOC), respectively, were tested for anxiolytic effects in N.I.H. Swiss-Webster mice using the light/dark transition, open-field and lick-suppression tests. Similar to the benzodiazepine (BZ) diazepam, 3α-OH-DHP (5-40 mg/kg) and 5α-THDOC (5-40 mg/kg) significantly increased the number of light/dark transitions. 3α-OH-DHP's effects were stereospecific as its diasteriomer, 3ß-OH-DHP was devoid of activity. The benzodiazepine antagonist CGS-8216 (10 mg/kg) blocked diazepam's (1.0 mg/kg) anxiolytic effects, but did not have any effect against 3α-OH-DHP (20 mg/kg). The data indicate that the pregnane steroids produce their anxiolytic effects through a separate mechanism than the BZs. 3α-OH-DHP (20 mg/kg), 5α-THDOC (20 mg/kg) and diazepam (1.0 mg/kg) increased activity in an open-field test. 3ß-OH-DHP had no effect in the open-field test. Furthermore, 3α-OH-DHP produced a 235% increase in punished responding in a lick-suppression test. These results demonstrate that the endogenous pregnane steroids possess anxiolytic effects that may be clinically relevant. (Supported by grants from CoCensys, Inc. and NS 24645 and NS 25986).

537.16

REGIONAL DISTRIBUTION OF A NEURONAL STEROID BINDING SITE IN RAT BRAIN D. Belelli* S. Wieland, N. C. Lan, R.E. Brinton and K.W. Gee. Dept. of Molecular Pharmacology and Toxicology, Sch. of Pharmacy, Univ. of Southern California, Los Angeles, CA 90033

The anatomical localization of the putative steroid recognition site associated with the $GABA_A$ receptor chloride ionophore was evaluated in rat brain by allosteric modulation of ^{35}S-t-butylbicyclophosphorothionate (TBPS) and ^3H-flunitrazepam (FLU) autoradiography.

All regions showing detectable specific ^{35}S-TBPS binding were affected by the most potent steroid 5α-pregnan-3α-ol-20-one (3α-OH-DHP). Detailed analysis of specific brain regions revealed regional variations in the potency of 3α-OH-DHP. For example, 3α-OH-DHP displaced ^{35}S-TBPS binding with nanomolar potency in the hippocampus but micromolar potency in the brainstem.

In addition, steroid coupling to the central benzodiazepine receptor was investigated by evaluating the ability of neuroactive steroids to enhance ^3H-FLU binding. A regionally dependent effect of 3α-OH-DHP on ^3H-FLU binding was observed since a lack of coupling between the steroid and the benzodiazepine sites was apparent in several brain regions.

Collectively these results provide initial evidence for a differential distribution of the benzodiazepine and steroid binding sites and suggest a unique regional distribution of a putative neurosteroid recognition site in rat brain. (Supported by a grant from CoCensys, Inc.)

537.17

TEMPERATURE DEPENDENCE AND STEROID MODULATION OF [^{3}H]FLUNITRAZEPAM BINDING TO RAT BRAIN MEMBRANES. R.J.Prince* and M.A.Simmonds. School of Pharmacy, University of London, London WC1N 1AX

The state of fluidity of lipid bilayers is dependent upon both the steroids present, notably cholesterol, and temperature. We have examined the effect of temperature upon [^{3}H]flunitrazepam (FNZ) binding to the $GABA_A$-benzodiazepine receptor and also upon the modulation of this receptor by the pregnane steroid alphaxalone.

The affinity of FNZ was measured at temperatures in the range 0-47°C using a displacement assay terminated by rapid filtration in the absence and presence of 100μM alphaxalone. Increasing temperature resulted in a decrease in affinity. A plot of reciprocal absolute-temperature against -log(Ki) showed a linear relationship in both cases. This is predicted by the van't Hoff equation ($\ln Keq = -\Delta H^\circ/RT + \Delta S^\circ/R$) and indicates that affinity changes may be due to thermodynamic considerations only. Linear regression yields values of -30.23 and -34.04 $kJ.mol^{-1}$ for ΔH° and 53.51 and 47.88 $J.K^{-1}mol^{-1}$ for ΔS° in the absence and presence of steroid respectively. The derived values for ΔH° are not significantly different at a confidence level of 95% indicating that steroid potentiation may be a temperature independent phenomenon.

537.18

PROPOFOL (PR), A NOVEL GENERAL ANESTHETIC, HAS SPECIFIC RECOGNITION SITES IN THE RAT BRAIN A. Concas, M.P. Mascia*, G. Santoro*, M. Serra*, E. Sanna and G. Biggio Dept of Experimental Biology, Chair of Pharmacology, Univ. of Cagliari, 09123 Cagliari, Italy

Recently, we have shown that PR, a novel general anesthetic enhances the function of $GABA_A$ receptor complex in the rat brain. In fact, like alphaxalone, barbiturates and benzodiazepines, PR increased ^{3}H-GABA binding and muscimol-stimulated $^{36}Cl^-$ uptake and reduced ^{35}S-TBPS binding. However, the additive effect produced by the concomitant addition of PR and the above drugs suggests different sites of action. To identify putative binding sites for PR we examined the binding of the radiolabelled PR (^{3}H-PR) in the rat brain. ^{3}H-PR binding to a crude synaptosomal preparation was specific (80-90% of specific binding), increased linearly with proteins concentration, reached equilibrium within 7 hr at 37 °C. In the rat cerebral cortex ^{3}H-PR binds to two population of binding sites (high affinity K_D 112 ± 8 nM; Bmax 7.8 ± 2 pmol/mg protein; low affinity K_D 6.4 μM; Bmax 55 ± 12 pmol/mg protein). Regional distribution studies showed that ^{3}H-PR binding was higher in the cerebellum, cerebral cortex and hippocampus and lower in the striatum and spinal cord. Finally, the finding that ^{3}H-PR binding is completely inhibited by pretreatment of membrane with proteinase K suggests the possible specific interaction of PR with some protein rather than other component of membrane.

537.19

BIOCHEMICAL EVIDENCE THAT "IN VIVO" ADMINISTRATION OF ABECARNIL ENHANCES GABAERGIC TRANSMISSION IN THE RAT CEREBRAL CORTEX. M. Serra*, M.C. Foddi*, C.A. Ghiani*, A. Concas, E. Sanna and G. Biggio Dept. of Experimental Biology, Chair of Pharmacol., Univ. of Cagliari, Cagliari 09123, Italy

The effect of abecarnil (AB), a new β-carboline possessing anxiolytic and anticonvulsant properties, was evaluated "in vivo" on the function of $GABA_A$ receptor complex. The intraperitoneal injection of AB produced in 30 min a dose dependent (0.25-20 mg/kg) decrease of [^{35}S]TBPS binding measured "ex vivo" in the rat cerebral cortex; this effect was abolished by the administration of the benzodiazepine receptor antagonist flumazenil (Ro 15-1788). AB, up to the maximal dose used (20 mg/kg), failed to induce ataxia and loss of righting reflex. Moreover, acute administration of AB (1 mg/kg) completely antagonized the convulsant activity and the increase of [^{35}S]TBPS binding induced by 350 mg/kg of isoniazid as well as the increase of [^{35}S]TBPS binding induced by foot-shock stress. Consistent with the above results the "in vitro" addition of AB, mimicking the action of benzodiazepines, increased [^{3}H]GABA binding, enhanced muscimol-stimulated $^{36}Cl^-$ uptake, reduced [^{35}S]TBPS binding and inhibited the binding of [^{3}H]Flunitrazepam. The results suggest that the enhancement induced by AB on GABAergic transmission can be detected biochemically in the membrane preparation "ex vivo".

OPIOIDS: BEHAVIOR I

538.1

ALFENTANIL DOSE-EFFECT RELATIONSHIPS FOR FOUR OPIATE EFFECTS IN THE RAT. M. B. Weinger, P. K. Yang*, S. S. Negus. Department of Anesthesiology, UCSD and VA Medical Centers, San Diego, CA 92161; Department of Neuropharmacology, Scripps Clinics, La Jolla, CA 92093.

Besides antinociception [AN] and sedation, opiates also cause catalepsy [CT] and muscle rigidity [MR]. However, MR dose-effect relationships have not been directly compared to AN or loss of righting reflex [RR]. To elucidate the mechanisms and sites of action of opiate anesthesia, the endpoints of opiate effect must first be described. We, therefore, studied four relevant opiate endpoints (AN, CT, MR, and RR) using the μ-agonist alfentanil [ALF] in the spontaneously ventilating rat.

Rats were randomized to receive a dose of ALF (0-500 μg/kg s.c.). For MR, 59 rats had EMG activity measured with percutaneous hindlimb electrodes. After ALF, data were recorded for 60 min. For AN and CT, 49 rats were studied for 120 min after ALF. CT was measured from the time the rat's forelimbs were placed on a 10-cm-high bar until either limb was removed. The nociceptive stimulus was tail immersion in 55°C water. AN latency was measured from tail immersion until purposeful tail movement (10-sec cutoff). For RR, 40 rats were studied for 120 min. RR was absent when a rat could not right itself in 15 sec. For each effect, data were converted to quantal responses by assigning any value >4 times baseline as a positive response. Data were converted to probit-log dose response curves, and slopes and ED50's were analyzed.

Slopes between the four dose-effect curves were not significantly different. The ED50 for RR differed significantly from AN and MR, and the ED50 for CT differed significantly from AN. The slopes and ED50's for MR and CT were similar, suggesting a common site of action. Catalepsy may be a useful model for spontaneous movement during opiate anesthesia. These techniques and data will help elucidate the pharmacology of opiate effects and their underlying receptor mechanisms.

538.2

SOCIAL DEFEAT IN FEMALE RATS: SENSITIVITY TO OPIATE SUPPRESSION OF TAILFLICK REFLEX AND ULTRASOUNDS. M. Haney, K.A. Miczek. Dept. Psychology, Tufts Univ., Medford MA 02155.

Female rodents are less sensitive to environmental stress-induced analgesia than males. We investigated the behavioral characteristics and pharmacological consequences of social defeat in female rats: (1) Do female rats emit ultrasounds during an aggressive encounter with a lactating opponent? (2) Do defeated females show a pattern of potentiation and cross-tolerance to morphine-induced analgesia? (3) Does gonadal hormone status modulate morphine sensitivity during or 1 week after a defeat experience? Morphine (1-10 mg/kg sc) or saline was administered to estrous and diestrous, Long-Evans rats exposed to 3 attack and threat encounters (ca. 1 min) over a 75 min period. The remainder of the 75 min, rats were threatened but protected from attack by wire mesh. Females emit ultrasonic calls during the physical and protected encounter. Morphine (6-10 mg/kg sc) selectively attenuated the high frequency (32-60 kHz) ultrasonic components without altering low frequency (22-32 kHz) or audible calls. Rats were analgesic within a similar dose-range; estrous state did not significantly modulate morphine's effects. Unlike males, (1) females actively escaped attack and rarely displayed submissive postures (crouch, supine) (2) morphine's analgesic properties were not potentiated during the defeat encounter. Rats treated with saline during the defeat were tolerant to morphine-induced analgesia one week later (1.5 fold rightward shift). The relatively attenuated opiate potentiation in defeated female rats may reflect the active rather than immobile response to attack.

538.3

OPIATE REGULATION OF AFFILIATIVE VOCALIZATIONS AND BEHAVIORS IN INFANT RHESUS MONKEYS. N.H. Kalin, S.E. Shelton* and C.T. Snowdon*. Dept. of Psychiatry and Psychology and VA Hospital, Univ. of Wisconsin, Madison, WI 53705.

We assessed the role of opiate systems in regulating affiliative behaviors and vocalizations. During maternal separation, infants emit coos. When separated and tested in proximity to conspecific adults with whom they cannot achieve physical contact, infants increase their cooing and also emit soft, low-frequency nasal whimpers (girns). Coos and girns differ on several acoustical parameters: duration (p <.001), frequency (p <.01), and number of harmonics, (p <.01). Behavioral and vocal responses are also modulated when infants are physically united with conspecifics after brief maternal separation. When united with an unfamiliar adult male, infants attempt to affiliate but are rejected. As attempts to achieve physical comfort fail, they express fear-related behavior, increase cooing (p <.01), and decrease girning. When reunited with their mothers, the mothers cradle them, and the infants girn more (p <.02) and cease cooing. These data suggest that both coos and girns are affiliative vocalizations but have different communicative functions. Pharmacological experiments reveal that opiate systems regulate coos and girns depending on environmental parameters. During reunion, endogenous opiate systems also appear to regulate infants' attachment behaviors.

538.4

SENSITIZATION OF OPIATE EFFECTS: PROGRESSIVE INCREASES IN FEEDING WITH REPEATED CENTRAL INJECTIONS OF DAGO BUT NOT DPDPE. R.A. WISE, and M.B. NOEL Center for Studies in Behavioral Neurobiology, Dept. Psychol., Concordia University, Montreal, Quebec, Canada. H3G 1M8

Ventral tegmental area (VTA) injections of morphine cause locomotion and potentiate feeding; the locomotor effects increase (sensitize) with repeated testing. In order to determine whether mu or delta receptors are involved in the feeding effects and whether sensitization occurs in relation to the feeding as well as the locomotor effects, we examined the effects of repeated administration of the selective mu agonist DAGO ([D-Ala^2,N-Me-Phe^4-Gly^5-Ol]-Enkephalin) and the selective delta agonist DPDPE ([D-Pen^2,D-Pen^5]-Enkephalin) in food deprived rats. Rats were implanted with stainless steel cannulae aimed at the VTA. Feeding behavior was quantified daily after 18 hours of food deprivation; food was presented in 18 meal segments consisting of five 45 mg food pellets per segment. VTA injections of DAGO and DPDPE caused dose-dependent acceleration of feeding; DAGO was 10 times more effective than DPDPE. While repeated injections of a low dose (0.05 nm) of DAGO resulted in progressively greater accelerations of feeding, repeated DPDPE (0.5 nm) injections did not. These data implicate mu opiate receptors in the facilitation of feeding and indicate that the potentiation of feeding, like the locomotor effects of mu opioids, is sensitized by repeated opioid treatment.

538.5

DIET AND OPIATE-INDUCED ANALGESIA IN RATS. R. B. Kanarek, E. S. White* and R. Marks-Kaufman*. Dept. of Psychology and School of Nutrition, Tufts Univ., Medford, MA 02155.

This series of experiments examined the effects of palatable foods on opiate-induced analgesia using a tail-flick apparatus in 42 adult male Sprague-Dawley rats. Rats were given ad lib access to Purina Chow (N=14), or given a choice of Purina Chow and either a 32% sucrose solution (N=14), or hydrogenated vegetable fat (N=14). Analgesic testing was conducted immediately preceding and at 30, 60, and 90 minutes following intraperitoneal injections of morphine sulfate (0.0, 5.0 and 10 mg/kg) or subcutaneous injections of ethylketocyclazocine (EKC) (0.0, 0.1, 0.3 and 0.6 mg/kg). No differences in analgesic responsiveness were observed as a function of diet preceding drug injections. However, dietary variables did alter opiate-induced analgesia. Sixty minutes following the injections of 10 mg/kg morphine, animals fed sucrose or fat had significantly longer mean tail flick latencies than rats fed only Purina Chow (Purina Chow = 1.5 sec; Purina + sucrose = 4.9 sec; Purina + fat = 3.3 sec). Similarly, at 30 minutes following injections of 0.3 mg/kg EKC, rats fed sucrose or fat had significantly longer mean tail flick latencies than their Purina Chow fed counterparts (Purina Chow = 0.7 sec; Purina + sucrose = 3.3 sec; Purina + fat = 2.7 sec). These data suggest that dietary variables interact directly with endogenous opioid systems to influence pain sensitivity.

538.6

THE RAPID DEVELOPMENT OF TOLERANCE TO MORPHINE IN GENETICALLY OBESE RATS. C.B. Goodman* and K.F.A. Soliman, College of Pharmacy, Florida A&M University, Tallahassee, FL 32307.

Endogenous opioids have been implicated in obesity in the genetically obese Zucker rat (fa/fa). The present study was designed to determine if tolerance would develop in the obese Zucker rat after two days treatment of morphine. Obese rats and their lean littermates were treated with morphine (10 mg/kg) for two days, while their corresponding controls received equivalent volumes of saline. Tolerance was assess by measuring pain sensitivity and plasma glucose level before and 30, 60, 120 minutes after morphine administration. The results show no tolerance was exhibited in the tail flick for both groups. The obese animals were shown to be more sensitive to morphine's antinociceptive effects. Moreover, in the hot plate test, both groups developed tolerance and the duration of analgesia was significantly prolonged ($p<0.05$) for 120 min in the obese rats when compared to their lean littermates. While no significant changes were observed in the plasma glucose levels of the obese animals, the lean group experienced a significant increase ($p<0.01$) in their plasma glucose levels for 60 mins. after the second day of morphine treatment. These results suggest that the acute development of tolerance may be mediated through a supraspinal mechanism. (Supported by NIH grants RR 0811, RR 03020 and NASA grant NAG 2-411).

538.7

ENHANCED DRINKING AFTER INJECTION OF THE *MU*-OPIOID AGONIST DAMGO INTO THE RAT VTA: SENSITIZATION AFTER CHRONIC AMPHETAMINE TREATMENT. A. Badiani, D. Rodaros and J. Stewart. Center for Studies in Behavioral Neurobiology, Dept. of Psychology, Concordia University, Montreal, QC, Canada, H3G 1M8.

Chronic pretreatment with amphetamine (AMPH) has been shown to enhance the effects of intra-ventral tegmental area (VTA) microinjections of morphine on locomotion (Stewart & Vezina, 1987) and food-intake measured over 2-5 h (Nencini & Stewart, 1990). In this experiment, highly selective μ- and *k*-opioid agonists, DAMGO and U-50,488H (U50), were compared for their effects on ingestive behaviors in 20 rats prepared with bilateral cannulae aimed at the VTAs. Two groups received daily i.p. injections of either saline (SAL) or 3 mk/kg of d-amphetamine (AMPH) in their home cages. After 10 days of chronic treatment, the rats were challenged, in a counterbalanced order, with U50 (1.0 nmol per side), DAGO (0.1 nmol per side) or saline (0.5 µl per side). 15 min after the microinjections, the rats were placed in test cages and feeding, drinking and other activities were recorded over 1 h. Tests occurred twice a week. DAMGO increased drinking by 64% in SAL-pretreated rats and by 185 % in AMPH-pretreated rats. Water-intake after microinjection of saline was the same in the two chronic treatment groups. DAMGO did not increase food-intake, measured over 1 h, in either group. U50 failed to affect both feeding and drinking. These results suggest that μ-opioid and dopaminergic pathways interact at the level of VTA to modulate drinking as well as other appetitive behaviors.

538.8

KAPPA OPIOID RECEPTOR MEDIATES FETAL RESPONSIVENESS TO MILK. W. P. Smotherman, R. R. Sheaffer*, S. Musselwhite* and S. R. Robinson*. Center for Developmental Psychobiology, SUNY-Binghamton, Binghamton, NY 13902-6000.

Fetal rats reliably express a facial wiping response to punctate tactile stimulation of the perioral area on days 20-21 of gestation (birth = 21.5 days). The wiping response is eliminated 60-s after intraoral infusion of milk. Pretreatment of fetuses with naloxone blocks the milk effect and restores the wiping response, indicating that milk activates a functional opioid system in the fetus. The κ opioid antagonist, nor-binaltorphimine HCl, also is effective in reversing the behavioral effects of milk, but the µ antagonist, β-funaltrexamine HCl, is not. These findings suggest that milk infusion triggers the release of endogenous opioids which bind to the κ receptor to alter fetal responsiveness to stimulation and effect global changes in fetal behavioral organization.

This research was supported by grant HD 16102 and RCDA HD 00719 to WPS.

538.9

POMC SYSTEM AND ALCOHOL PREFERENCE. J.C. Froehlich*, G. Wand*, S. Ochs and T.-K. Li*. Dept. of Med., Indiana Univ. Sch. of Med., Indianapolis, IN 46202; Dept. of Med., Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

We have previously reported that differences in activity within the central enkephalinergic system are associated with genetic differences in oral ethanol preference. We now report that rats that have been genetically selected for high or low alcohol drinking (alcohol-preferring or P, and alcohol nonpreferring or NP lines, respectively) differ in activity within the proopiomelanocortin (POMC) system. Ethanol-naive P and NP rats were sacrificed and the anterior pituitary (AP), neurointermediate lobe of the pituitary (NIL), and hypothalamus (H) were dissected out. Total RNA was extracted and northern analyses were performed using a POMC cDNA probe. POMC mRNA for each sample was normalized to the amount of 18S RNA present. Plasma content of β-endorphin and corticosterone were analyzed by RIA. Densitometric analyses showed that POMC mRNA was approximately 2-fold higher in the AP and NIL of P rats compared with NP rats. Plasma levels of β-endorphin and corticosterone were also higher in rats of the P line than in the NP line. The results suggest that a genetic predisposition towards alcohol drinking may be associated with increased activity or "tone" of the POMC system (AA07611; AA08312; AA08553; AA01298).

538.10

EFFECTS OF SELECTIVE SIGMA AGENTS ON LICKING AND OPEN-FIELD BEHAVIORS R.B. Murphy, L.H. Schneider, R. Bock*, and J.D. Davis, Cornell Univ. Medical College, Dept. of Psychiatry, Bourne Lab, White Plains, NY 10605 and Dept. of Chemistry and Ctr. Neural Sci., New York Univ., NY, NY 10003 and Univ. Illinois, Dept. Psychol, Chicago IL 60680

We previously reported that peripherally-administered sigma agents do not modulate sucrose sham feeding in the rat in a manner commensurate with their putative involvement with the ascending dopamine (DA) systems (Soc. Neurosci. Abstr.16:1028,1990). However, peripherally administered sigma agents do produce a significant shift in the mean of the interlick interval distribution toward longer time which is not observed when selective DA agents are administered centrally or peripherally. We report here the effects of intraventricular and bilateral intranigral administration of the selective sigma agents (+)-SKF 10,047 (SKF) and di-o-tolylguanidine (DTG) on the microstructure of ingestion of 50% sweetened milk, as well as on various open field behaviors. Male, 18-h deprived S/D rats received DTG (4 ug/rat) or SKF (10 ug/rat) intraventricularly; no significant effects on amount consumed or microstructure were found, other than a significant lengthening of the duration of feeding in DTG rats. In a separate group in which DTG (700 ng/rat) was infused bilaterally into the substantia nigra, a small but significant reduction in amount ingested was observed as compared with CSF. Rats which received DTG (700 ng/rat) or SKF (4 ug/rat) intranigrally were also examined in the open field. The only parameter effected was numbers of squares crossed, which were significantly increased in DTG but not in SKF or CSF rats. In summary, central administration of selective sigma agents failed to produce the shift in interlick interval distribution, in contrast to their peripheral administration. The central results further support the lack of involvement of DA systems in sigma-mediated behaviors. We hypothesize that the major effect of the sigma agents is on hindbrain sigma sites accessible to peripheral circulation which control lick rate. **[SUPPORTED BY: NIDA DA 05728 (RBM), NINDS NS24781 (LHS), and NIH DK 41563 (JDD)]**

538.11

Eigenmannia virescens: A NEW OPIATE ASSAY? R.D. Olson, B.B. Rowland*, S.M. Samuel*, A.M. Vasquez*, G.A. Olson, and A.J. Kastin. Department of Psychology, University of New Orleans, New Orleans, LA 70148.

Several species of African and South American fish are able to produce an electric organ discharge (EOD) rate that is both highly stable and individually unique. In Experiment 1, 75 fish (*E. virescens*) were injected IP (5 ul) with either diluent, naloxone (2 mg/kg), morphine (10 mg/kg), a single injection containing naloxone and morphine, or naloxone followed 10 seconds later by morphine. Testing consisted of a 10 minute pre-injection baseline period and a 30 minute post-injection test period. Morphine was found to significantly decrease ($p<.05$) EOD rate relative to diluent and naloxone, which did not differ. A single injection of naloxone and morphine produced results similar to separate injections of naloxone and morphine; in both cases, naloxone antagonized the effects of morphine, suggesting that separate injections are not necessary. Experiment 2 evaluated doses of morphine (0, 5, 10, 20, and 40 mg/kg IP), with results indicating that 40 mg/kg significantly reduced ($p<.05$) the EOD rate relative to all other groups and even terminated the EOD rate completely in some subjects; 20 mg/kg significantly decreased ($p<.05$) the EOD rate relative to the remaining doses, which did not differ from one another. The results suggest that *E. virescens* might serve as a new opiate assay.

538.12

NALOXONE ENHANCES ACQUISITION OF A SPATIAL TASK IN NON-BREEDING MEADOW VOLES IN A SEXUALLY DIMORPHIC MANNER. L.A.M. Galea, L. Saksida*, M. Kavaliers, and K.- P. Ossenkopp. Departments of Psychology and Oral Biology, Univ. Of Western Ontario, London, Ontario, Canada, N6A 5C2.

Endogenous opioid systems have been implicated in spatial task learning. Polygynous meadow voles, *Microtus pennsylvanicus*, display pronounced sex differences in the ability to learn spatial tasks. In the present study, we examined the effects of the opiate antagonist, naloxone (1mg/kg), in spatial task learning (Morris water maze) of non-reproductive adult male and female meadow voles. Females treated with naloxone acquired the task faster than any other group, although all groups learned the task in five acquisition days. Contrary to previous studies with adult reproductive voles, no sex differences favoring males were detected in acquisition of the spatial task. Results from this study suggest that there may be miminal sex differences in water maze learning in sexually immature voles. These findings also suggest that spatial learning may be differentially modulated by the endogenous opioid systems in male and female voles.
(Supported by NSERC grants to MK and KPO).

538.13

EFFECTS OF ß-FUNALTREXAMINE AND NALOXONAZINE ON ALFENTANIL-INDUCED MUSCLE RIGIDITY AND ANTINOCICEPTION IN THE RAT. S.S. Negus and M.B. Weinger, Dept. of Anesthesiology, UCSD and VA Medical Centers, San Diego, CA 92161

Alfentanil is a potent and short-acting opioid agonist that is selective for mu opioid receptors. As with other mu agonists, alfentanil produces such clinically useful effects as antinociception, while also producing such unwanted side effects as muscle rigidity. The purpose of the present study was to compare the pharmacological nature of alfentanil-induced muscle rigidity and antinociception by evaluating the susceptibility of these effects to antagonism by ß-funaltrexamine (ß-FNA) and naloxonazine (NXZ). ß-FNA has been characterized as a selective and irreversible mu antagonist, whereas NXZ has been characterized as a selective and long-acting antagonist of the mu-1 subtype of the mu receptor. Rats were pretreated with either ß-FNA (vehicle, 10.0 mg/kg , 20 mg/kg) or NXZ (vehicle, 7.5 mg/kg, 15.0 mg/kg) approximately 20 hours before evaluating the effects of alfentanil. Muscle rigidity was measured by recording the electromyographic activity from the gastrocnemius muscle, whereas antinociception was measured using the warm-water tail dip procedure. Alfentanil dose-effect curves were generated for each pretreatment using a between-subjects design. Both ß-FNA and naloxonazine antagonized alfentanil-induced muscle rigidity and alfentanil-induced antinociception. These results indicate that mu receptors in general, and mu-1 receptors in particular, are capable of mediating both the antinociception and the muscle rigidity produced by alfentanil in the rat.

538.14

THE INFLUENCE OF CONTINUOUS INFUSION OF NALOXONE AND ITS WITHDRAWAL UPON SPONTANEOUS AND AMPHETAMINE -STIMULATED LOCOMOTOR ACTIVITY. D.N.C. Jones* and S.G. Holtzman. Dept. of Pharmacology, Emory Univ. School of Medicine, Atlanta, GA 30322.

Several reports in the literature demonstrate an opioid receptor mediated modulation of dopaminergic function. Therefore, the present study was designed to investigate the influence of both the continuous infusion of the opioid antagonist, naloxone (NX) and its subsequent withdrawal upon spontaneous locomotor activity and amphetamine (AMPH) -induced hyperactivity in rats. NX filled (1 mg/kg/hr) or sham osmotic pumps were implanted s.c. in rats (male, 280-290g at start of experiment) under light anesthesia and similarly removed following a 7-day infusion period. Spontaneous locomotor activity (fine and gross movements) was assessed on days 0 (pre-infusion), 3,6,8 and 14. A cumulative-dose-response curve to AMPH (0.1-6.4mg/kg s.c.) was constructed either on day 6 or day 8 (24hr following the removal of the pumps). During the infusion of NX, rats exhibited a lower level of both gross and fine locomotor activity than did sham animals, although this reached statistical significance for the latter measure only ($F=4.86$, $P<0.05$). There were no differences between the treatment groups 24hr and 7 days after pump removal. Cumulative administration of AMPH on either day 6 or 8 produced dose dependent increases in both fine and gross locomotor movements. On day 6, however, the response of NX-infused rats to AMPH was significantly attenuated ($F=6.15$, $P<0.05$, gross counts only) compared with that of sham-infused animals, with no apparent differences between the groups following saline administration. 24hr following pump removal, there were no longer any significant differences betwwen the response of the two groups of rats to amphetamine. The finding that NX attenuates AMPH-induced hyperactivity agrees with those of others and is possibly explained by an attenuation of the AMPH-induced release of dopamine (Hooks et al.,this meeting). This study extends the range of conditions investigating NX/AMPH interactions to include continuous NX treatment and reveals that the influence of NX was not shown to persist beyond cessation of the infusion. (Supported in part by Grant DA00541 and RSA DA00008)

538.15

THE EFFECT OF ALFENTANIL ON DIAPHRAGMATIC ELECTROMYOGRAPHIC RECORDINGS IN THE RAT. C. M. Campbell*, M. B. Weinger, M. Quinn*, S. S. Negus, R. L. Lieber (sponsor). VA Medical Center, San Diego, CA 92161.

Muscle rigidity and respiratory depression are two undesirable side effects of opiate anesthesia. Opiate-induced increases in thoracic muscle tone contribute to impaired ventilation. However, the effect of high-dose opiates on diaphragmatic muscle tone and function has not been described. We therefore studied the effects of alfentanil (ALF) on diaphragmatic electromyographic (EMG) activity in the spontaneously ventilating rat.

5 - 7 days before the experiment, a differential electrode was surgically implanted in the diaphragm. A 100-Hz - 3-kHz band-pass filter was employed to remove electrocardiographic and low-frequency respiratory artifact. Baseline EMG activity was recorded for at least 15 min. Rats were then injected with ALF (0.5 mg/kg s.c.), and the EMG was observed for the next 30 min. Data were analyzed by computer to determine the effects of ALF on inspiratory and expiratory diaphragm activity.

Before ALF, diaphragm EMG activity increased during inspiration and decreased during expiration. ALF disrupted this phasic activity by augmenting the expiratory phase EMG. Thus, after ALF, the diaphragmatic EMG activity level stayed relatively constant and high for the entire 30-min experiment. Further study will clarify the mechanism of the effect and the role of diaphragmatic "rigidity" in opiate-induced respiratory depression.

538.16

EFFECTS OF ACUTE NALTREXONE ON LOCOMOTOR ACTIVITY IN AUTISTIC CHILDREN. G.S. Asleson,* B.H. Herman, I.F. Borghese*, R.P. Allen* & A. Arthur-Smith Brain Res. Cen., Children's Natl. Med. Cen. & Depts. Psychiat. and Peds, George Washington USM, Washington DC, USA 20010 & Dept. Psychiat., JHUSM, Baltimore, MD 21214

We have estimated that about 90% of autistic children are hyperactive as measured by the Conner's Parent Teacher Rating Scale (CPTRS). Here we demonstrate that three methods for measuring activity all indicate that acutely administered naltrexone significantly decreases the hyperactivity of autistic children. Placebo (P1,P2) or naltrexone (Nal) (0.5, 1.0, 1.5, and 2.0 mg/kg) was administered once per week to autistic children (3-12 y.o.), and Ss were tested on the day of drug. Activity was measured in the BRC Social Proximity Test (BRCSPT) by determining the number of squares S crossed in a playroom and by recording activity by an ambulatory activity monitor (AAM). Parents rated 24h activity of their child on the CPTRS. The BRCSPT consists of three 10 min sessions, two in the presence of a familiar volunteer-one without toys and one with toys and a third with the mother. In the BRCSPT, Nal significantly decreased AAM scores in comparison with P1($N=3$, $p<.005$; M± SEM, P1=330 ± 45,1.0 =169 ± 54, 1.5=151 ± 34, 2.0= 186 ± 45). Similarly, Nal significantly decreased number of squares entered in the BRCSPT ($N=7$, $p < .05$; M ± SEM, P1 = 31 ± 2, 0.5 = 25 ± 1,1.0 = 23 ± 1,1.5= 24 ± 2, 2.0= 26 ± 2, P2= 28 ± 2. In the CPTRS, Nal significantly reduced parents' ratings of hyperactivity ($N=8$, $p<.05$; M ± SEM, P1=13 ± 3, 0.5 = 9 ± 2, 1.0 = 8 ± 2; 1.5 = 7 ± 2, 2.0 = 7 ± 1, P2 = 12 ± 2. Supported by FDA, NICHD, Du Pont (to BHH).

539.1

HIGH-AFFINITY SELECTIVE LIGANDS FOR MAPPING CNS D-1 DOPAMINE RECEPTORS. M.P. Kung, J. Billings, S. Chumpradit, C.D. Unsworth, P.B. Molinoff, H. Kung. Depts. of Radiology, Psychiatry & Pharmacology, Univ. of Pennsylvania, Phila., PA 19104

Selective D1 ligands with high affinity and high specific activity for mapping CNS dopamine D1 receptors were developed. Substitution with iodine at the 3' or 4' position of 5-phenyl ring of SCH23390 gave two compounds, R-TISCH and R-FISCH, respectively. Both R-[^{125}I]TISCH and R-[^{125}I]FISCH displayed high affinity and selectivity for CNS D1 dopamine receptors in rat striatal membranes (Kd = 0.20 nM for R-TISCH and 0.75 nM for R-FISCH). The binding of both ligands also showed a stereoselective preference for the R(+) over the S(-) isomer. Kinetic studies indicate that the dissociation rate of R-FISCH (k_{-1} = 0.353 min^{-1}) is faster than that of R-TISCH (k_{-1} = 0.082 min^{-1}).The high binding affinity in conjunction with the slower off-rate suggest that R-TISCH may be responsible for better target-to-nontarget ratio (striatum/cerebellum) than R-FISCH observed *in vivo*. R-TISCH also displayed high affinity for 5-HT$_2$ receptors (Kd = 0.28 nM); whereas R-FISCH appeared to have a low affinity for 5-HT$_2$ receptors. Regional distribution of R-TISCH and R-FISCH in rat brain, as measured by *ex vivo* autoradiography, displayed highest binding in regions known to have a high concentration of D-1 receptors. Initial *in vivo* imaging study of [^{123}I]TISCH in humans displayed a high concentration of this ligand in the basal ganglia. These results indicate that R-TISCH and R-FISCH are both suitable for mapping CNS D-1 receptors and are potentially useful for *in vitro* and *in vivo* pharmacological studies. (Supported by NIH grants NS-24538 and NS-18591.)

539.2

LAMINAR DISTRIBUTION OF DOPAMINE RECEPTOR SUBTYPE mRNAS IN THE MACAQUE ANTERIOR CINGULATE CORTEX. S.C. Sealfon, W.G. Janssen*, L.A. Snyder, G.W. Huntley, Y. Shiffman*, P.R. Hof, A. Prikhozhan*, J.H. Morrison. Fishberg Res. Cntr. for Neurobiology, Dept. of Neurology, and Dept. of Geriatrics. Mount Sinai Medical School. New York, NY 10029

The anterior cingulate cortex is a key cortical component of the circuits implicated in emotion, reward and affective responses to pain. In the primate, the anterior cingulate cortex receives a dense mesocortical dopaminergic projection that terminates in both superficial and deep layers. With the molecular cloning of the genes for multiple dopamine receptors, it has now become feasible to investigate, using subtype-specific probes, the laminar distribution of the dopamine receptor-expressing neurons in the anterior cingulate cortex.

cDNAs for the primate D_1 and D_3 receptors were isolated via PCR and subcloned into Bluescript plasmid. ^{35}S-labeled cRNA was synthesized for *in situ* hybridization, which was done in 20 μM thick sections of anterior cingulate and prefrontal cortex from paraformaldehyde perfused macaque brain. Both probes labeled neurons in lamina that receive dopaminergic imput and the precise laminar pattern and density of labelling was distinct for the D_1 and D_3 receptor. Preliminary data suggest that D_1 mRNA was most abundant in layer V, intermediate in layer III and detectable in layer VI. D_3 mRNA was greatest in layer III and was also relatively high in layers II and V. We are now cloning cDNAs for the primate D_2, D_4 and D_5 receptors. Quantitative analysis of the anterior cingulate laminar distribution of these subtypes will be presented. Supported by NIH grants K11 DK01854, MH 45212 and AG06647.

539.3

TIME COURSE OF ENHANCED D1 AGONIST-INDUCED METABOLIC RESPONSES FOLLOWING ACUTE DOPAMINE DEPLETION. J.M. Trugman and C.L. James*. Dept. of Neurology, Univ. of Virginia, Charlottesville, VA 22908

One key aspect of dopaminergic denervation supersensitivity is the enhanced efficacy of D1 agonists to stimulate movement; the increased ability of D1 agonists to stimulate regional cerebral glucose utilization (RCGU) in the substantia nigra pars reticulata (SNr) in rats with chronic dopamine depletion provides a metabolic correlate to the heightened motor response. The present study examined the time course of D1 agonist-induced metabolic responses following acute dopamine depletion with reserpine (5.0 mg/kg ip). RCGU effects were assessed with [14C]-2-deoxyglucose autoradiography. The D1 agonist SKF 38393 (30 mg/kg) increased SNr RCGU by 18 % in naive rats. In comparison, SKF 38393 increased SNr RCGU by 38%, 68% and 60% at 6, 12 and 24 hours post-reserpine ($p<.05$); the response returned to control values at 5 days. D1 and D2 receptor number in the striatum and SNr, as measured by antagonist ligand binding, were unchanged 24 hours post-reserpine. We conclude that the metabolic effects of D1 stimulation are clearly enhanced within 12 hours of reserpine injection and that the enhanced effects are not mediated by an increase in D1 receptor number. The data suggest that supersensitive responses to D1 stimulation correlate temporally with striatal dopamine depletion.

539.4

DIFFERENCES OF D_1 AND D_2 DOPAMINE RECEPTOR DENSITY BETWEEN THE NEUROLEPTIC NON RESPONSIVE (NNR) AND NEUROLEPTIC RESPONSIVE (NR) LINES OF MICE - A QUANTITATIVE RECEPTOR AUTORADIOGRAPHIC (QRA) STUDY. **Y. Qian, B. Hitzemann* and R. Hitzemann. Departments of Psychiatry and Neurobiology, SUNY @ Stony Brook, NY 11794 and Psychiatry Service, VA Medical Center, Northport, NY 11768.**

QRA was used to examine D_1 and D_2 receptor density in the NR and NNR lines of mice, which have differed by an order of magnitude in their sensitivity (ED_{50}) to catalepsy induced by neuroleptics with a high D_2/D_1 receptor activity profile. D_1 receptor density (^{3}H-SCH23390 binding) was not different in any aspect of the caudate-putamen (CPu), the nucleus accumbens (NA) and substantia nigra zona reticulata (SNr). D_2 receptor density (^{3}H-Spiroperidol binding) was not different in the dorsomedial CPu or NA but was 20 to 30% lower in the NNR line in the caudal aspect of the lateral CPu. The NNR line also showed a 20 to 50% elevation of D_2 receptor density across all the midbrain DA cell groups (A8,A9 and A10). Overall, the data show that selection of mice for response and non-response to neuroleptic-induced catalepsy is associated with changes in D_2 but not D_1 receptor density.

539.5

DENSITY OF DOPAMINE (DA) CELLS IN THE NEUROLEPTIC RESPONSIVE (NR) AND NEUROLEPTIC NON-RESPONSIVE (NNR) LINES OF MICE: RELATIONSHIP TO D_2 AUTO-RECEPTOR DENSITY. **B. Hitzemann*, Y. Qian and R. Hitzemann. Departments of Psychiatry and Neurobiology, SUNY @ Stony Brook, Stony Brook NY 11794 and Psychiatry Service, VAMC, Northport NY 11768.**

The NR and NNR lines of mice have been selectively bred for 14 generations to yield animals more and less responsive to neuroleptic-induced catalepsy. A principal correlated response to selection, appears to be an increase of D_2 somatic autoreceptor density in the NNR (see Qian et al. 1991, this meeting). This response was first noted in S_4 and has persisted through S_{11}. The trivial explanation for these data is that DA cell density is increased in the NNR line. We now report that DA cell density in actually decreased in the NNR line. The decrease is most marked (30 to 50%) in the cell sparse zone of the rostral substantia nigra zona compacta (SNc) and the ventral tegmental area. A 15 to 25% decrease is seen in the densocellular zone of the SNc and in the retrorubal field. These data suggest that the specific activity of D_2 somatic autoreceptors is markedly enhanced in the NNR line.

539.6

NEUROLEPTIC INDUCED CATALEPSY AMONG EIGHT INBRED STRAINS OF MICE. **S.J. Kanes, B. Hitzemann* and R. Hitzemann. Departments of Psychiatry and Pharmacology, SUNY at Stony Brook, Stony Brook, NY 11794-8101 and Psychiatry Service, VAMC, Northport NY 11768.**

Our laboratory has used selective breeding to investigate the genetic factors associated with response and non-response to neuroleptic induced catalepsy (see e.g. Qian et al., 1991) The study of multiple inbred strains provides a different strategy to understand such genetic factors. Eight inbred strains have been tested: LP, CBA, C57BL, C3H, A, AKR, BALB/C, DBA/2. The ED_{50} values (mg/kg) among the eight strains for haloperidol-induced catalepsy are: LP 9.5, CBA 4.5, C57BL 3.8, C3H 1.9, A 2.3, AKR 0.82, BALB/C 0.45, DBA/2 0.48. ED_{50} values for the specific D_1 antagonist, SCH 23390 are: LP 0.33, CBA 0.20, C57/BL 0.16, C3H 0.28, A 0.70, AKR 0.36, BALB/C 0.20, DBA/2J 0.32. There was no correlation between haloperidol and SCH-23390 induced catalepsy (r^2=.28,p >0.05). Studies are in progress to determine pre and post-synaptic D_2 receptor density among lines, focusing on those regions shown to be different between the neuroleptic responsive(NR) and non-responsive(NNR) lines.

539.7

D1 AND D2 DOPAMINE (DA) RECEPTORS IN MICE WITH GENETICALLY DIFFERENT MESOTELENCEPHALIC DA SYSTEMS. I. Laszlovszky[1] and C. Vadasz[1,2].
[1]Nathan Kline Institute, Orangeburg, NY 10962 and [2]New York University Medical Center, 550 First Ave., New York, NY 10016.

The aim of this work was to test the hypothesis that in the mesotelencephalic DA system densities of striatal D1 and D2 receptors are correlated with strain-dependent variations in mesencephalic tyrosine hydroxylase activity (TH/MES). In order to provide reliable estimates for D1 and D2 receptor properties by controlling the intra- and inter-assay variability, a homogeneous pool of reference striatal membrane was prepared, which was subjected to Scatchard analysis in each batch of assays. K_d and B_{max} data for striatal samples of BALB/c, C57BL/6 and CXBI mouse strains were corrected by factors derived from the references. Statistical analysis indicated that each strain significantly differed in TH/MES. However, no significant strain differences were found for striatal D1 and D2 receptor binding with ^{3}H-Spiperone and ^{3}H-SCH 23390, respectively. Our results demonstrate that genetic factors determine a highly significant variation of TH/MES that is not correlated with significant genetic changes in D1 or D2 receptor affinities or with densities in the striatal projection area.

539.8

THE PHARMACOLOGICAL PROFILE OF U-86170F, A DOPAMINE AUTORECEPTOR AGONIST. D.L. Evans, R.A.Lahti, and L.M.Figur*. CNS Diseases Research, The Upjohn Company, Kalamazoo, MI 49001

Schizophrenia is believed to be due to an overactive dopaminergic system in the brain. Since dopamine (DA) autoreceptors regulate the synthesis, release, and firing rate of these neurons, DA autoreceptor agonists may be useful in treating schizophrenic symptoms.

U-86170F, a 3-amino-tetrahydroquinolinone-derived compound, binds to the high affinity agonist state of the D2 receptor (Ki=7.5 nM). In addition, it is a potent DA autoreceptor agonist in several *in vivo* models. This compound reversed the effects of γ-butyrolactone (GBL) on striatal DA levels (ED_{50} = 0.3 mg/kg) and exhibited autoreceptor agonist-like activity in the α-methyl-p-tyrosine/prolactin model (ED_{50} = 0.1 mg/kg IP and 0.4 mg/kg PO). In reserpinized mice, U-86170F caused an increase in locomotor activity, but unlike apomorphine, it had a ceiling effect. When administered IV once every hour for 2 weeks, U-86170F reversed the effects of GBL on striatal DA levels and plasma prolactin, as well as suppressed striatal homovanillic acid levels.

The pharmacological profile of U-86170F suggests that it is a potent DA autoreceptor agonist with limited postsynaptic activity. This unique autoreceptor agonist may be useful in the treatment of schizophrenic symptoms.

539.9

DOPAMINE (D2) AGONIST RECEPTOR BINDING PROPERTIES OF [^{3}H]U-86170. L.M.Figur*, D.L.Evans, and R.A.Lahti. CNS Diseases Research, The Upjohn Company, Kalamazoo, MI 49001

Use of [^{3}H]U-86170 as an agonist (Ag) ligand in conjunction with [^{3}H]raclopride, an antagonist (Ant) ligand, has introduced a novel approach to predicting the intrinsic activities (IA) of dopaminergic compounds.

Saturation studies using [^{3}H]U-86170 in rat striata and cloned $D2_i$ cells (C.Chio *et al.*, Nature, 343:266, 1990) revealed Kd's of 1.72 and 0.99 nM, respectively. The Bmax in $D2_i$ clones was 60-70% of that obtained with [^{3}H]raclopride, and [^{3}H]U-86170 binding was inhibited by GTP, indicating that it binds to the high affinity Ag state of the D2 receptor.

Evaluations of several dopaminergic agents in [^{3}H]raclopride + GTP binding (low affinity Ag state) and [^{3}H]U-86170 binding (high affinity Ag state) were made. Ratios of the Ki values (LowAg/HiAg) correlated well with electrophysiologically derived IA values (M.Piercey *et al.*, Society for Neuroscience, Abs 109.11, 1990). The ratio of the Ki values of an agent for these two receptor states provides a useful measure of IA for dopamine Ag's and partial Ag's. In addition, a correlation exists between a compound's effect on rat striatal homovanillic acid levels and its IA.

Using an Ag and an Ant ligand in cloned D2 receptor membrane preparations provides a simple and effective means of identifying dopamine Ag, partial Ag, and Ant activity.

539.10

U-86170F, A POTENT DOPAMINERGIC AGONIST THAT DOES NOT INDUCE TOLERANCE WITH CHRONIC ADMINISTRATION. M.F.Piercey, M.W.Moon*, R.A.Lahti, W.E.Hoffmann, and J.T.Lum. CNS Research, The Upjohn Co., Kalamazoo, MI, 49001.

U-86170F(4H-Imidazo[4,5-i,j]quinolin-2[1H]-one,5-[dipropylamino]-5,6-dihydro-,[-], monohydrobromide, hemihydrate) is a novel potent dopamine (DA) agonist that binds to both DA and serotonin (5-HT) receptors. We have examined its effects on firing rates of DA neurons in substantia nigra (SNPC, Bunney *et al.*, JPET 155:560, 1973) and 5-HT cells in dorsal raphe (DR, Aghajanian *et al.*, JPET 137:178, 1970). U-86170 dose-dependently depressed DA neurons with an ED_{50} of 8 μg/kg i.v., by a haloperidol-sensitive mechanism. Higher doses depressed 5-HT neurons (ED_{50}=107 μg/kg i.v.). The (+)-enantiomer was inactive. Animals treated for 10 days with U-86170F (1.8 mg/kg/day) had potencies (ED_{50}=4.0 +/- 1.8 μg/kg) similar to those chronically treated with vehicle (ED_{50}= 3.0 +/- 1.0 μg/kg i.v.). In contrast, animals chronically treated with apomorphine were less sensitive to APO (68 +/- 35 μg/kg) than were vehicle-treated controls (27 +/- 12 μg/kg). It is concluded that U-86170 is a potent DA agonist which could be useful in the long-term treatment of Parkinson's Disease.

539.11

SCH 39166, A D-1 ANTAGONIST WITHOUT SEROTONERGIC ACTIVITY. R.D. McQuade, C.E. Tedford, R.A. Duffy *, L.A. Taylor, M.A. Hunt **, J.K. Wamsley + and A. Barnett, Schering-Plough Research, Bloomfield, NJ 07003 and Neuropsychiatric Research Center, Fargo, ND 58103 +

Previous data have clearly indicated that the benzonaphthazepine, SCH 39166, is a potent and selective D-1 antagonist. This drug is currently undergoing clinical trials as a potential antipsychotic agent. SCH 23390, the predecessor of SCH 39166, also was a selective D-1 antagonist, which in addition, exhibited moderate affinity for serotonin (5HT) receptors, especially the 5HT-2 and 5HT-1_c subtypes. Thus, the in vitro binding of SCH 39166 to the 5HT-2 and 5HT-1_c receptors was determined by its ability to inhibit the binding of ^{3}H-ketanserin to membranes from rat frontal cortex and ^{3}H-mesulergine to membranes from porcine choroid plexus, respectively. SCH 39166 inhibited the binding of ^{3}H-ketanserin with a K_i of 167.0 nM, while it inhibited the binding of ^{3}H-mesulergine with a K_i of 1328.0 nM. In contrast, SCH 23390 inhibited the binding to 5HT-2 and 5HT-1_c receptors with K_i's of 20.0 and 30.7 nM, respectively. The less active stereoisomer of SCH 39166, SCH 39165, exhibited a K_i of 381.0 nM at 5HT-2 and 566.0 nM at 5HT-1_c receptors. These data indicate that SCH 39166 does not exhibit the stereoselectivity at 5HT receptors which it does at D-1 sites.

The interaction of SCH 39166 with D-1 and 5HT receptors was also studied using in vivo autoradiography. In these studies, the animals are injected with ^{3}H-SCH 39166 and localization of the radioligand to brain tissue is measured via autoradiography. These studies indicate that ^{3}H-SCH 39166 binds to the striatal-nigral pathway, with a specificity indicative of a D-1 interaction. There is little or no apparent binding to frontal cortex or choroid plexus, demonstrating an absence of activity at 5HT-2 and 5HT-1_c receptors. These data confirm our earlier in vitro autoradiographic findings and together with the binding data strongly argue that SCH 39166 does not interact with 5HT receptors.

539.12

EFFECT OF THE D-1 DOPAMINE RECEPTOR ANTAGONIST SCH 39166 ON SEIZURES INDUCED BY LITHIUM+PILOCARPINE. P. Barone, G. Cicarelli*, M. Trampus, E. Ongini and G. Campanella*. Dept. of Neurology-2nd Sch. of Med., Univ. of Naples and Research Labs of Schering-Plough, Comazzo MI, Italy.

Recent evidence indicates that the two dopamine receptor subtypes, D-1 and D-2, exert opposing roles in the control of epilepsy propagation. Blockade of D-1 receptor elevates, while D-2 receptor blockade reduces the threshold for pilocarpine-induced seizures. Pretreatment with lithium chloride (LiCl) is known to potentiate the seizure activity induced by cholinomimetics. The present study investigates the effect of a novel D-1 receptor antagonist, SCH 39166, on seizures induced by lithium+pilocarpine. Male Sprague Dawley rats were treated with LiCl (4 mEq/kg ip) 24 hr prior to the intraperitoneal injection of either saline or SCH 39166 (0.01-0.3 mg/kg) or the D-2 antagonist raclopride (1-10 mg/kg). After 15 min rats were given either 10 or 15 mg/kg ip of pilocarpine, the ED50 and ED100 for convulsions, respectively. SCH 39166 prevented, while raclopride potentiated convulsive activity, as revealed by behavioural and electroencephalographic alterations and severe brain damage. These results suggest that the use of D-1 selective neuroleptics might reduce the risk of convulsions in patients under treatment with the combination of lithium and neuroleptics.

539.13

DIZOCILPINE (MK 801), BUT NOT NBQX, ALTERS DOPAMINE (DA)-MEDIATED CHANGES IN STRIATOPALLIDAL NEURONAL ACTIVITY AND BEHAVIOR. M.D. Kelland, R.C. Boldry, K.-X. Huang, T.N. Chase and J.R. Walters. ETB, NINDS, NIH, Bethesda, MD 20892, USA.

Altering basal ganglia glutamate (GLU) transmission may have potential for treating Parkinson's disease. Using single-unit recording techniques, and a stereotypy rating scale, we are examining the role of GLU in regard to apomorphine (APO)-induced electrophysiological and behavioral responses.

APO (0.3 mg/kg, i.v.) significantly reduced the firing rate of 9/10 Type I caudate neurons. Following MK 801 (NMDA antagonist; 0.1 mg/kg, i.v.), APO inhibited fewer cells (3/10), while remaining cells were excited or unaffected, resulting in no net change in rate. Globus pallidus (GP) neuronal waveforms can be identified as Type I (-/+) or Type II (+/-). APO excited Type II GP cells (10/11), and inhibited Type I GP neurons (10/12). MK 801 caused a net blockade of APO effects on Type II cells (4/10 excited, 3/10 inhibited), but had equivocal effects on Type I GP neurons. MK 801 increased locomotion, but completely inhibited the stereotyped sniffing and licking induced by i.v. APO.

NBQX (AMPA antagonist; 10 mg/kg, i.v.) reduced the firing rate of 4/5 Type II GP neurons, but did not alter their responsiveness to APO.

Ketamine-anesthesia (150 mg/kg, i.p.), did not share APO-blocking properties with MK 801 (only neuronal activity tested). Conversely, MK 801 (18.4 mg/kg, i.p.) did not induce anesthesia in rats.

These data suggest that normal glutamate transmission is necessary to observe DA-mediated electrophysiological effects and stereotypy. The ability of MK 801 to block APO stereotypy may be due to the observed alterations in striatal/GP output patterns and/or other sites of action of MK 801. While the locomotor stimulant effects of MK 801 indicate potential for treating Parkinson's disease, reduction of the DA-mediated effects addressed here suggests otherwise.

539.14

D1 AGONIST HAS OPPOSITE EFFECTS ON NEURONAL TRANSMISSION IN THE BASAL GANGLIA OF TWO ANIMAL MODELS OF PARKINSON'S DISEASE: EFFECTS BLOCKED BY NMDA ANTAGONIST. K.-X. Huang and J.R. Walters. NINDS, Bethesda, MD 20892.

Unilateral 6-OHDA-induced lesion of the substantia nigra dopamine neurons and subchronic treatment with reserpine are two strategies used for producing animal models of Parkinson's disease (PD). We have compared the responses of substantia nigra pars reticulata (SNpr) neurons to the D1 agonist SKF-38393 in these animal models using extracellular recording techniques in locally anesthetized, gallamine paralyzed rats. Effects of MK-801 were also examined since NMDA antagonists are of interest in PD. In rats treated with reserpine (1 mg/kg/day, sc) for 6 days, SKF-38393 (10 mg/kg, iv) significantly increased the firing of SNpr neurons (ave. increase: 88 ± 21%) compared with control (18 ± 8%, n=7). This effect was reversed by the D1 antagonist SCH-23390, and was persistent: SKF-38393 induced a similar change in rats treated with reserpine for 6 days then allowed a five-day washout period. As shown previously, in 6-OHDA-lesioned rats studied 9-26 weeks after lesion, SKF-38393 inhibited SNpr firing rates (ave. decrease: 75 ± 10% below baseline, n=5). Time course differences do not account for these opposite effects: seven days after 6-OHDA lesion SKF-38393 did not induce effects similar to those seen after reserpine. MK-801 (0.15 mg/kg, iv), 5 min before SKF-38393, blocked both the stimulatory and the inhibitory effects of SKF-38393 in reserpinized and lesioned rats, respectively. Results suggest that monoamine depletion and dopamine cell degeneration both affect D1 neuronal transmission, but in opposite ways, and raise questions about which model is more relevant to PD. They also support a role for tonic NMDA transmission and point to considerable plasticity in D1 mechanisms regulating striatal output.

539.15

AN EEDQ RESISTANT SUBPOPULATION OF D1 DOPAMINE RECEPTORS MAY BE INVOLVED IN THE MEDIATION OF REPETITIVE JAW MOVEMENTS (RJM) IN RATS.
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New York,N.Y.10016
We have,in 1983,shown that an oral behavior in rats manifested by RJM and tongue protrusion,can be induced in a dose dependent manner by the D1 agonist SKF 38393 and abolished by specific D1 antagonist SCH 23390. Inactivation of D1 receptors with EEDQ reduces 80-85% of D1 dopamine receptor binding but has no significant effect on the rate of D1 agonist induced RJM. Scatchard analysis of caudate D1 receptor binding in control and EEDQ treated rats has revealed changes in density but not in affinity(Bmax 101.7+7.5 pmol/gm wet weight, Kd 1.16+0.21nM;and Bmax 16.5+2.7 Kd 0.90+0.13, respectively).We also wish to report that following a single injection of EEDQ,80-85% of D1 receptors is also lost in mpFc,olfactory tuberculum and substantia nigra. In the caudate the remaining population of D1 receptors is resistant to subsequent EEDQ challenge. In radioligand binding studies dopamine displacement of 3H-SCH 23390 binding to caudate homogenates resulted in 4 fold shift to the right(Allfit analysis)in the presence of 5.0 μMGpp(NH)p in control but not in EEDQ treated rats. It thus appears that RJM in rats may be mediated by a subpopulation of Gpp(NH)p-independent as well as EEDQ resistant D1 receptors in rats.

539.16

FUNCTIONAL COUPLING OF SOMATO-DENDRITIC AUTORECEPTORS IN THE RAT MESO-LIMBIC DOPAMINERGIC PROJECTION. S. Ahlenius. Department of Neuropharmacology, Astra Research Centre, S-151 85 Södertälje, Sweden.

Adult male rats were injected with the enantiomers of sulpiride (SULP)(0.2-5.0 μg) or 3-PPP (1.25-80 μg) into the nucleus accumbens or into the ventral tegmental area (VTA). These compounds present a range of intrinsic activities from a full DA agonist [(+)3-PPP], a partial agonist [(-)3-PPP] to an antagonist [(-)SULP]. In the accumbens region, SULP enantiomers and (-)3-PPP produced a suppression of locomotor activity (LMA), whereas (+)3-PPP appeared inactive. In the VTA, 3-PPP enantiomers and (+)SULP produced a suppression, whereas (-)SULP produced an increase, of LMA. These findings demonstrate that stimulation of somato-dendritic autoreceptors is of greater importance than stimulation of autoreceptors on nerve terminals for the suppression of LMA produced by systemically administered DA receptor agonists, and that a partial agonist like (-)3-PPP acts by a combination of post-synaptic receptor blockade and autoreceptor stimulation. Finally, a blockade of somato-dendritic autoreceptors by (-)SULP produced a stimulation of LMA, which may explain the behavioral activation that can be found after systemic administration of this or other DA receptor blocking agents, whereas the unexpected suppression of LMA produced by (+)SULP administration onto cell body autoreceptors suggests the possibility of weak intrinsic activity of this compound.

539.17

DO PRESYNAPTIC DOPAMINE AUTORECEPTORS INHIBIT DOPAMINE RELEASE VIA ACTIVATION OF POTASSIUM CHANNELS? Takahiko Tanaka*, Steven R.Vincent, George G. Nomikos and Hans C. Fibiger. Div. of Neurological Sciences, Dept. of Psych. Univ. of British Columbia, 2255 Wesbrook Mall, Vancouver, B.C., Canada, V6T 1Z3

Somatodendritic dopamine (DA) autoreceptors inhibit DA cell firing by activation of potassium (K^+) channels via a G protein mediated mechanism. However, the signal transduction mechanisms by which presynaptic DA autoreceptors in the striatum inhibit DA release are not known. The purpose of this study was to examine the role of K^+ channels in DA autoreceptor function in the striatum using in vivo microdialysis. Local application of the DA agonists quinpirole (QPR, 10 μM) and BHT-920 (1 μM) reduced extracellular DA in the striatum. This was blocked by the DA antagonist (-)-sulpiride (SLP, 10 μM), which itself increased extracellular DA. Local infusion of the K^+ channel blocker quinine (QIN, 1mM) caused a large increase in DA release which stabilized after 90 min. QIN completely blocked the SLP-induced DA increase but did not block the effect of QPR. SLP blocked the effect of QPR in both the presence and absence of QIN.

These results confirm that autoreceptors in the striatum regulate DA release. In addition, blockade of K^+ channels increases DA release. The findings suggest that endogenous DA inhibits DA release via a presynaptic receptor coupled to a K^+ channel. However, the results also indicate that DA receptor agonists act via additional mechanisms that are independent of K^+ channel activation to inhibit release.

539.18

BIOCHEMICAL EFFECTS OF (-)-STEPHOLIDINE ON THE PRESYNAPTIC DOPAMINE RECEPTORS. G.Z. Jin, L.J. Chen*, B.C. Sun*, X. Guo*, G. Hu* and H.Y. Huang*. Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 200031, China.

(-)-Stepholidine (SPD), is a novel DA receptor antagonist on postsynaptic DA receptors, but it possesses agonistic action on rotational behaviour in the 6-OHDA-lesioned rats. This work attempts to investigate whether SPD exhibits antagonistic or agonistic action on presynaptic DA receptors in the striatum. It has been shown that SPD profoundly augumented the activity of TH in rat with an elevation of DOPA accumulation measured by HPLC. SPD could not reverse the inhibition on DOPA-accumulation against the enhancement of GBL. SPD, however, could potentiate the effect of GBL. SPD increased the DA release and DOPAC level in rat measured by in vivo voltammetry. SPD enhanced the [^{3}H]-DA release from rabbit slice by electric stimulation. Quinpirole inhibited the [^{3}H]-DA release. The inhibition of AC activity by NO437 and the stimulation on high affinity GTPase activity by NO437 on rat synaptosome were antagonized by SPD. These results clearly denote that SPD possesses antagonistic effect on presynaptic receptors (D-2).

539.19

CHRONIC SCH 23390 ACCELERATES THE RECOVERY OF D1 DOPAMINE (DA) RECEPTORS IN THE RAT STRIATUM (ST) AND S. NIGRA (SN) AFTER IRREVERSIBLE BLOCKADE. O.Giorgi,M.G. Pibiri,M.R. Murgia and M.G.Corda. Department of Experimental Biology, Section of Neuroscience, University of Cagliari, Italy.

The present study was designed to investigate the repopulation of 3H-SCH 23390 binding sites in the ST and SN after the irreversible inactivation induced by N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ, 10 mg/kg, SC) in rats chronically treated with SCH 23390 (0.05 mg/kg, SC, three times a day for three weeks). The process of D1 receptor repopulation could be adequately described by a model that assumes a constant rate of receptor production (zero order) and a first order receptor degradation rate. As expected, the repeated administration of SCH 23390 significantly enhanced the steady state density of D1 DA receptors in the ST (+30%) and in the SN (+24%). Chronic treatment with SCH 23390 also increased the production rates of D1 receptors in the ST (control: 6.6 fmoles/mg prot/h, treated: 7.8 fmoles/mg prot/h) and in the SN (control: 1.2 fmoles/mg prot/h, treated: 1.8 fmoles/mg prot/h). By contrast, the receptor degradation rate constants were unchanged: ST control, 0.0073 ± 0.0007/h, ST treated, 0.0066 ± 0.0005/h; SN control, 0.0047 ± 0.0004/h, SN treated, 0.0056 ± 0.0005/h. These data are consistent with the view that an increase in the receptor production rate is at the basis of the upregulation of D1 receptors in the ST and in the SN induced by chronic SCH 23390. The acceleration in the receptor production rate may be due to an increased expression of the genes that control the production of D1 DA receptors.

539.20

DEVELOPMENTAL DIFFERENCES IN D_1 AND D_2 DOPAMINE RECEPTOR DEPLETION AND TURNOVER IN RATS AFTER IRREVERSIBLE ANTAGONIST TREATMENT. C.A. Crawford, J.K. Rowlett, S.A. McDougall*, N. Elkins* & M.T. Bardo. Department of Psychology & Center of Membrane Sciences, University of Kentucky, Lexington, KY 40506.

Previously, our lab reported developmental differences in the behavioral responses to treatment with the irreversible dopamine (DA) blocker, N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ). We found that preweanling rat pups did not show the adult-typical attenuation of dopamine mediated behaviors after EEDQ treatment. In the present study, we investigated potential age-dependent differences in DA receptor subtype depletion and subsequent receptor recovery after treatment with EEDQ. Rats received a single injection of EEDQ (7.5 mg/kg) or vehicle when 10, 16 or 39 days old. Rats were sacrificed and their striata removed for assay 24, 48, 96 or 192 hrs after EEDQ treatment. Saturation experiments were done using [^{3}H]SCH 23390 (for D_1 binding) and [^{3}H]spiperone (for D_2 binding) in tissue homogenates. Results showed that EEDQ produced an initial depletion of D_1 and D_2 receptors in all age groups. This effect tended to be less profound in both 10- and 16-day old rats when compared to the 39-day old rats. For all ages, evidence for receptor recovery was obtained.

539.21

STRIATAL DOPAMINE RECEPTOR AND DOPAMINE LEVEL DEPLETION IN NEONATAL AND ADULT RATS FOLLOWING IRREVERSIBLE RECEPTOR MODIFICATION J.K. Rowlett, C.A. Crawford, S.A. McDougall* & M.T. Bardo Department of Psychology, University of Kentucky, Lexington, KY 40506.

The present study assessed the effects of the receptor alkylating compound N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) on D_1 and D_2 dopamine receptors, dihydroxyphenylacetic acid (DOPAC) and dopamine (DA) levels in 16 day old and adult rat striatum. Dopamine receptor binding was assessed in homogenates using [^{3}H]SCH 23390 (for D_1 binding) and [^{3}H]spiperone (for D_2 binding). DOPAC and DA levels were measured using high-performance liquid chromatography. Results revealed that for both dopamine receptor subtypes, EEDQ induced a dose-dependent depletion that was of lesser magnitude in 16 day olds than in adults. DOPAC values for 16 day old rat pups showed no effect of EEDQ, while DOPAC levels were increased for adult rats. DA values also were not altered by EEDQ treatment in 16 day old rat pups. However, adult rats showed a significant decrease in DA levels at all doses tested (7.5, 15, 25 mg/kg). The EEDQ-induced decrease in DA levels in adults was also evident in animals pretreated with selective D_1 and D_2 antagonists. These results suggest that EEDQ may produce receptor-independent neurotoxicity, and that neonatal rats are less sensitive than adults to both the receptor inactivating and DA depleting effects of EEDQ.

539.22

LEAD (Pb) AFFECTS DOPAMINE D1 AND D2 RECEPTOR DEVELOPMENT IN A BIPHASIC AND REGIONALLY DIFFERENT MANNER IN STRIATUM AND NUCLEUS ACCUMBENS. **D.V. Widzowski, and D.A. Cory-Slechta. Environ. Health Sci. Center, School of Med. and Dent., Univ. of Rochester, Rochester, N.Y. 14642.**

Alterations in dopaminergic (DA) systems have been suggested as one neurochemical mechanism for Pb-induced behavioral deficits. Previous work has shown DA receptor number (Bmax) to be elevated in the striatum (ST), but decreased in the nucleus accumbens (NA) after postnatal Pb exposure, suggesting regional differences in response to lead. Work in this laboratory has demonstrated alterations in DA (D1 and D2) Bmax after either postnatal (birth to day 21) or postweaning (day 21 onward) Pb exposure with an apparent biphasic dose-effect function; postnatal Pb exposures produced inverse U-shaped functions, while postweaning Pb exposure produced U-shaped curves. The present studies were undertaken to further characterize the biphasic and regional effects of Pb on DA receptors. For postnatal (PN) exposure, lactating dams received 0, 100, 350, 1000 or 2000 ppm Pb lead acetate in drinking water; postweaning (PW) Pb exposures were 0, 50 or 250 ppm Pb acetate. Rats were sacrificed on 7,14,21 or 60 days of age for PN exposure, and on days 28 or 60 for PW exposure. Results confirmed biphasic effects of lead following both postnatal and postweaning Pb exposure. For Pn-exposed rats on day 7, both ST and NA exhibited inverse-U shaped dose-response functions for both D1 and D2 receptors, with ST curves somewhat left-shifted relative to NA, suggesting a more pronounced effect of Pb on striatum. At neither day 21 or 60 was there evidence of differential Pb accumulation in ST vs NA, suggesting that in PN-exposed rats, differential Pb levels were not the mechanism of the differential sensitivity. By 60 days of age in PN-exposed rats, U-shaped rather than inverse U-shaped Bmax curves were apparent, including a significant increase in D1 receptors at the highest exposure level. Pb was still present in a dose-related manner at day 60 in both ST and NA, suggesting Pb might still be actively affecting the tissue. **This work was supported by NIH grant ES05017.**

CATECHOLAMINES: DOPAMINE II

540.1

DOPAMINE ACTIONS ON MEMBRANE CONDUCTANCES IN RAT VENTRAL TEGMENTAL AREA (VTA) NEURONS. Z.G. Jiang and R.A. North. Vollum Institute, Oregon Health Sciences University, Portland, OR 97201

Whole cell recordings were obtained from 120 VTA neurons in rat midbrain slices. Pipets contained potassium as the main cation, and ATP and GTP. First, dopamine (DA 30 μM, EC_{50} = 22 μM) caused an outward current at -50 mV in 56% of the cells; this was typically about 50 pA. The current reversed to inward at -105 mV, close to E_K. The current was associated with an increase in instantaneous conductance at -60 mV of 2 nS (27% of control, n = 14). Second, a voltage-dependent sustained outward current that was sensitive to TEA (10 mM) and barium (100 μM) was increased by dopamine (by 18% at 0 mV). Third, dopamine reduced a hyperpolarization-activated cation current (I_h) (by 22% at -120 mV), with no obvious shift of its voltage-dependence. Dopamine had no effect on a transient outward current (I_A). Thus, dopamine inhibits VTA principal neurons both by increasing two potassium conductances and reducing I_h.

540.2

VOLTAGE-CLAMP ANALYSIS OF CALCIUM-DEPENDENT INWARD CURRENTS IN CULTURED MESENCEPHALIC DOPAMINE NEURONS. L.-X. Liu, G. Kapatos, and L.A. Chiodo. Cellular & Clinical Neurobiology Prgm., Dept. Psychiatry, Wayne State Univ. Sch. Med., Detroit, MI 48235.

DA neurons were identified by preloading with 5,7-DHT (15-50 μM for 1 hr) and visualization under UV fluorescence. This resulted in a blue-violet fluorescence within the soma and dendrites. To study only calcium (Ca^{2+})-dependent inward currents, the extracellular solution contained 140 mM tetraethylammonium Cl, 10 mM $CaCl_2$, 1 mM $MgCl_2$, and 10 mM glucose (pH 7.4). The patch pipette solution contained 10 mM EGTA, 135 mM CsCl, 1 mM $MgCl_2$, 1 mM GTP, 2 mM ATP, 14 mM creatine phosphate, 50 U/ml creatine phosphokinase. Both solutions were buffered to pH 7.4 with 10 mM HEPES.

Depolarizing voltage steps from a holding potential of -90 mV resulted in inward currents which were observed at membrane potentials between -40 and 20 mV. These currents were not observed when Ca^{2+} was removed from the external solution. The whole-cell current displayed an initial peak outward component which rapidly inactivated to a sustained current of lower amplitude which lasted for the duration of the voltage step (500 msec). This current response suggests the presence of at least two Ca^{2+} currents in cultured DA neurons, one which is transient and a second which is sustained. Studies designed to isolate and characterize these inward currents are in progress. It was noted that the presence of inward currents was correlated with the age of the cultures. Cultures were studied between 8 and 19 days, however, inward Ca^{2+} currents were only present in culture which were at least 13 days old. (Supported by MH41557 and NS26081).

540.3

EFFECTS OF PRENATAL ALCOHOL EXPOSURE ON SUBSTANTIA NIGRA DOPAMINE NEURONS. R. Shen, A.S. Freeman and L.A. Chiodo. Cellular & Clinical Neurobiology Prgm., Dept. Psychiatry, Wayne State Univ. Sch. Med., Detroit, MI 48235.

Fetal Alcohol Syndrome is a disorder which is often associated with altered spontaneous motor activity and increased behavioral impulsivity. Some of the effects detailed in the literature are reminiscent of behaviors thought to be mediated, in part, by dopaminergic (DA) neuronal systems within the brain. In an attempt to better understand the neurobiological underpinnings of this syndrome, we have begun to study the effects of *in utero* exposure of rats to ethanol on the subsequent physiology and pharmacology of mesencephalic DA neurons in adulthood. Pregnant rats received a liquid diet containing 0%, 17.5%, and 35% ethanol-derived calories from day 8 of pregnancy until delivery. Total caloric intake was the same for all groups. At birth, pups were transferred to foster dams.

Single-unit extracellular recordings were made from identified DA neurons located within the substantia nigra, two to three months after weaning. DA neurons in rats treated with 17.5% and 35% ethanol exhibited slightly higher spontaneous firing rates and burst-related action potentials compared to controls. DA neurons in these rats also were dramatically less sensitive to the inhibitory effect of intravenous administration of the mixed D1/D2 receptor agonist apomorphine. This was observed as both a significant increase in the ED_{50} (144 µg/kg for the combined 17.5% and 35% ethanol groups *vs.* 16 µg/kg in controls) as well as a dramatic reduction in efficacy, suggesting a functional down-regulation of DA receptors. Thus, prenatal exposure of rats to ethanol affects the physiology and pharmacology of nigral DA neurons ultimately observed in the adult animals. (MH41557 and P50 AA07606).

540.4

EFFECTS OF SIGMA LIGANDS ON THE POPULATION ACTIVITY OF MIDBRAIN DA NEURONS. J. Zhang[1], L.A. Chiodo[1], J.G. Wettstein[2] and A.S. Freeman[1]. [1]Department of Psychiatry, Wayne State University School of Medicine, Detroit, MI 48201 and [2]Institut de Recherche Jouveinal, 94265 Fresnes, France.

Sigma receptors have been hypothesized to play a role in the etiology of psychosis and in the therapeutic effectiveness of antipsychotic drugs. Several sigma ligands also produce psychotomimetic effects although the receptor specificity of these responses are in question. Midbrain A9 and A10 dopaminergic (DA) systems are believed to mediate the side effects and therapeutic effects, respectively, of antipsychotic drugs. In the present study, we evaluated the effects of three sigma ligands, (+)-pentazocine (PZ), DTG and JO 1784, on the numbers of spontaneously active A9 and A10 DA neurons encountered per electrode track in anesthetized rats.

Acute injections were given 1 hr prior to electrophysiological recordings. Chronic treatment consisted of 21 daily injections with a 20 hr withdrawal. Each treatment group contained 10 rats. Acute i.p. PZ (10 mg/kg), DTG (2 mg/kg) and s.c. JO 1784 (1 and 10 mg/kg) did not alter the numbers of spontaneously active DA neurons per electrode track. Chronic treatment with PZ (1 and 10 mg/kg) or DTG (0.2 and 2 mg/kg) increased the numbers of A10 DA cells per track. At 1 mg/kg, chronic JO 1784 had no effect on the number of DA cells per track. At 10 mg/kg, chronic JO 1784 moderately decreased the number of active A9 DA cells per track and increased the firing rate of A10 DA cells. Thus, repeated but not acute treatment with these sigma ligands altered the population profile of active DA neurons. These effects differ from those of typical and atypical antipsychotic drugs which inactivate A10 DA neurons upon chronic administration. (Supported by MH42136.)

540.5

RELEASE OF CHOLECYSTOKININ FROM RAT MIDBRAIN SLICES. A.S. Freeman, L.A. Chiodo, S.I. Lentz, K. Wade and M.J. Bannon. Department of Psychiatry, Wayne State University School of Medicine, Detroit, MI 48201.

Cholecystokinin octapeptide (CCK-8) is present within a majority of rat midbrain dopamine (DA)-containing neurons. Exogenous CCK-8 can modulate DA neuronal electrophysiology, in part, by direct actions on the somatodendritic region of these cells. Thus, if CCK-8 is released somatodendritically from DA neurons, it may influence DA cell function as has been shown for DA itself. In the present study, RIA (sensitivity, 1 fmole/tube) was used to measure the *in vitro* release of CCK-8 from midbrain slices (400 µm). The slices obtained from a 2 mm coronal slab of trimmed rat midbrain were used in each assay tube. All CCK immunoreactivity was established by HPLC to be authentic CCK-8.

Low levels of CCK-8 were detected in the basal incubation medium. Thirty mM potassium caused about a 3-fold increase in the release of CCK-8. This stimulated release was abolished in calcium-free medium. The D2 DA receptor agonist quinpirole (1-100 µM), but not the D1 agonist SKF 38393 (10-100 µM), attenuated the potassium-stimulated release of CCK-8 without altering basal release. The quinpirole effect was prevented by the D2 antagonist l-sulpiride (10 µM) and was not affected by concurrent incubation with SKF 38393 (10 µM). These results show that CCK-8, like DA, can be released from midbrain slices, presumably from DA/CCK-8-containing neurons. This finding is in accordance with the possibility that CCK-8 plays a role in the regulation of DA neuronal function at the level of the cell body, where it might influence DA cell excitability. (Supported by MH41557, MH42136 and MH43026.)

540.6

DOPAMINE NEURON ONTOGENY: MORPHOLOGICAL AND ELECTROPHYSIOLOGICAL CHARACTERISTICS. D.K. Pitts[1,2], J. Rubin[2], A.S. Freeman[2] and L.A. Chiodo[2]. [1]Dept. of Pharm. Sci., Coll. Pharmacy & A.H.P. and the [2]Cell. & Clin. Neurobiol. Prgm., Sch. Med., Wayne State Univ., Detroit, MI 48202.

Antidromically (AD) identified rat nigrostriatal dopamine (DA) neurons undergo significant physiological and pharmacological changes during postnatal (PN) development (Pitts et al. Synapse 6:309, 1990; Tepper et al. Dev. Brain Res. 54:21, 1990). The distribution of tyrosine hydroxylase-immunopositive (TH+) neurons and the electrophysiological characteristics of AD identified mesoaccumbens DA (MADA) neurons during PN development were examined in the present study. As previously described (Tepper et al. Neurosci. Abstr. 431:11, 1990), on PN day (PND) 1 TH+ neurons and fibers were found scattered throughout the substantia nigra with a subsequent reduction in TH+ somata/fibers ventrally by PND 14 resulting in the appearance of a more adult-like non-DAergic pars reticulata. In addition, at PND 1 separate posterior-lateral and anterior-medial groups of TH+ neurons were observed which may ultimately contribute to the adult mesencephalic DA neuron population. Preliminary studies of AD identified MADA neurons at PND 14-16 suggest that there are fewer cells with a bursting discharge pattern and that the burst duration is shorter relative to adullts. (Supported by MH47857 [DKP], MH42136 [ASF], MH41557 [LAC].)

540.7

EFFECTS OF 8-HYDROXY-2-(DI-N-PROPYLAMINO)TETRALIN (8-OH-DPAT) ON THE ELECTRICAL ACTIVITY OF DOPAMINE NEURONS IN THE RAT VENTRAL TEGMENTAL AREA. E. Esposito, M. Pessia* and S. Prisco*. Istituto di Ricerche Farmacologiche "Mario Negri", Consorzio "Mario Negri" Sud, S. Maria Imbaro (CH), Italy.

A number of studies have shown that serotonin (5-HT) containing neurons in the raphe nuclei exert an inhibitory influence upon dopamine (DA) neurons in the substantia nigra, pars compacta (SNc). The selective 5-HT1A receptor agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) has been shown to increase the firing rate of DA neurons in the SNc, probably as a consequence of its inhibitory action on 5-HT containing neurons in the dorsal raphe nucleus. Neuroanatomical data indicate that DA neurons in the ventral tegmental area (VTA) receive a serotoninergic input from the raphe nuclei, however the functional role of serotonin in the control of the activity of mesolimbic DA system is still unclear.

In the present study, extracellular single unit recordings were performed in vivo on male Sprague Dawley rats, anesthetized with chloral hydrate. Dopamine neurons in the VTA were recorded using single barrel micropipettes and were identified by their location, waveform, firing rate and pattern. Intravenous injection of 8-OH-DPAT (10-160 µg/Kg; cumulative dose) caused excitation in about 40% of the neurons studied; in 20% of the cells recorded a slight inhibition was observed; the remaining cells were unaffected by 8-OH-DPAT. These findings indicate that the activity of a subpopulation of DA neurons in the VTA is under the control of the serotoninergic system. Experiments are under way to establish whether the effect of 8-OH-DPAT on VTA DA neurons is mediated by an inhibitory action on 5-HT-containing neurons in the nucleus raphe dorsalis (DR) and/or medianus (MR).

540.8

GAMMA-HYDROXYBUTYRATE INCREASES THE FIRING RATE OF A9 DOPAMINE NEURONS IN UNANESTHETIZED RATS.
M. Diana, A. Mura, A.L. Muntoni, M. Pistis, S. Aramo & G.L. Gessa.
Dept. of Neurosci. "B.B. Brodie", Univ. of Cagliari, ITALY.

Gamma-hydroxybutyrate (GHB) is a normal metabolite of the mammalian brain and is found in discrete cerebral regions such as the substantia nigra (SN). However GHB receptors are virtually absent from the SN while they are found in the striatum and hyppocampus. For these and other reasons GHB is a molecule of great interest especially in light of recently reported evidence which ascribes to GHB a role in the treatment of alcohol abuse (Gallimberti et al 1990 Lancet). Since electrophysiological effect of GHB have always been explored in anesthetized rats and anesthesia alters many drug-induced effects we studied the effect of GHB in unanesthetized rats. Male Sprague-Dawley (200-350 gr) rats were temporarily anesthetized with alothane then incannulated with a tracheal catheter for artificial respiration and in the femoral vein for intravenous administration of drugs. D-tubocurarine (4mg/kg iv) was administered and once muscular paralysis was obtained the rats were placed on a stereotaxic frame (Kopf) Low doses of GHB (50-400 mg/kg) dose dependently increased the firing rate of A9 dopaminergic neurons from about 10 to about 50% above baseline. When GHB was administered intraperitoneally in higher doses (750 mg/kg) only a mild reduction of firing rate was observed. In contrast, in chloral hydrate anesthetized rats, 200 mg/kg of iv GHB failed to modify neuronal activity while 400 mg/kg iv completely suppressed firing rate. The results indicate: 1) anesthesia alters the electrophysiological effects of GHB. 2) GHB mimics the effects of ethanol upon dopaminergic neurons in both unanesthetized and anesthetized rats.

540.9

Effects of Coadministration of SCH 23390 and SKF 38393 with Haloperidol on the Number of Spontaneously Active Dopamine Neurons in the SNC and VTA. D.E. Bregna*, S.E. Degelmann*, M.R. Szewczak. Dept. of Biological Research, Hoechst-Roussel Pharmaceuticals Inc., Somerville, N.J. 08876.

Extracellular single unit recordings were used to determine the effect of acute or chronic coadministration of either the D1 antagonist, SCH 23390, or the D1 agonist, SKF 38393, with the D2 antagonist, haloperidol on cell populations of the SNC and VTA areas in anesthetized rats. Acute administration of haloperidol caused a significant increase in active DA cells in both SNC and VTA; chronic 21 day administration resulted in a significant decrease in both areas. Acute and chronic treatment with SCH 23390 or SKF 38393 had no effect on the number of active DA neurons in either SNC or VTA. Acute coadministration of either SCH 23390 or SKF 38393 with haloperidol resulted in increases in both SNC and VTA similar to results seen with haloperidol alone. Chronic coadministration of SCH 23390 at 5 mg/kg or SKF 38393 at 3 mg/kg with haloperidol at 0.5 mg/kg resulted in a significant decrease in the number of active DA cells in both SNC and VTA similar to the effect of haloperidol alone. However, chronic coadministration of SKF 38393 at 10 mg/kg with haloperidol at 0.5 mg/kg resulted in a selective decrease in the number of spontaneously active DA neurons in the VTA and not the SNC. Thus, it appears that chronic stimulation of D1 receptors with SKF 38393 prevents haloperidol induced depolarization blockade of DA neurons in the SNC. These results substantiate the idea that stimulation of D1 receptors play a significant role in modulating expression of the D2 receptor.

540.10

DOPAMINE ACTIONS ON NUCLEUS ACCUMBENS NEURONS: WHOLE CELL RECORDING IN CULTURE. W.-X. Shi and S. Rayport. Dept. Psychiatry and Ctr. Neurobiol. & Behav., Columbia Univ.; Dept. Neuropathology, NYS Psychiatric Institute, NY 10032.

To examine the possible actions of dopamine (DA) at mesolimbic synapses, we made perforated-patch or whole cell tight seal recordings from cultured nucleus accumbens neurons. We used an intracellular solution containing 140 mM KGluconate, 0.1 $CaCl_2$, 2 $MgCl_2$, 1 EGTA, 2 ATP, 0.1 GTP, and 10 HEPES, pH 7.25. Neurons were dissociated from neonatal rats and maintained *in vitro* for up to 3.5 months. DA (50-100 μM) was applied by local perfusion to 74 cells; 31 cells responded to DA. Of these cells, 26% were hyperpolarized or showed an outward current under voltage clamp that reversed at more hyperpolarized levels and was due to an increase in membrane conductance. In the other 74% of DA-responding cells, DA produced either depolarization or an inward current that was diminished at hyperpolarized levels but could not be reversed.

In the same cells, we also examined the modulatory effect of DA on synaptic interactions between nucleus accumbens cells; 47 of the 74 cells received synaptic input from other neurons. Of these cells, 40% showed spontaneous EPSPs, 21% showed IPSPs, and the remainder showed both. In 20% of those cells which received PSPs, DA (100 μM) enhanced synaptic input by increasing PSP frequency, while in 12% of the cells, DA reduced PSP frequency or PSP size. These results reinforce the notion that DA has a wide range of actions in the nucleus accumbens, likely reflecting the activation of different DA receptors.

540.11

BLOCKADE OF SOMATODENDRITIC AUTORECEPTORS ON NIGRAL DOPAMINE NEURONS CONTRIBUTES TO THE FIRING RATE-INCREASING EFFECTS OF DOPAMINE ANTAGONISTS. M.L. Pucak and A.A. Grace. Depts. of Behavioral Neuroscience and Psychiatry, Center for Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA 15260.

Systemic administration of the dopamine (DA) antagonist haloperidol (HAL) increases the firing rate of nigral DA neurons. This excitation has been suggested to result from the blockade of DA receptors located on striatal neurons projecting to the substantia nigra. HAL also may exert local effects within the nigra by blocking somatodendritic autoreceptors which are located on the DA neurons themselves, thus removing a tonic inhibition mediated by dendritically released DA; however, the importance of this mechanism is controversial. We have examined the relative significance of these two mechanisms by comparing the response of nigral DA neurons to intravenously administered HAL in anesthetized intact rats with the response observed in rats with hemisections of the striatonigral projection. In control rats, HAL (cumulative dose=4-6.4 mg/kg) caused >20% increases in firing rate in 4/5 DA neurons (average increase=24.8±6.1%). After hemisections, such increases were observed in 3/3 DA neurons (average increase=42.2±14.6%). Thus, the striatonigral projection does not entirely mediate the HAL-induced excitation of DA neurons, suggesting that HAL acts at least partially via blockade of DA receptors within the substantia nigra. In order to assess whether the local effects of HAL were mediated via blockade of D_2 DA cell autoreceptors or via blockade of D_1 receptors located on the terminals of the striatonigral projection, the D_2-specific DA antagonist sulpiride was administered. Sulpiride at a dose of 32 mg/kg was less effective than HAL at increasing DA neuron firing rate in intact rats; 2/5 DA neurons recorded from control rats showed >20% increases in firing rate upon administration of sulpiride (average increase=11.6±4.9%). However, 2/2 DA neurons recorded from rats with hemisections of the striatonigral projection showed >20% increases in firing rate (average increase=37.4±14.9%). Thus, blockade of somatodendritic autoreceptors appears to make a significant contribution to the increased firing rate of DA neurons in reponse to systemic administration of DA antagonists, suggesting that at least some DA neurons are tonically inhibited by dendritically released DA. Supported by USPHS MH45156, NS19608, and MH09873 (to MLP).

540.12

ON THE EFFECTS OF THE DOPAMINE D3-RECEPTOR PREFERRING AUTORECEPTOR ANTAGONISTS (+)-AJ76 AND (+)-UH232 ON DOPAMINE RELEASE AND METABOLISM. Nicholas Waters, Montford Piercey# and Arvid Carlsson*.Dept. of Pharmacology, Univ of Göteborg P.O box 33031, S-400 33 Gothenburg, Sweden, # CNS research, The Upjohn Company, Kalamazoo, MI 49001, U.S.A

Recently, the existence of a third dopamine (D3) receptor (Sokoloff et. al. 1990) was revealed. The DA autoreceptor antagonists (+)-AJ76 and (+)-UH232 were shown to possess the highest preference for the D3 site of all DA antagonists screened. (+)-AJ76 and (+)-UH232 induce weak locomotor stimulation. Like classical dopamine receptor antagonists (+)-AJ76 and (+)-UH232 blocks the stimulatory effects of apomorphine and d-amphetamine. In biochemical experiments (+)-AJ76 and (+)-UH232, increase the synthesis and metabolism of dopamine in the striatal and limbic brain regions. However, neither (+)-AJ76 nor (+)-UH232 produces hypomotility or catalepsy. In this study we compare the effects of these two compounds on DA release and metabolism in the nucleus accumbens and in the caudate nucleus with raclopride and haloperidol, in the microdialysis model. We show that (+)-AJ76 is more effective on DA release than raclopride(max increase ≈200% of ctrl.) and haloperidol(max increase ≈200% of ctrl.). We found that (+)-AJ76 has a limbic preference, maximal DA release in n.accumbens is ≈500% but in the n.caudate ≈300%. The limbic preference is confirmed in electrophysiological studies, ED50 in VTA is 148 μg/kg i.v. and in the SNPC 316 μg/kg. (+)-UH232 releases dopamine in a fasion more like classical dopamine antagonists (maximal DA incerease ≈200% of controls) and we show no limbic preference for this compound.

Sokoloff, P., Giro, B., Martres, M.-P., Bouthenet, M.-L. & Schwartz, J.-C. Nature (1990) 347, 146-151

540.13

THE DOPAMINE (D3 - PREFERRING) AUTORECEPTOR ANTAGONISTS (+)-AJ76 AND (+)-UH232 PRODUCE CONDITIONED PLACE-PREFERENCE IN THE RAT. Kjell Svensson , Marianne Thorngren *, Lena Wollter ,* and Arvid Carlsson * Department of Pharmacology, Univ. of Göteborg, P.O. Box 33031, S-400 33 Göteborg, Sweden

The preferential dopamine (DA) autoreceptor antagonists (+)-AJ76 and (+)-UH232 increase the synthesis and turnover of DA in brain limbic and striatal regions (Svensson et al., Arch. Pharmacol., 334:234, 1986). Sokoloff et al.(Nature, 347: 146, 1990) have recently shown that they possess a high preference for the DA D3 receptors. In contrast to classical DA receptor blockers, (+)-A J76 and (+)-UH232 produce a mild behavioral arousal over a wide dose range in habituated rats. Interestingly , in animals displaying a high baseline activity (e.g. d-amphetamine or cocaine-pretreatment), (+)-UH232 did not produce an excessive stimulation but blocked the hyperactivity. Various behavioral models were used in order to investigate the possible positive reinforcing properties . Both compounds were shown to be inhibitory in the intracranial self-stimulation paradigm (Kling-Petersen et al., this meeting) and (+)-AJ76 but not (+)-UH232 partially generalized to the to the cocaine cue (Hoffmann et al., this meeting). In the present study, we investigated the effects of (+)-AJ76 and (+)-UH232 in a three compartment conditioned place preference (CPP) paradigm. A clearcut dose-dependent (12.5-52 μmol/kg, sc) place preference was observed for both (+)-AJ76, (+)-UH232 and d-amphetamine (0.25-4 mg/kg, sc). Caffeine produced a weak preference at 5 mg/kg, sc but was inactive at 20 mg/kg , sc . We are at present studying the effects of other weak stimulants (such as DA reuptake inhibitors) and also the interactions of the preferential D3 receptor antagonists with raclopride, d-amphetamine and cocaine in the CPP paradigm. (Supported by The Upjohn Company, Kalamazoo, MI).

540.14

EFFECTS OF THE DOPAMINE (D3- PREFERRING) AUTORECEPTOR ANTAGONIST (+)-AJ76 AND OTHER WEAK CENTRAL STIMULANTS IN THE INTRACRANIAL SELF-STIMULATION MODEL. Torben Kling-Petersen and Kjell Svensson, Dept of Pharmacology, Univ of Göteborg, POB 330 31, 400 33 Göteborg, SWEDEN

The preferential dopamine autoreceptor antagonist (+)-AJ76, exerts a weak stimulatory effect when tested in locomotor activity experiments using habituated animals. However, (+)-AJ76 also blocks d-amphetamine induced hyperactivity. Thus, the behavioral effects of (+)-AJ76 is highly dependent on the baseline activity of the animal. Interestingly, Sokoloff et al. (Nature, 347, pp:146-151 (1990)) recently showed that (+)-AJ76 possesses a high preference for the newly cloned dopamine D3 receptor.

Various behavioral models have been utilized in order to investigate possible positive reinforcing properties of (+)-AJ76. In the intracranial self-stimulation (ICSS) paradigm in the rat, bipolar electrodes aimed at the median forebrain bundle delivers mono-phasic, cathodal current of varying intensities. When testing (+)-AJ76 in the ICSS paradigm, the results depend on the ICSS method employed: In a response rate independent threshold determination method, (+)-AJ76 (0.9-52.0 μmol/kg, s.c.) shifts the response curve to the right, ie acts inhibitory. The subthreshold method is designed to pick up weak stimulatory effects. It presents the animal to a non-rewarding current intensity, but can produce increased lever pressing when combined with a stimulant. In this model (+)-AJ76 exhibits weak stimulatory properties in 50% of the animals tested.While being mainly inhibitory in the ICSS model, (+)-AJ76 was shown to partially generalize to the cocaine cue (Hoffman et al, this meeting) and to produced conditioned place preference in the rat (Svensson et al, this meeting). We are presently investigating the interactions of (+)-AJ76 with cocaine, d-amphetamine and the effects of the DA reuptake inhibitors nomifensine and GBR-12909 in the ICSS paradigm. (Supported by The Upjohn Company, Kalamazoo, MI.)

540.15

ANTAGONISM OF SYNTHESIS MODULATING DOPAMINE AUTORECEPTORS IN VITRO BY (+)-AJ76, (+)-UH232 AND PD-128483. M.P. Galloway, E.A.Novak*, B.N.Mathews* Cellular & Clinical Neurobiology Program, Lafayette Clinic and Dept. Psychiatry, Wayne State Univ Sch Med, Detroit MI

Dopamine (DA) synthesis can be regulated in situ by stimulation of nerve terminal DA autoreceptors (AR). Using striatal brain slices, we have examined the ability of different classes of DA antagonists to affect the inhibition of DA synthesis induced by DA agonists in vitro. Stimulation of DA ARs was measured as the inhibition of K^+ stimulated DOPA accumulation by a fixed dose of either quinpirole or 7-OHDPAT. The rank order for reversal of 1 μM QUIN was (+)-UH232 > (+)-AJ76 = clozapine > (-)sulpiride. Although (+)-AJ76 was equipotent with CLOZ against QUIN, (+)-AJ76 was more efficacious than CLOZ when tested against 7-OHDPAT (1 μM). PD 128483 (10 μM) partially reversed the effect of QUIN under these conditions. Under basal conditions (K^+ = 5 mM) to assess agonist characteristics, only (-)-UH242 and PD 128483 and (-)-3PPP, but not (+)-AJ76, (+)-UH232, or (+)-UH242, exhibited AR agonist properties. Under depolarizing conditions (K^+ = 30mM), neither PD 128483 nor (-)-3PPP inhibited DOPA accumulation. Compounds do not interact with the DA transporter since neither PD 128483 nor the aminotetralins alone increased extracellular DA and none of the aminotetralins (3 μM) blocked the amphetamine (3 μM) induced release of DA. The effects of PD 128483 at DA autoreceptors appear to mimic those of the partial agonist (-)-3PPP. Furthermore, our results confirm that (+)-AJ76 and (+)-UH232, putative D-3 receptor antagonists, are antagonists at synthesis modulating DA AR's. Supported by DA-04120 and Michigan Department of Mental Health.

540.16

DECREASED SENSITIVITY OF APOMORPHINE (APO) FOR STRIATAL DOPAMINE (DA) AUTORECEPTORS IN TRANSGENIC HPRT⁻ MICE. D. Tang*, K. Harada*, K.Y. Lee, M. Goldstein and M.R. Kuehn*. Neurochem. Res. Lab., New York Univ. Med. Ctr, N.Y., N.Y. 10016 and Dept. for Genetics, Univ. Ill. Coll. Med., Chicago, Ill. 60612

To determine whether decreased striatal DA levels and increased DA turnover in transgenic HPRT⁻ mice is associated with sensitivity changes of DA agonists for DA autoreceptors, we have analyzed the effects of Apo on the γ-butyrolactone (GBL)-elicited enhanced synthesis of striatal Dopa. Basal striatal Dopa synthesis following inhibition of aromatic L-amino acid decarboxylase by NSD 1015 is not altered in HPRT⁻ mice as compared with HPRT⁺ controls. The cessation of dopaminergic impulse flow by administration of GBL increases the striatal Dopa synthesis to the same extent in HPRT⁻ and in control mice. Administration of the DA agonist Apo (0.1 mg/kg) reverses the GBL-elicited enhanced synthesis of striatal Dopa, but the % reversal is significantly lower in the transgenic HPRT⁻ mice than in controls. These results suggest that DA autoreceptors which regulate synthesis and release of DA are desensitized in HPRT⁻ mice. It is postulated that desensitization of DA autoreceptors might be responsible for the increased turnover of striatal DA in HPRT⁻ mice and may play a role in the pathophysiology of Lesch-Nyhan disease. Supported by NIMH 02717 and NINCDS 06801.

540.17

1-(SPIRO-1'-ALKYLPHENYL)BENZAZEPINES, NOVEL DOPAMINE D1 RECEPTOR SELECTIVE ANTAGONISTS. Paul P. Ehrlich*, James R. Campbell*, Robert Schoenleber*, Donald R. Britton, Robert MacKenzie and John W. Kebabian. Neuroscience Research D47U, Pharmaceutical Discovery, Abbott Laboratories, Abbott Park, Il 60064

Various 7-substituted-8-hydroxy-3-methyl-1-(spiro-1'-indan) and (spiro-1'-1',2',3',4'-tetrahydronaphthalene)-2,3,4,5-tetrahydro-1H-3-benzazepines were synthesized and evaluated as D1 antagonists.

The *in vitro* D1 and D2 binding assays and D1 functional activity were assessed according to previously established protocol.

The *in vivo* ED50 values were obtained by use of a behavioral assay which was based on the 6-hydroxydopamine unilaterally lesioned rat rotational model.(U. Ungerstedt and G.W. Arbuthnott, *Brain Research*, **1970**, *24 :* 485). Antagonist activity was measured by the ability of the test compound to block rotation induced by stimulation by A68930, a selective D1 agonist, (M.P. DeNinno et. al. *J. Med. Chem.* **1990**, *33* : 2948), by simultaneous subcutaneous injection of test drug.

Of the compounds generated four of the most promising candidates were shown to be the 7-bromo-spiro-1'-indan, 7-bromo-spiro-1'-naphthalene, and the corresponding 7-methoxy derivatives. These showed excellent *in vitro* activity, however, only the spiro-1'-indans showed good *in vivo* activity.

540.18

THE D1 DOPAMINE RECEPTOR ANTAGONIST SCH 23390 EXERTS AGONIST-LIKE EFFECTS ON RAT NUCLEUS ACCUMBENS NEURONS. S.R. Wachtel and F.J. White. Wayne State Univ. Sch. Med., Dept. Psychiatry, Cellular and Clinical Neurobiology Program, Neuropsychopharmacology Laboratory, Lafayette Clinic, Detroit, MI 48207

Previous behavioral and electrophysiological studies have demonstrated that acute dopamine (DA) depletion abolishes many effects of D2 DA receptor agonists unless D1 agonists are also administered. This enabling role of D1 DA receptor stimulation has also been indicated by the ability of D1 antagonists to prevent the unconditioned behavioral effects of D2 agonists. The present experiments investigated the ability of the selective D1 DA receptor antagonist SCH 23390 to alter the inhibitory effects produced by iontophoretic administration of the selective D2 DA receptor agonist quinpirole on rat nucleus accumbens (NAc) neurons. In contrast to its effects on D2 agonist-elicited behaviors, systemic administration of SCH 23390 (0.5 mg/kg, i.v.) failed to block subsequent inhibition of NAc neurons produced by iontophoretic administration of quinpirole (10 mM, 8-32 nA). Similarly, iontophoretic administration of SCH 23390 (50 mM, 5-40 nA) failed to block the inhibition of NAc neurons by iontophoretic quinpirole. In fact, SCH 23390 further inhibited the firing of NAc neurons. When administered alone, iontophoretic SCH 23390 produced a current-dependent inhibition of NAc activity similar to that observed with the D1 agonist SKF 38393. Furthermore, like D1 agonists, low iontophoretic currents of SCH 23390 (4 nA) "enabled" the inhibitory effects of quinpirole in rats acutely depleted of DA by pretreatment with the tyrosine hydroxylase inhibitor α-methyltyrosine. These finding are similar to previous behavioral reports indicating that low doses of SCH 23390 elicit D1 agonist-like grooming behavior in rodents. (Supported by MH-40832 and DA-04093)

540.19

ELIMINATION OF D1 AND D2 DOPAMINE (DA) RECEPTOR INTERACTIONS IN THE RAT STRIATUM BY CHRONIC BLOCKADE OF EITHER D1 OR D2 RECEPTORS X.-T. Hu and F.J. White. CCN Program, Dept. Psychiat., Wayne St. Univ. Sch. of Med., Lafayette Clinic, Detroit, MI 48207

Electrophysiological and behavioral studies were conducted to determine whether coincident supersensitivity of D1 and D2 receptors is necessary for elimination of the D1 and D2 receptor synergistic interactions within the dorsal (caudate-putamen, CPu) and the ventral (nucleus accumbens, NAc) striatum of rats. Selective supersensitivity of D1 or D2 receptors was induced by daily repeated administration (s.c.) of the selective D1 antagonist SCH 23390 (SCH) or the selective D2 antagonist eticlopride (0.5 mg/kg each) for 3 weeks with 1 week drug withdrawal. Single cell recordings indicated that the inhibitory responses of CPu and NAc neurons to iontophoresis of the selective D1 agonist SKF-38393 (SKF) (but not the selective D2 agonist quinpirole) were significantly enhanced in SCH (but not saline or eticlopride)-pretreated rats. In contrast, the inhibitory effects of quinpirole (but not SKF) were significantly enhanced in eticlopride (but not saline or SCH)-pretreated rats. In addition, while SKF usually potentiates the quinpirole-induced inhibition on CPu and NAc activity in rats with normosensitive DA receptors, such synergistic action was eliminated in either D1 or D2 sensitized rats. Also, the necessity of D1 receptor activation for D2 receptor-mediated neuronal inhibition, which is typically observed in rats acutely depleted of DA, was abolished following D2 receptor supersensitivity. Behavioral experiments indicated that the stereotyped responses to acute administration of quinpirole were significantly enhanced following chronic blockade of D2 receptors. Although acute DA depletion significantly reduced quinpirole (2 mg/kg, s.c.)-elicited stereotypies in saline-pretreated controls, supersensitive stereotyped responses to this D2 agonist remained in eticlopride-pretreated AMPT rats. These findings indicate that either D1 or D2 receptor supersensitivity affects the synergistic interactions of adjacent D1 and D2 receptors located on rat striatal neurons (Supported by USPHS Grants DA-04093 and MH-40832 to FJW).

540.20

EVIDENCE FOR DOPAMINE D2 AUTORECEPTORS IN NEONATAL AND INFANT RAT PUPS. C.A. Moody, H. Rabine*, L.P. Spear, Dept. of Psychology and Center for Developmental Psychobiology, SUNY, Binghamton, NY 13902-6000.

The presence of functional dopamine (DA) D2 autoreceptors was assessed in neonatal (postnatal day 3-P3) and infant (P10) rat pups. 35 min prior to sacrifice pups received a 750mg/kg s.c. injection of gamma-butyrolactone (GBL) to block impulse flow in DA neurons. 30 min prior to sacrifice all pups received a 100mg/kg i.p. injection of the dopa decarboxylase inhibitor 3-hydroxybenzylhydrazine, with pups in the quinpirole group receiving a s.c. injection of 0.5mg/kg quinpirole immediately thereafter. Following decapitation, forebrain and striatal regions were dissected on ice from P3 and P10 brains, respectively, and frozen for later assessment of DOPA concentrations via HPLC/ec. At both ages GBL treatment increased DOPA levels, with this increase being blocked by quinpirole. This agonist induced reduction in DOPA levels provides evidence that D2 presynaptic autoreceptors are functional in terms of inhibiting DA synthesis in young animals. In addition, preliminary findings suggest that the D1 agonist SKF-38393 may also inhibit DA synthesis early in life. Further investigations are being conducted to determine if this is indeed an apparent D1 autoreceptor effect or is merely a function of direct endproduct inhibition by this catechol compound.

540.21

BHT 920 SELECTIVELY STIMULATES A SUBTYPE OF D-2 RECEPTORS COUPLED WITH VOLTAGE-DEPENDENT K^+CHANNELS.
A. Valerio*, M. Pizzi, M.O. Carruba*, S. Espositi*, M. Benarese*, M. Memo, P.F. Spano. Dept. Biom. Sci. & Biotech. , Section Pharmacol. Exper. Ther. School of Medicine, Univ. Brescia, I-25124.

Radioactive rubidium efflux was used to measure potassium (K^+) permeability in a study designed to assess both the presence and the sensitivity to ions and drugs of the K^+ channels located in the presynaptic nerve terminals of rat striatum.Our data provide evidence for the existence of at least two different K^+ channels which participate differently in the regulation of K^+ fluxes under both resting and stimulated conditions. The two different components of K^+ fluxes were found on the basis of their kinetic properties and sensitivity to calcium ions. We also found that both the K^+ channels present in the isolated nerve terminals are sensitive to dopamine and various, but not all, D-2 dopaminergic drugs. In particular, the azepine derivative BHT 920 discriminates between the two channels behaving as selective opener of the voltage -dependent, calcium-insensitive K^+ channels. Our results support the conclusion that presynaptic nerve terminals of rat striatum possess two distinct categories of K^+ channels: a voltage-dependent and a calcium-activated K^+ channel. The two different K^+ channels serve as effectors of two distinct subtypes of dopamine D-2 receptors.

540.22

NEUROCHEMICAL EFFECTS AND SELECTIVE DISTRIBUTION OF AMISULPRIDE IN THE MESOLIMBIC DOPAMINERGIC SYSTEM AFTER CHRONIC TREATMENT.
M.C Maubrey*, A.M. Gardier, J.H. Trouvin, J. Margarit* and C. Jacquot, Fac Pharmacie, SDI 6313, 92296 Chatenay-Malabry, *Lab Delagrange, 91380 Chilly-Mazarin, France

We have previously shown that low-dose (2mg/Kg, i.p.) amisulpride (Ami), an atypical neuroleptic and D2 receptor antagonist, increases the concentration of 3MT (an index of dopamine release) in the nucleus accumbens (NAc) without modifying DOPAC or HVA concentrations. No effect was observed in the striatum (St).(Maubrey et al., 1988, Ann. Psychiatr, 3; 284-297). In this study, we determined whether the 3MT increase persisted after 14 days of treatment and whether the localized effect of Ami in the NAc was associated with a preferential distribution of the neuroleptic in this structure.
Wistar rats treated with Ami for 14 days (2mg/kg/day, i.p.) were sacrificed 60 min, after the last injection. DA, DOPAC, HVA and 3MT concentrations in the NAc and the St were determined by means of HPLC coupled with electrochemical detection. Trough Ami concentration was determined simultaneously in the NAc and the St, following chloroform extraction, by HPLC coupled with fluorimetric detection, 24 hours after the last administration.
Chronic treatment with Ami increased 3MT concentration in the NAc (+18%, $p < 0,05$), but not in the St. This DA releasing effect localized to the NAc was associated with a higher concentration of Ami in the NAc than in the St (218,3ng/g versus 99,7ng/g, respectively). These results support a selective involvements of the NAc in the effect of low-dose Ami and suggest that Ami may have a high affinity for a sub-type of DA receptors located preferentially in the NAc and involved in the release of DA.

540.23

DOPAMINE LEVELS IN CULTURED RAT MESENCEPHALIC NEURONS.
S.Schinelli,M.Paolillo*,M.Quartieri* and G.L.Corona*, Institute of Pharmacology, University of Pavia, 27100 Pavia, ITALY.

Dopaminergic neurons account for only a few per cent of the cells dissociated from embryonic midbrain and therefore the measurement of endogenous dopamine (DA) and its metabolites requires a very sensitive assay. We have developed an HPLC method coupled to coulometric detection to measure the levels of DOPAC and DA in rat mesencephalic cultures. Papain dissociated mesencephalic cells from 14-15 days old rat embryos were seeded in 24 multiwell plates at a density of 300000 cells/well, grown in 15% horse serum and then incubated up to 2 weeks in vitro. Cellular content of DA and DOPAC was monitored at 3,6,9 and 12 days in vitro (DIV); DA and DOPAC levels increased linearly with time of incubation ranging respectively from 2.5 pmoles/well and 0.12 pmoles/well at 3 DIV to 38.1 pmoles/well and 2.3 pmoles/well at 12 DIV. The DA/DOPAC ratio remained constant during the time of development in vitro. The levels of DA were significantly increased when mesencephalic neurons were cocultured with target striatal cells and furthermore cellular DA content in cocultures was directly correlated to the number of target striatal cells.

540.24

Anatomical Heterogeneity of Locomotor Responses to D1/D2 Agonists and Amphetamine in the Nucleus Accumbens.
W. D. Essman, P. McGonigle and I. Lucki, Depts. of Psychiatry and Pharmacology, University of Pennsylvania, Philadelphia, PA, 19104.

The nucleus accumbens (N.Acbs) plays an important role in the locomotor effects of dopaminergic agonists. The present studies provide evidence for an anterior/posterior regional heterogeneity for induction of locomotion by coinjections of the D1 agonist SKF 38393 (S) and the D2 agonist quinpirole (Q), or the dopamine releaser d-amphetamine (A), into the N.Acbs. Two groups of male Sprague-Dawley rats were implanted with guide cannulae (ML: ±1.5; DV: -5.8) aimed at different sections of the N.Acbs (AP: +2.0; +2.5). Following assessment of the locomotor stimulating effects of coadministration of various doses of S/Q or 32 ug/side A, animals were sacrificed and cannulae placements histologically determined. Subjects were divided into anterior (ANT; AP:>+2.3) and central (CEN; AP:<+2.3) placements based on the atlas of Paxinos and Watson (1986).

All groups were acclimated to the activity chambers prior to examining drug effects (saline control values: range = 25.1 - 69.4 counts/hour (c/h)). Rats in the CEN group (N=19) showed a consistent and robust locomotor response to maximally-effective S/Q combinations (Mean = 1,320.5 ± 165.9 c/h). Fifteen rats showed a high locomotor response (>500 c/h) to S/Q, whereas only 3 rats showed a moderate response (100-500 c/h) and one rat failed to respond to the S/Q combinations (<100 c/h). In contrast, twenty rats with ANT placements displayed inconsistent locomotor effects to S/Q combinations (Mean = 507.8 ± 130.0 c/h). Ten rats failed to show increases in locomotor activity; two rats showed moderate increases in locomotion; and eight rats displayed high locomotor activity. The pattern of enhanced locomotor response in animals with central versus anterior accumbens placement was also evident in the response to A (CEN: 642.6 ± 80.7 c/h; ANT: 387.2 ± 45.6 c/h). These data suggest that the receptors mediating the locomotor responses to dopamine agonists in the N.Acbs are located in the central to posterior portion of this nucleus. Supported by USPHS grant GM 34781.

540.25

A COMBINED GOLGI-STAINING AND IMMUNO-CYTOCHEMICAL STUDY OF NEURONS CONTAINING DARPP-32, A DOPAMINE-REGULATED PHOSPHOPROTEIN, IN ANTERIOR CINGULATE CORTEX.
C. C. Ouimet, Psychology Department, Florida State University, Tallahassee, FL 32306.

Many neurons in layer VI of the rat cerebral cortex contain DARPP-32, a dopamine and cyclic AMP-regulated phosphoprotein enriched in cells containing the D_1 dopamine receptor. The present study was undertaken to determine whether DARPP-32-containing neurons in anterior cingulate cortex are of a single morphological type. Male Sprague-Dawley rats (150-250 g) were anesthetized with sodium pentobarbital (60 mg/kg) and perfused with fixative. Brain sections were immunolabeled for DARPP-32 and then Golgi-stained. After the neurons were drawn, the silver stain was removed with sodium thiosulfate. At least 5 morphological cell types, including modified pyramids, an aspiny neuron, an inverted pyramid, a bipolar cell and a horizontally oriented cell were then classified as immuno-reactive. These results indicate that DARPP-32 is present in many cell types in layer VI and suggests that dopamine and cAMP modulate layer VI cells that have different functions.

540.26

BIOCHEMICAL AND PHARMACOLOGICAL MODULATION OF THE DOPAMINE SENSITIVE ADENYLATE CYCLASE IN HOMOGENATES OF BOVINE RETINA.
M. Schorderet, S. De Raad* and S. Ofori, Dept. of Pharmacology, CMU, CH 1211 Geneva 4 and School of Pharmacy, B.E.P., CH 1005 Dorigny-Lausanne.

The effects of various biochemical factors or pharmacological agents on the dopamine (DA)-induced accumulation of cAMP mediated by the stimulation of DA D-1 receptors were investigated. The procedure of Markstein et al. (J. Neural Transm. 69, 179, 1987) was modified as follows in order to optimize the response to DA: 1)100 µl of retinal homogenate were added to 350 µl of the buffer (48.8 mM Tris-HCl, 2 mM $MgCl_2$, 0.45 mM EGTA, 0.1 mM IBMX, pH 7.4) containing DA-agonists or -antagonists or forskolin, except in the controls. The mixture was kept at ice-temperature during 13.5 min before a rapid conditioning of 1 min at 30°C. The final 3 min incubation was then started with 50 µl of a solution of 5 mM ATP/ 0.1 mM GppNHp. After centrifugation (3400 rpm), proteins and cAMP (Brown et al., Biochem. J., 121,561, 1971) were measured in the pellets and in the supernatants, respectively. Under these optimal conditions, reproducible dose-response curves to DA or forskolin were obtained. In addition, the system was successfully tested to study the DA D-1-agonist properties of a new ergoline (CY 208-243) and to reassess the D-1 antagonist properties of clozapine. These results would confirm the usefulness of bovine retinal homogenates for 1)the study of DA-sensitive adenylate cyclase; 2)the screening of DA D-1 agonists or antagonists.

540.27

A DOPAMINE MICROCULTURE SYSTEM FOR IN VITRO DRUG TOXICITY TESTING. J. van Lange, H.M. Akbari, X.P. Hou, and E.C. Azmitia.
Dept. of Biology, New York Univ., Washington Sq. East, NY, NY 10003.

Primary neuronal cultures have become an increasingly popular experimental method in examining the inhibitory or stimulatory effects of neuroactive agents. We have already established the validity and usefulness of a primary fetal serotonin (5-HT) microculture system for drug toxicity studies in our laboratory. In this study, we establish a similar culture system with dopamine (DA) neurons to compare the effects of various drugs on these two neurochemical systems. A mesencephalic tegmental slice containing the majority of DA neurons was dissected from gestational day 14 fetal rat brains (age established by crown-rump measurement) in Minimal Essential Medium(MEM) with antibiotics. Slices were mechanically dissociated and plated onto poly-d-Lysine coated 96-well microculture plates (NUNC). The optimal initial cell plating density was found to be 0.8-1.0 x 10^6 cells/cm^2. Cell viability and fiber outgrowth were assessed using ^{3}H-DA(50nM) uptake after 5 days culture. Nonspecific accumulation was determined by the addition of 5x10^{-5}M nomifensine, a specific dopamine uptake blocker.

We assessed the specificity of our culture system by examining the effects of both DA and 5-HT specific drugs on DA fiber outgrowth inhibition or enhancement. Drugs were added on day (D) 2 of culture and ^{3}H-DA uptake done on D5. Cocaine, MDMA, nomifensine and fenfluramine were tested. DA uptake development was most affected by nomifensine which reduced ^{3}H-DA uptake to 50% of control after a single application at 1x10^{-6}M concentration. Fenfluramine, a serotonergic releaser, did not inhibit uptake development unless added concentrations exceeded 10^{-5}M. Interestingly, cocaine is not as potent as nomifensine in inhibiting DA fiber outgrowth, indicating that the release properties of cocaine distinguish it from the reuptake blocker. We are currently investigating other drugs' effects on cultured dopamine neurons. Supported by NIDA contract # 271-A7-A144.

TRANSMITTERS IN INVERTEBRATES: MOLLUSCS II

541.1

IDENTIFICATION OF A NETWORK OF NEURONS IN THE ABDOMINAL GANGLION OF *APLYSIA* THAT ARE IMMUNOREACTIVE FOR THE R15α PEPTIDES. J. Koester
Center for Neurobiology and Behavior, Columbia University, N. Y., NY 10032

The endogenously bursting, peptidergic neuron R15 expresses the R15 gene, which encodes 3 neuropeptides: R15α1, R15β, and R15γ. The mRNA precursor transcribed from the R15 gene is alternatively spliced in other neurons, resulting in an mRNA that encodes a partially overlapping set of peptides: R15α2, R15β, and R15γ (Buck et al., 1987). The R15α1 peptide has been shown to cause water retention (Weiss et al., 1989) and both the R15α1 and R15α2 peptides have cardioexcitatory effects. We have previously found that in the abdominal ganglion only 5 cells are strongly immunoreactive (IR) for the R15α1 and R15α2 peptides. Two have previously been identified: R15 (which contains the R15α1peptide) and the cardioexcitatory neuron RB_{HE} (which contains the R15α2 peptide). The purpose of this study was to identify the other 3 IR neurons, all of which stain selectively for the R15α2 peptide.

The 3 remaining R15α2 – IR neurons have been identified. They are named L40, $L9_A$, and $L9_B$. The 2 L9 cells both project out the siphon nerve, and preliminary evidence suggests that they are the L9 gill motoneurons. The L40 cell is a newly characterized interneuron that projects to the head ganglia. It excites the other 4 IR cells by strongly exciting Int XIII, which directly excites R15, RB_{HE}, and the 2 L9 cells. Moreover, L40 is excited by L10, which also excites the other 4 cells that are IR for the R15α peptides.

Thus, all 5 of the neurons in the abdominal ganglion that are strongly IR for the R15α peptides appear to belong to the same functional network. Previous work has implicated R15, L10 and RB_{HE} in the control of water balance and kidney function. We are attempting to test this hypothesis and to determine the roles of the L40 and L9 cells in this context.

541.2

INNERVATION OF THE HEART OF *APLYSIA* BY SEROTONIN- AND R15α2 PEPTIDE- IMMUNOREACTIVE TERMINALS, AND BY NEURON L2. M.E. Skelton and J. Koester, Center for Neurobiology and Behavior, Columbia University, N.Y., NY 10032

Neuron L10 has been postulated to increase renal excretion by (1) opening the renal pore and inhibiting the LUQ cells that cause pore closure, and (2) exciting cardioexcitatory neuron RB_{HE}. The latter presumably increases filtration which takes place across the wall of the heart and/or the cristae aorta. The RB_{HE} cell has been shown to contain serotonin and R15α2 peptide. The purpose of this study was to (1) study the innervation of the heart by RB_{HE}; (2) to look for inputs to the heart that might counteract the L10-RB_{HE} effect, analogous to the way that the LUQ cells antagonize L10's effect at the renal pore.

Histofluoresence studies suggest that *Aplysia* heart is rich in serotonergic nerve endings (Taxi and Gautron, 1969). We have confirmed the serotonergic nature of these endings using fluorescent-labeled antibodies. However, double-label experiments revealed that R15α2 peptide immunoreactivity (IR) and serotonin-IR overlap only partially. Serotonin-IR is found in nerve terminals throughout the heart, while R15α2-IR is coextensive only with the serotonin-IR endings in the auricle and in the AV valves. This result indicates that either RB_{HE} segregates the R15α2 peptide to selected parts of its terminal field, or that the heart receives serotonergic input from neurons other than RB_{HE}.

We also found, based on extracellular recording and dye filling, that one of the LUQ cells (L2) has extensive nerve terminals in the auricle. Firing L2 has no effect on heart beat or on its modulation by other neural inputs. Our working hypothesis is that L2 activity modulates the filtration characteristics of the heart wall. Because L2 is inhibited by L10, we hypothesize that its actions in the heart oppose the actions of L10 that are mediated by RB_{HE}.

Current work is directed towards identifying more precisely the sources of the serotonin-IR and the R15α2-IR in the heart and further characterizing the role of cardioactive neurons in *Aplysia*.

541.3

PEDAL PEPTIDE MODULATES A SODIUM CURRENT IN NEURONS L2-L6 OF *APLYSIA*. W. L. Pearson & P. E. Lloyd, Comm. on Neurobiol. & Dept. of Pharmacol. & Physiol. Sci., Univ. of Chicago, Chicago, IL 60637.

Application of pedal peptide (Pep) has been shown to depolarize the left upper quadrant (LUQ) neurons (L2-L6) of *Aplysia*. Several Pep-induced ionic currents contribute to this depolarization. Using voltage clamp and ion substitution techniques, we have isolated and characterized one of the Pep-induced currents as a Na^+ current ($I_{Na(Pep)}$).

Pep (2.5 μM-15 μM) was bath applied to axotomized LUQ cells bathed in "Ca^{++}-free" saline. Voltage-jump experiments showed that Pep induced a large inward current that reversed near 20 mV. At negative potentials the current displayed pronounced rectification, so that the I-V curve was unsloped below -30 mV. Substituting tetramethylammonium for Na^+ in the saline reduced the current and shifted the reversal potential to lower potentials, indicating that the current is carried primarily by Na^+. Addition of divalent cations to the saline blocked $I_{Na(Pep)}$. For example, 2.5 mM Mn^{++} reduced $I_{Na(Pep)}$ by 50%. Ca^{++} and Co^{++} produced a similar reduction of $I_{Na(Pep)}$. At physiological Ca^{++} concentrations, $I_{Na(Pep)}$ is largely blocked.

The biophysical properties of $I_{Na(Pep)}$ are very similar to the properties of cyclic nucleotide-gated currents characterized in a wide variety of cells, including other *Aplysia* neurons. Ionophoretic injection of cyclic nucleotides evoked a current resembling $I_{Na(Pep)}$. Treating LUQ cells with IBMX, a phosphodiesterase inhibitor, enhanced $I_{Na(Pep)}$, indicating that a cyclic nucleotide may be involved in modulating the activity of this current. Supported by NS 23569.

541.4

ABSENCE OF ACETYLCHOLINESTERASE (AChE) ACTIVITY AT CHOLINERGIC NEUROMUSCULAR SYNAPSES IN *APLYSIA*. P.E. Lloyd Dept. Pharm. Physiol.Sci., Comm.Neurobiol. Univ. Chicago, Chicago IL 60637.

Intrinsic buccal muscle 5 (I5, also the ARC) is innervated by 2 cholinergic neurons which have axons in buccal nerve 3 (N3). The metabolism of ^{3}H-choline (^{3}Ch)and ^{3}H-acetylcholine (^{3}ACh) in I5 was studied. I5 contains a high affinity uptake system for ^{3}Ch which is active into the nM range. N3 stimulation increases ^{3}Ch uptake, which is then found entirely as ^{3}ACh (presumably in cholinergic terminals). When I5 is loaded with ^{3}ACh in this manner, subsequent N3 stimulation depletes only ^{3}ACh. Thus stimulation of N3 (even with contractions blocked) selectively affects ^{3}ACh metabolism. Several lines of evidence suggest that these neuromuscular synapses have very low endogenous AChE activity. First, when N3 is stimulated and the bath surrounding I5 analysed, over half the label is recovered as ^{3}ACh in the absence of any AChE inhibitor. Second, stimulation-dependent increase of released label is not changed by the addition of cold choline (≤100μM) indicating that little hydrolysis of ^{3}ACh and subsequent uptake of ^{3}Ch occurs. Finally, ^{3}ACh is hydrolysed very slowly by I5, and eserine (100μM) slows this hydrolysis only slightly suggesting that it may not be due to a true AChE. Thus, unlike vertebrate neuromuscular junctions, I5 terminals have little endogenous AChE activity. However it is possible that AChE activity could be provided by blood. Supported by NS 23569.

541.5

IN-SITU HYBRIDIZATION WITH NON-RADIOACTIVE PROBES IN WHOLEMOUNTS OF APLYSIA GANGLIA. J.K. Ono & R.E. McCaman. Dept. of Biol. Sci., Calif. State Univ., Fullerton, CA 92634 and Div. of Neurosciences, Beckman Research Inst. of the City of Hope, Duarte, CA, 91010.

The large identifiable neurons in the CNS of Aplysia californica may be useful for determining the subunit composition of ion channels that have been cloned. We have developed in situ hybridization preparations of wholemounts of Aplysia ganglia to facilitate identification of specific neurons containing particular mRNA transcripts of interest. These neurons may later be used in live preparations to test for the presence of specific types of ion channels. cDNA probes to neuronal actin and FMRFamide, previously cloned from Aplysia, were labeled with digoxigenin.

Results with these probes for abundant mRNAs indicate that the staining with the digoxigenin-based method is similar in appearance to immunohistochemically treated wholemounts of ganglia. Additional studies are planned to determine whether this procedure has the sensitivity to detect rarer Aplysia mRNAs. (Supported by CSUF Mini-Grant and NIH AREA Grant to JKO and Beckman Research Funds to REM).

541.6

EFFECTS OF APLYSIA BAG CELL PEPTIDES ON cAMP METABOLISM DISTINGUISH MULTIPLE FUNCTIONAL CLASSES. R. Sanger Redman and R.W. Berry. Dept. Cell, Molecular and Structural Biology, Northwestern Univ., Chicago, IL 60611.

The secretion of egg-laying hormone (ELH) by the bag cell neurons of Aplysia is modulated via cAMP by peptides which are secreted with ELH. We have examined each of the secretory products of the proELH precursor and related peptides of the atrial gland for effects on bag cell cAMP metabolism. We find that neither ELH itself nor the acidic peptide alter bag cell cAMP metabolism. In contrast, each of the Bag Cell Peptides (BCP's) and the structurally-related atrial A and B peptides do. As previously reported, α-bag cell peptide reduces bag cell cAMP at 15°, but elevates cAMP at 20°. Since only inhibition by this peptide is observed in adenyl cyclase assays and its effect on whole cells at 20° is converted to inhibition in low Ca^{++}/high Mg^{++} media, we suggest that the peptide evokes release of an excitatory agent at the higher temperature. However, its actions are not altered by tetrodotoxin, suggesting that presynaptic excitation is not involved. The effects of γ-BCP are qualitatively identical to those of α-BCP, although its potency is lower, and the inhibition of adenyl cyclase by these peptides is additive. In contrast, β-BCP and the atrial peptides elevate bag cell cAMP in a temperature-independent fashion. The effect of β-BCP persists in low Ca^{++}/high Mg^{++} medium, suggesting a direct action on bag cells, whereas the atrial peptides elevate bag cell cAMP when secretion is blocked at 15°, but not at 20°. Thus these structurally similar peptides differ substantially in their functional characteristics: α- and γ-BCP mediate temperature-dependent direct inhibition/indirect excitation; β-BCP mediates temperature-independent direct stimulation; and the atrial peptides mediate temperature-dependent direct/indirect stimulation.

541.7

INPUT RESISTANCE AND MEMBRANE POTENTIAL SHIFTS FOLLOWING TONIC NEUROTRANSMITTER APPLICATIONS TO *APLYSIA* SILENT NEURONS. A. R. Sheppard and R. G. Villanueva*. J.L. Pettis VAMC and Loma Linda Univ., Loma Linda, CA 92357.

Many studies identify modulation of specific channels or synthesis pathways by neurotransmitters (NXs), but leave open the question of the integrated effects on membrane properties. To do so, we measured membrane potential (V_m) and input resistance (R_{in}) in cells L1, R1 and R2 of the *Aplysia* abdominal ganglion by current clamps at 30 sec intervals. Bath perfusions of NX were for 30 min at 21°C. In general, dopamine, gamma-aminobutyric acid, and glutamate depolarized the cells, while R_{in} increased, decreased, and decreased, respectively. Serotonin effects (5 to 50 μM) were not well characterized, despite some significant shifts (2–10 mV, 50% in R_{in}). Acetylcholine had the least consistent effects. We saw few reliable correlations between the changes in R_{in} and V_m, although sometimes we saw the strong correlations expected if changes in one channel type were predominant. Effects shortly after NX application were sometimes reversed by slower trends, suggesting competition between processes with different time courses. Our results illustrate the complexity of integrated cell responses to modulation by tonic chemical stimulation. (Supported by U.S. DOE, Office of Energy Management, DE-AI01-90CE35035.)

541.8

THE ACTIONS OF MOLLUSCAN CARDIOACTIVE PEPTIDES ON THE HEART OF THE NUDIBRANCH ARCHIDORIS MONTEREYENSIS. B.L. Wiens and P.H. Brownell. Oregon State University, Corvallis, OR. 97331

The heart of the nudibranch mollusc *Archidoris montereyensis* is regulated by at least five motor neurons; two of these neurons, one excitatory and the other inhibitory, are uniquely potent modulators of cardiac activity. We are currently trying to identify the transmitters that mediate the actions of the powerful heart excitor. Previously, we demonstrated that the molluscan cardioactive transmitters serotonin (5-HT), dopamine, small cardioactive peptide B (SCP_B), and FMRFamide excite an isolated preparation of the *Archidoris* heart. Immunocytochemical studies indicate that 5-HT, SCP_B, and FMRFamide-like substances are present in axonal processes innervating cardiac and vascular tissues, although these transmitters were not detected in the identified heart excitor motor neuron. In this study we have assayed the activity of 4 additional peptides, R15α1 and 2, myomodulin, and substance P, recently shown to act on or innervate regions of other molluscan cardiovascular systems. The R15α2 peptide, present in *Aplysia's* heart excitor neuron RB_{HE}, is the most potent excitatory transmitter active on the heart of *Archidoris*. At threshold concentrations (<5 nM) this peptide increases the amplitude of heart contractions, and at progressively higher concentrations it also affects the rate in a dose-dependent manner. Myomodulin, though less potent (thres.= <500 nM), also increases the rate and amplitude of contractions. R15α1 and substance P do not appear to affect the heart's activity (highest concentrations tested 10 and 100 uM, respectively). Immunocytochemical labelling should reveal whether R15α2 and myomodulin-like transmitters are contained within the heart excitor neuron of *Archidoris* and its terminals on the heart. Thus, *Archidoris* should continue to be a useful model for studying the functional relationship between a small set of motor neurons, a larger number of cardio-excitatory transmitters, and regulation of cardiovascular processes. (Supported by NIH pre-doctoral felloship #MH09818, and Sigma Xi).

541.9

THE ONTOGENY OF NEURONAL SYSTEMS EXPRESSING SCP-LIKE AND SEROTONIN-LIKE ANTIGENS IN *BERGHIA VERRUCICORNIS*. S.C. Kempf, A. Saini*, and A. Jones*. Dept. of Zoology and Wildlife Science. Auburn University, Auburn, AL 36849

At 6 d after ovaposition these embryonic neuronal systems are much simpler that those found in near competent larvae. In 6 d embryos, only 5 neuron perikarya are positively labeled for 5-HT and one for SCP. the 5-HT positive neurons appear to be positioned centrally over the cerebral commissure rather than in the cerebral ganglia. 5-HT positive axons are present in the velum and in commisssures and connectives associated with the cerebral and pedal ganglia. SCP positive axons are present in the cerebral commissure and ganglia and extend from the single labeled neuron toward a cerebral ganglion. 12 d after ovaposition, competent larvae possess bilaterally symmetric pairs of 5-HT positive neurons apparently located in both the cerebral and pedal ganglion pairs. Numerous axonal processes are present, associated with the cerebral and pedal ganglia and also in the velum, visceral mass and foot. The SCP positive neuronal system now consists of bilaterally symmetric pairs of neurons that appear to be located in the left and right pleural ganglia. Axons extend from these neurons to their respective cerebral ganglia as well as through the circum-esophageal loop. Many SCP and 5-HT positive axons are parallell and directly adjacent to each other. Within the cerebral and pedal neuropils, SCP positive axons mingle closely with 5-HT positive axons.

541.10

IMMUNOLOCALIZATION OF HISTAMINE, OCTOPAMINE AND GABA IN THE CENTRAL NERVOUS SYSTEM OF LYMNAEA

T. Karhunen and P. Panula
Department of Anatomy, University of Helsinki, Finland

The distribution of histamine, octopamine and GABA in the subesophageal ring ganglia of the gastropod mollusc Lymnaea palustris was studied immunocytochemically in carbodiimide-fixed cryostat sections.

Octopamine-like immunoreactivity was distributed in only few large neurons predominantly in the buccal, cerebral and pedal ganglia. A total of 8-10 large neurons and a cluster of smaller positive cell bodies were found. Histamine was also found in the buccal, cerebral and pedal ganglia and in the visceral and large parietal ganglia. The number of histamine-positive cells was distinctly larger especially in the distal parts of the cerebral ganglia and the fiber tracts were more widespred in the connectives and nerves than those immunoreactive for octopamine and GABA. No colocalization of these three substances were found.

541.11

MET-ENKEPHALIN-LIKE IMMUNOREACTIVITY IN THE CNS AND HEART OF *LYMNAEA STAGNALIS*. N.M. Ewadinger, N.I. Syed, R.L. Ridgway, A.G.M. Bulloch and K. Lukowiak. Dept. of Med. Physiology, Univ. of Calgary, Calgary, Alberta, Canada T2N 4N1.

Using indirect immunocytochemistry on whole-mount tissues, we constructed a neuronal map showing met-enkephalin-like immunoreactivity in the CNS of *Lymnaea*. A total of 31 neurons, distributed among all except the pleural ganglia and left pedal ganglion, were consistently stained. In control experiments, no specific staining was observed when either the primary or secondary antiserum was omitted, or when non-immune serum replaced the primary antiserum. Preabsorption of the primary antiserum with met-enkephalin peptide (60 mM) eliminated specific staining.

Among the immunoreactive neurons were two giant, well-characterized peptidergic neurons: VD1 and RPD2. Their identity was further confirmed in double-labelling experiments which combined Lucifer yellow fills with immunocytochemistry. These cells are strongly electrically coupled and are known to be involved in cardiorespiratory and whole-body withdrawal behaviours. Since these neurons innervate the heart, we examined this target organ for the presence of met-enkephalin-like immunoreactivity and found staining of neuronal processes, particularly in the auricle. These results suggest that a met-enkephalin-like peptide is present in *Lymnaea* which may have a functional role in cardiovascular control.

Supported by MRC (Canada).

541.12

THE MECHANISM OF GLUTAMATE ACTION IN THE BUCCAL CPG OF HELISOMA TRIVOLVIS E.M. Quinlan and A.D. Murphy Department of Biological Sciences, University of Illinois at Chicago, Chicago, Illinois 60607.

The buccal CPG of Helisoma is comprised of three subunits (S1, S2, S3). Glutamate and its agonists mimicked the effect of S2 on identified S2 postsynaptic neurons, suggesting that the S2 neurons are glutamatergic. It appears that inhibition from S2 plays a critical role in the temporal linkages among subunits. Neurons that receive inhibition from S2 are hyperpolarized by glutamate and quisqualate, but not ACPD. These hyperpolarizations and the S2 IPSPS were not blocked by antagonists for ionotropic (e.g. CNQX) or metabotropic (e.g. AP4) glutamate receptors. These data suggest either a novel ionotropic glutamate receptor or an ACPD/AP4 insensitive metabotropic receptor. S2 excitation appears to be mediated by a kainate-like receptor. AMPA, glutamate and kainate depolarized neurons that receive excitation from S2. S2-evoked EPSPs and depolarizations produced by glutamate, AMPA, and kainate were blocked by CNQX. Glutamate, kainate and AMPA also stimulated rhythmic S2 activity. If kainate was used to evoke rhythmic S2 activity, addition of CNQX inactivated S2. If S2 activity was evoked by other neuroactive substances, CNQX blocked the S2-evoked EPSPs but not the S2 IPSPS, demonstrating that rhythmic S2 activity can occur in the absence of kainate-like excitatory influences. (NIH R01 NS26145)

541.13

A PECK OF PICKLED PEPTIDES. W. Lesser and M.J. Greenberg. Whitney Lab, St. Augustine, FL 32086.

Two series of cDNA clones have been sequenced from the garden snail *Helix aspersa*: clone HF1 and clone HF4. Clone HF1 encodes only the two tetrapeptides FMRFamide and FLRFamide; clone HF4 has sequences for eight longer peptides but no tetrapeptides. The peptides can be identified and quantified in tissue extracts with HPLC and RIA, and their distributions within a particular tissue can be visualised with immunohistochemistry. The two tetrapeptides and seven of the longer peptides end -RFamide while the eighth peptide encoded in clone HF4 has the sequence pQDPFLRIamide. HPLC/RIA analyses of heart extracts suggest that only the tetrapeptides are present in the heart. However, the heart stains with antisera raised against EFLRIamide implying that pQDPFLRIamide and the other longer peptides are also present.

Are the tetrapeptides the only FMRFamide analogues present in the heart? If so, then why does the EFLRIamide antiserum stain the heart? If the longer FMRFamide analogues are present in the heart why are they not detected by HPLC/RIA?

Funded by NIH grants F32 HL08371 and HL28440.

541.14

CHARACTERIZATION OF SQUID (*LOLIGO*) FMRFAMIDE RECEPTORS. G.J. Chin and K. Payza. Laboratory of Developmental Neurobiology, NIH, Bethesda, MD 20892 and LBG, St. Elizabeth's Hospital, NIMH, Washington, DC 20032.

We have continued our studies (Neurosci. Abstr. 16:550) of the receptor for the invertebrate neuropeptide FMRFamide by using a radioligand to characterize the binding site and by using detergents to solubilize the receptor. ^{125}I-daYFnLRFa binds to optic lobe membranes with a K_d of 0.15 nM and a B_{max} of 0.25 pmol/mg membrane protein at the optimal pH of 6.5. Binding is displaced potently by FMRFa (IC_{50} = 1-10 nM) and weakly by FMRF-OH (10 μM). GTP, GTPγS and GDP reduce binding by up to 85 % with IC_{50} = 5-10 μM while 100 μM ATP and GMP have no effect. FMRFa and pb2 stimulate adenylate cyclase in optic lobe membranes while pb5 is a partial antagonist. Both non-ionic (Triton X-100, octyl glucoside) and ionic (CHAPS, Zwittergent 3-10) detergents selectively solubilize the receptor. FMRFa (100 μM) inhibits labeling of the solubilized receptor by pb2 and pb5 while FMRF-OH, serotonin, and leu-enkephalin (all at 100 μM) do not.

HYPOTHALAMIC-PITUITARY-ADRENAL REGULATION II

542.1

EFFECTS OF REPEATED RESTRAINT STRESS IN RATS ON NEUROENDOCRINE AND MOLECULAR MARKERS OF THE BRAIN'S RESPONSE Yoshifumi Watanabe, Jesus Angulo, G. Bing[1], D.Filer[1], E.A. Stone[1] and B.S. McEwen Lab. Neurendocrinol. Rockefeller University, New York, N.Y. 10021; and [1]Department of Psychiatry, NYU School of Medicine, New York 10016, N.Y.

We have investigated effects of daily restraint stress on acute and chronic measures of the brain's response. Acute restraint stress elevated serum corticosterone levels; the response duration was reduced by repetition of the stress 3h/d. The attenuation occurred within the first 7d, and continued out to 28d. By 28d, rats showed no significant reductions in body, thymus and spleen weights and no increase in adrenal weights. Nevertheless, tyrosine hydroxylase mRNA in locus ceruleus increased by 30-40% during 7-28d of 3h/day stress. Sustained increases also occurred in the mRNA for oxytocin in the paraventricular nucleus; measurements of vasopressin mRNA are in progress. There are also rapid and delayed responses to restraint stress in cerebral cortex; just 1h of restraint stress produced marked increases in mRNA for 4 immediate early genes (nur77, c-fos, zif-268 and tis-7); in the hippocampus, 1h of restraint stress led to an increase of zif-268 but not c-fos mRNA. Repeated restraint stress of 7-10d duration decreases the cyclic AMP response to noradrenaline in cerebral cortex by a glucocorticoid-dependent mechanism (Stone et.al., Eur J. Pharm. 1987). The multiple changes induced by stress imply that many neurochemical systems are involved, some of which may be linked through causal cascades while others may involve modulatory effects of circulating hormones. (MH41256 [BMc];AF05R 89-0208 and MH 45265 [EAS]).

542.2

ALTERED ADRENOCORTICOTROPIN, CORTICOSTERONE AND OXYTOCIN RESPONSE TO STRESS DURING CHRONIC SALT LOAD. H.S. Chowdrey*, D. S. Jessop*, H. Patel* and S. L. Lightman. Neuroendocrinology Unit, Charing Cross and Westminster Medical School, London W6, U.K.

A chronic osmotic stimulus (12 days drinking 2% saline) can influence the hypothalamo-pituitary axis by inhibiting the secretion of basal and adrenalectomy-elevated plasma adrenocorticotropin (ACTH) in rats (1). To determine whether the stress response is also impaired, we measured plasma levels of ACTH, corticosterone and oxytocin in saline-treated rats following a 5 minute restraint stress. Morning plasma ACTH levels were markedly decreased from 972 ± 165 pg/ml in the control animals to 349 ± 114 pg/ml in animals given 2% saline. The stress-induced release of ACTH seen in the control rats (from 972 ± 165 to 1439 ± 105 pg/ml, n=6) was completely abolished in rats treated with 2% saline for 12 days (unstressed 349 ± 114 pg/ml, stressed 205 ± 27 pg/ml). A stress-induced release of corticosterone was also observed in control rats (from 28.8 ± 7.9 to 99.5 ± 8ng/ml) but not in saline-treated rats (stressed 36.0 ± 8.2ng/ml). A normal ACTH response to stress was obtained in adrenalectomised rats on isotonic saline (from 2338 ± 436 to 4265 ± 195pg/ml, n=9)) while ACTH levels in both unstressed and stressed rats on 2% saline were reduced (1175 ± 213 and 2338 ± 369pg/ml respectively, n=9). In contrast to ACTH and corticosterone, the oxytocin response to stress was intact in animals on 2% saline. These results demonstrate that a chronic osmotic stimulus activates an inhibitory mechanism over the release of ACTH and has differential effects on the ACTH/corticosterone and oxytocin responses to stress.

Reference: 1. Jessop, D.S., Chowdrey, H.S. and Lightman, S.L. Brain Research 523: 1 (1990).

452.3

CORTICOTROPIC-RELEASING HORMONE (CRH) IMMUNOREACTIVITY (IR) IN THE PARAVENTRICULAR NUCLEUS (PVN) IS INFLUENCED BY TESTOSTERONE. E.W. Rodriguez, D.J. Magnuson, T.S. Gray, R.J. Handa. Dept. of Cell Biology, Neurobiology and Anatomy. Loyola University, Chicago. Maywood, IL 60153.

The plasma corticosterone response to novelty stress is greater following long term gonadectomy of male rats. To further characterize the role of androgens in the regulation of the hypothalamo-pituitary-adrenal (HPA) axis we castrated (gx'd) male Sprague/Dawley rats and treated them with testosterone (T). Control animals were castrated and sham treated or left intact. After 3-4 weeks of treatment, animals were perfused through the ascending aorta with paraformaldehyde, and CRH- or vasopressin- (VP) IR was examined in 20 μm-thick frontal sections. The number of CRH-IR cells was significantly greater by 68% in the PVN in gx'd animals compared to intact controls. T replacement restored CRH-IR cell numbers to that of intact animals. No differences were seen in VP-IR cells in the PVN or in CRH-IR cells in the bed nucleus of the stria terminalis. These data suggest that androgens can influence the HPA axis at the level of the PVN.

542.4

INDIVIDUAL DIFFERENCES IN THE HYPOTHALAMIC-PITUITARY-ADRENAL RESPONSE TO STRESS: RELATIONSHIP TO TESTOSTERONE. V. VIAU & M.J.MEANEY. Douglas Hospital Research Ctr., Depts. of Psychiatry and Neurology/ Neurosurgery, McGill Univ., Montreal H4H 1R3, Canada.

Male rats typically show smaller ACTH and corticosterone (B) responses to stress compared to females. This sex difference has been attributed, in part, to inhibitory affects of testosterone (T) on HPA activity. Considering that variations in circulating levels of T exist between male rats, we examined the extent to which T levels could account for individual differences in HPA response to restraint stress. ACTH and B responses were determined following 10 min of restraint stress in intact and gonadectomized males. The intact males were grouped on the basis of high or low basal T levels; 4.0 ± 0.6 vs 1.2 ± 0.3 ng/ml, respectively. The peak ACTH and B responses following stress were significantly lower in the high T group compared to the low T and GDX animals: ACTH; 267.2 ± 74.3, 614.6 ± 31.3 and 497.2 ± 63.2 pg/ml, respectively. B; 31.9 ± 1.9, 66.6 ± 11.5 and 73.1 ± 16.0 μg/dL, respectively. In the high T group, ACTH and B levels returned to pre-stress values by 10 and 60 min, respectively, following the termination of stress. However, in the low T and GDX groups, both ACTH and B levels remained elevated up to 60 min post-stress. There was a negative correlation between the level of T and the integrated ACTH and B responses to stress (r = .85 and .76, respectively; $p < 0.5$). No significant differences in pre-stress levels of ACTH and B were observed as a function of T. Finally, animals either showed an acute increase or decrease in T levels following stress. Interestingly, elevated ACTH responses to stress were associated with rats that showed a positive T response. These findings suggest that T is a potent regulator of HPA activity during stress, as slight variations in T levels are associated with profound differences in the pattern of ACTH and B responses to stress. Thus, in the male rat, individual differences in HPA responses to stress are, at least partially, related to the gonadal status of the animal.

542.5

ABSENCE OF CORTISOL NEGATIVE FEEDBACK REGULATION OF PULSATILE ACTH IN THE OVINE FETUS NEAR TERM. E.M. Apostolakis, L.D. Longo, S.M. Yellon. Div. Perinatal Biology, Depts. Physiol, Pediat, Ob/Gyn, Loma Linda Univ, Sch of Med, Loma Linda, CA 92350

During late gestation, as a part of the endocrine cascade which triggers parturition, plasma cortisol increases in the fetal sheep to surge within 48h of birth. Plasma ACTH also increases, suggesting an absence of negative feedback near term. To investigate the cortisol negative feedback regulation of pulsatile ACTH secretion, we continuously infused cortisol (CI, 1 μg/h/kg est BW, i.v.) for 96 h in 6 chronically catheterized fetuses beginning on d 133 and again on d 142 of gestation. Fetal blood (2 ml) was obtained every 5 min for 2 h (0900-1100 h) before infusion and 24 h later. As determined by RIA, pulse characteristics using previously defined criteria (*Biol Repro 42 Suppl:178A,1990*) were:

Fetal Plasma ACTH (pg / ml; x ± SE)

CI	Days	FREQ	AMP	PEAK	NADIR	x ± SE
0	133	1.7 ± .03	49 ± 11	74 ± 13	38 ± 4	48 ± 4
+	134	1.3 ± .03	41 ± 6	106 ± 13*	65 ± 9*	68 ± 13
0	142	1.4 ± .04	49 ± 27	87 ± 29	38 ± 26	82 ± 26
+	143	2.4 ± 05**	32 ± 5	118 ± 13**	86 ± 10**	89 ± 22*

*p < 0.05 vs 133 d; **p < 0.05 vs 142 d, ANOVA with repeated measures

Increased cortisol was associated with enhanced ACTH pulse peak and nadir at 134 vs 133 d and 143 vs 142 d. The data indicate an absence of cortisol negative feedback regulation of basal pulsatile ACTH secretion in fetal sheep during late gestation. Moreover, the data imply either a positive feedforward mechanism or independent regulation of plasma ACTH and cortisol (NR06042, HD03817).

542.6

STRESS-INDUCED HYPERSECRETION OF ACTH IN ADRENALECTOMIZED RATS REPLACED WITH CONSTANT CORTICOSTERONE IS NORMALIZED BY AN ACUTE INCREASE IN CORTICOSTERONE. L. Jacobson and R.Sapolsky, Dept. of Biological Sciences, Stanford Univ., Stanford, CA 94305-5020.

Constant corticosterone (B) levels that normalize basal AM plasma ACTH in adrenalectomized (ADX) relative to sham-ADX (SHAM) rats do not normalize ACTH responses to stress [Akana et al. Endocrinology 122 (1988):1337]. To determine if this enhanced ACTH response to stress is due to lack of stress-induced B secretion, 300 g male rats with indwelling arterial & sc catheters were subjected to hypoxia stress (10% O_2) 5 d after sham-ADX or ADX & 35% B sc pellet replacement (B-PELLET). B-PELLET rats received B or DMSO vehicle sc 5 min before gas flow began; SHAMs received DMSO. Although hypoxia-induced PaO_2 was comparable in SHAM & B-PELLET rats (ca. 42 mm Hg), vehicle-treated B-PELLET rats secreted significantly more ACTH than did SHAMs. However, ACTH responses to hypoxia were similar between SHAM rats & B-PELLET rats given acute B doses that mimicked the plasma B increase in SHAMs. We conclude that stress-induced hypersecretion of ACTH in B-PELLET rats is due at least in part to the lack of fast feedback inhibition by stress-induced increases in B.

542.7

STRESS-INDUCED RELEASE OF CORTICOTROPIN-RELEASING FACTOR FROM THE MEDIAN EMINENCE OF THE RAT IN VIVO. E. Merlo Pich, M. Lorang, W. Vale[1], G.F. Koob and F. Weiss. [1]Salk Institute and Dept. of Neuropharmacology, Scripps Clinic and Research Foundation, 10666 N. Torrey Pines Rd, La Lolla, CA 92037.

Corticotropin-releasing factor (CRF)-like immunoreactivity was measured by radioimmunoassay in perfusate collected under halothane anesthesia from microdialysis probes implanted into the median eminence region of rats. Procedures optimizing recovery were developed in vitro prior to the start of in vivo experiment. Perfusate was collected every 20 min starting at two hours after implantation. Basal levels were estimated to be about 108±26 pg/ml (n=6). Increasing the potassium concentration in the perfusion medium to 60 mM resulted in a 3-fold increase of CRF. A significant increase of CRF was also observed after injection of 1.5 M hypertonic saline (1.8 ml/100 g bw ip) to the anesthetized rats. Post-mortem histological analysis revealed that probe implantation produced a relatively small lesion in the median eminence and an enlarged third ventricle, suggesting the cerebrospinal fluid was also sampled. No CRF levels was detected when the microdialysis probe was implanted in the posterior hypothalamus under both basal and stimulation conditions. These data are in agreement with previous evidence of in vitro release of CRF from hypothalamic tissue (Smith et al. 1986) and in vivo secretion into hypophysial portal blood in urethane-anesthetized rats (Plotsky & Vale 1984). These findings also suggest that CRF is released in response to depolarization of nerve terminals in the medio-basal hypothalamus and median eminence and that CRF is released after certain physiological stressors.

This research was supported in part by grant DK 26741 to G.F.K.

542.8

RAPID CO-PRODUCTION OF VASOPRESSIN (VP) AND CORTICOTROPIN RELEASING HORMONE (CRH) WITHIN SELECT PARAVENTRICULAR NEUROSECRETORY CELLS FOLLOWING BEHAVIORAL STRESS. J.A. Barrett, A.J.Silverman and D.D.Kelly, Depts. of Psychiatry and Anatomy & Cell Biology, Columbia University, & NYS Psychiatric Institute, NY, NY 10032

VP is a potent synergist of CRH in the release of ACTH by the anterior pituitary. These two peptides can be localized within the same parvocellular neurons in adrenalectomized rats and in intact rats exposed to behavioral stress for 3-10 days or have been isolated for 4wks. To evaluate whether the degree of stress exposure affects the proportion of PVN cells that exhibit the property of CRH/VP co-production, we used a double label protocol with antibodies against CRH and the glycopeptide portion of VP neurophysin (AG Robinson;AM16166). Fischer 344 male rats were assigned to 5 experimental groups (N=6). Four groups were exposed to varying durations of behavioral stress (Hou-Yu et al, NS Abstr. 12:783,'89) prior to sacrifice: 2, 12, 24 and 48hrs. In the PVN of no-stress controls many pale CRH-positive cells were detected, 3% of which stained for VP. Stress exposure significantly increased the proportion of CRH/VP co-staining cells in all groups to 10-11%, regardless of whether stress duration was 2 hrs or 2 days. (Planned weighted comparison: p=.007). Although it is clear that the proportion of CRF/VP co-producing cells was insensitive to stress duration, it is not clear whether the same subpopulation of co-producing cells were observed in all groups.NS23858

542.9

DEXAMETHASONE BLOCKADE DOES NOT ABOLISH ULTRADIAN RHYTHMS IN CORTICOSTERONE SECRETION BY THE RAT ADRENAL. M.S. Jasper and W.C. Engeland. Sect. of Neurobiology/ Dept. of Surg., Brown Univ./RI Hospital, Providence, RI 02903.

An ultradian rhythm in corticosterone (B) secretion has been demonstrated in the rat using intra-adrenal microdialysis (FASEB J. 4:A827,1990). To determine if rhythmic corticosterone secretion is dependent on physiological presentation of ACTH, intradrenal microdialysis was performed on dexamethasone (DEX) blocked rats, with and without replacement of ACTH by constant infusion. Under pentobarbital anesthesia, rats were prepared with two jugular vein catheters and an adrenal probe constructed from cellulose fibers (9 kD MW cutoff). Experiments conducted 2 days post-surgery consisted of continuous collection of dialysate at 10 min intervals from 1000 to 1800 hr. All rats were administered DEX (25 µg/100g s.c.) two hours before sampling. In one group of rats (n=4), ACTH was not replaced. In the other group (n=3), ACTH was infused i.v. at 75 pg/min/100g., from 1100 hr to 1800 hr, producing a submaximal B secretion rate. Dialysate B was measured by RIA. Peak detection was performed by manually drawing a baseline through troughs in the secretory profile, and using the PC-Pulsar criteria for significance. Peaks were detected in B secretory rate in all animals. The mean pulse amplitude was increased by the infusion of ACTH (2.0±0.4 vs. 29.9±5.4 pg/min, $p<0.001$). Interpulse interval was not affected by ACTH. To determine if periodic components in B secretion persisted after DEX blockade, power spectra were calculated, and the significance of peaks was assessed by the permutation rank test. Significant periodicities were detected in all animals. When individual spectra were averaged in each group, both groups showed peaks near 100 min. These data suggest that ultradian rhythmic variation in adrenal B secretion can occur in the absence of physiological variation in ACTH stimulation; however ACTH appears to modulate the amplitude of the B pulses. Supported in part by NIH grant DK38951.

542.10

ELECTRICAL STIMULATION IN THE SUPRACHIASMATIC NUCLEUS DEPRESSES THE ACTIVITY OF NEURONS IN THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS REGION. M.L.H.J. Hermes*, R.M. Buijs and L.P. Renaud. Neuroscience Unit, Ottawa Civic Hospital, Ottawa, Canada, K1Y 4E9.

There is extensive evidence that the suprachiasmatic nucleus (SCN) contains the circadian oscillator responsible for 24-hour rhythms in many behavioural, endocrine, and autonomic functions. The mechanism by which information from the SCN is transmitted to other brain regions regulating these functions is unknown. Recently, by means of infusion techniques, it was demonstrated that peptides present within projections of the SCN may be involved in the control of these daily rhythms, in particular that of plasma corticosterone levels. This rhythm is considered to involve parvocellular neurons in the hypothalamic paraventricular nucleus that secrete CRF into the median eminence (ME) portal pituitary circulation.

To further understand the way by which the SCN may impose rhythms on centrally controlled processes we undertook electrophysiological recordings from neurons situated in the region of the paraventricular hypothalamic nucleus in pentobarbital anesthetized male Long-Evans rats and examined their response to electrical stimulation of the SCN. Of a total of 31 spontaneously active neurons, 4 could be identified as projecting to the ME. Following SCN-stimulation, peristimulus histograms of the ME-projecting neurons displayed a distinct 40-80 msec reduction is excitability. Of the 27 neurons that were not projecting to the ME, 23 were likewise depressed, one was stimulated and 3 were not responsive. The data suggest that in this experimental situation, the SCN exerts a predominantly depressant influence on neurosecretory and other unidentified neurons in the area of the hypothalamic paraventricular nucleus. Supported by MRC and Heart and Stroke Foundation.

542.11

INHIBITION OF PLASMA CORTICOSTERONE LEVELS BY THE SUPRACHIASMATIC NUCLEUS. R.M. Buijs[12]*, T.P. van der Woude[1], A. Kalsbeek[1] and J.J. van Heerikhuize[1]. [1]Neuroscience Unit, Ottawa Civic Hospital, Ottawa, K1Y 4E9, [2]Netherlands Inst. for Brain Research, 1105 AZ Amsterdam.

The suprachiasmatic nucleus (SCN) is established as the (main) circadian parameter of the mammalian brain. As such it synchronizes a number of humoral and behavioral rhythms to the daily dark-light cycle, of which plasma corticosterone (B) for example, is shown to rise shortly before the onset of the dark period. A number of studies have shown that the SCN is involved in the regulation of this process. Recently we provided evidence that vasopressin from the SCN inhibited B release when infused in the paraventricular-dorsomedial hypothalamic nucleus. To investigate further the role of the SCN this nucleus was lesioned bilaterally whereafter the animals received an indwelling jugular vein catheter allowing unrestrained blood sampling. Plasma B levels of SCN-lesioned animals during the light period were not different from those obtained during the dark period but were 4 times higher than those obtained from intact animals during the light period. No difference was observed in plasma B levels between SCN lesioned animals and intact animals during the dark period.

After receiving a mild stress the plasma B excursion in lesioned animals was much greater than that of intact animals during the light period but was comparable to that obtained from intact animals during the dark period. These data show that the SCN serves a inhibitory role on plasma B release but only so during the light period.

542.12

AGING, STRESS AND STRAIN-DEPENDENT DIFFERENCES IN HYPOTHALAMIC CATECHOLAMINES, PITUITARY CRH RECEPTORS AND PLASMA ACTH, PROLACTIN AND CORTICOSTERONE. Y. Tizabi, G. Aguilera[1], R. Li[1,2], R.J. Wyatt[2], D. Kirch[2] and G.M. Gilad[2]. Dept. of Pharmacol., Coll. of Med., Howard Univ.; [1] DEB, NICHD, NIH; [2] NPB, NIMH Neurosci. Cent. at St. Elizabeths, Washington, DC 20059.

Neuroendocrine regulation is known to be impaired in aging and this impairment may be genetically determined. Neuroendocrine changes were, therefore, characterized in the shorter lived and hyper-reactive to stress Wistar Kyoto (WKY) strain and compared to the Brown Norway (BN) strain. We found: 1. catecholamines, similar in the young strains, were reduced (20%) and were less responsive to restraint stress in aged WKY; 2. CRH binding, similar in the strains, was reduced (26%) in aging; 3. ACTH levels, 67% higher in WKY, were not changed in aging and both strains were similarly responsive to stress; 4. corticosterone, similar in the strains, was reduced (50%) in aging, but the stress-induced increase was relatively higher and was maintained longer compared to young; 5. prolactin, two fold higher in young WKY, was reduced (60%) in aged BN, but the stress response remained similar in both strains. The data support the premise that during aging regulatory neuroendocrine mechanisms deteriorate and the rate is genetically determined.

542.13

CAPILLARY VOLUME AND FILLING IN THE NEUROHYPOPHYSIS AND HYPOTHALAMUS. R.B. PAGE, T. RUTHERFORD, AND R. M. BRYAN. Division of Neurosurgery, College of Medicine, Pennsylvania State University, Hershey,PA. 17033.

We tested the hypothesis that selective filling occurs in restricted microvascular regions supplied by capillary loops in the median eminence by measuring percent capillary volume and filling in the median eminence and selected hypothalamic nuclei. 0.5 ml of FITC dextran (150 mg/kg) was injected via a femoral venous catheter over 4 seconds into six restrained, awake, male Long-Evans rats. The animals were decapitated 10 seconds after injection. Six micron thick cryostat sections were taken through the median eminence, neural lobe, SON, PVN, and arcuate nucleus. Sections were examined and photographed by fluorescence microscopy, stained for alkaline phosphatase and rephotographed by light microscopy. Percent capillary volume and filling were calculated for each region. In the median eminence capillary volume averaged 28%. The internal plexus accounted for more than 50% of the total capillary volume. Volume percent filling averaged 96% in the median eminence and 93% in its internal plexus. In the SON, PVN, and ARC capillary volume percent averaged 15%, 7% and 4% respectively. Capillary percent filling averaged 75%, 75% and 86%. These results do not support the hypothesis that selective recruitment of capillary loops occurs in the internal zone of the median eminence, but do allow the possibility of capillary recruitment in the Paraventricular Nucleus.

543.1

LHRH IS MORE POTENT THAN ENDOTHELIN-1 (ET-1) IN STIMULATING CHANGES IN INTRACELLULAR CALCIUM AND LH SECRETION FROM SINGLE PROESTROUS RAT GONADOTROPES. S.R.Rawlings*, J.Flores*, G.Block & D.A.Leong. Dept. Medicine & NSF Centre for Biological Timing, University of Virginia, Charlottesville, VA 22908.

A recent study using a rapid perfusion system showed that ET-1 and luteinizing hormone (LH)-releasing hormone (LHRH) were equipotent in stimulating LH release from dissociated gonadotropes of castrate rats, a result that parallelled their effects on intracellular free Ca^{2+} ion concentration ($[Ca^{2+}]_i$) in single gonadotropes (Stojilković *et al.*, Science 248, 1990). This result, and the presence of ET-1-immunoreactivity in the hypothalamus, has suggested a potential role for ET-1 in reproductive function.

Dynamic fura-2 video-imaging was employed to investigate the effects of ET-1 and LHRH on $[Ca^{2+}]_i$ in acutely dissociated proestrous rat gonadotropes post-identified using an LH reverse hemolytic plaque assay (RHPA). The RHPA was also employed to measure the cumulative LH release from single gonadotropes over a 2 hour period. The Ca^{2+} responses to ET-1 and LHRH were highly variable from cell to cell, although they both showed response profiles typical of factors that stimulate an $Ins(1,4,5)P_3$ and DAG-generating system. LHRH was, however, more potent at stimulating Ca^{2+} responses as summarized below:

Ca^{2+} Response	[LHRH]	[ET-1]
No response	-	$\leq 10^{-10}$M
Low amplitude rise	$<10^{-11}$M	10^{-10}M - 10^{-9}M
Ca^{2+} oscillation	10^{-11}M - 10^{-9}M	10^{-8}M - 10^{-7}M
Spike & plateau	10^{-9}M - 10^{-8}M	$\geq 10^{-6}$M

In the RHPA the ED_{50} for LHRH and ET-1-stimulated plaque number (a measure of gonadotrope secretion responsiveness) was 10 pM and 3 μM respectively. In conclusion, the potency of ET-1 to stimulate proestrous gonadotropes is significantly lower than the known regulator of gonadotrope function LHRH, calling into question any role of ET-1 in the *in vivo* regulation of LH release during the estrous cycle.

543.2

TRANSFORMING GROWTH FACTOR-β1 SYNTHESIS IN THE ANTERIOR PITUITARY GLAND IS REDUCED DURING ESTROGEN-INDUCED INCREASE IN LACTOTROPIC GROWTH. D.K. Sarkar and K.H. Kim*, Department of Veterinary and Comparative Anatomy, Pharmacology and Physiology, and Department of Biochemistry/Genetics and Cell Biology, Washington State University, Pullman, WA 99164.

We have previously shown a marked growth inhibitory effect of transforming growth factor-β1 (TGF-β1) on pituitary lactotropes. To determine whether changes of lactotropic growth reflect variations in TGF-β1 synthesis in the anterior pituitary (AP), we determined the level of immunoreactive TGF-β1 and TGF-β1 mRNA in the AP. Animal models used are ovariectomized and estrogen-treated ovariectomized Fischer 344 female rats (Gottschall *et al.*, Proc Soc Exp Biol Med, 181:78, 1986). A significant population of cells was identified to be TGF-β1 producing in the AP by using both immunohistochemical procedures and *in situ* hybridization techniques for TGF-β1 mRNA. The AP concentration of TGF-β1 was also measured by a specific radioimmunoassay for the peptide growth factor and was found to be reduced 6-fold following 7 days of estrogen administration in ovariectomized rats. When the relative level of TGF-β1 mRNA in the AP was measured using the Northern blot hybridization procedure, the estrogen-treated rats showed a significantly lower level of TGF-β1 mRNA than the control ovariectomized rats. The estrogen-treated rats, as compared to the controls, also showed a 3-fold higher concentration of AP prolactin and a 2-fold increase in AP weight. These data suggest that TGF-β1 synthesis in the AP is reduced during estrogen-accelerated lactotropic growth, and that the peptide growth factor may be important in controlling lactotropic growth and function.

543.3

GnRH INDUCES OSCILLATORY $[Ca^{2+}]_i$ AND ELECTRICAL RESPONSE IN RAT PITUITARY GONADOTROPHS. M. Kukuljan*, S.S. Stojilković*, D.A. Doron, E. Rojas* and K.J. Catt*. ERRB/NICHHD and LCBG/NIDDK, NIH, Bethesda, MD 20892.

Pituitary gonadotrophs secrete LH and FSH in response to GnRH, which induces release of Ca^{2+} from intracellular stores and oscillations of $[Ca^{2+}]_i$. Both this process and the regulation of voltage-activated Ca^{2+} channels are thought to be involved in the control of GnRH-induced gonadotropin secretion. Basal and receptor-regulated changes in $[Ca^{2+}]_i$ and membrane potential were monitored by fluorescence analysis and the perforated patch technique in individual rat pituitary gonadotrophs. All measurements were conducted at 20 to 25° C. Most gonadotrophs exhibited episodes of spontaneous oscillations in basal $[Ca^{2+}]_i$ that were abolished by addition of EGTA or Ca^{2+} channel blockers. Under control conditions, gonadotrophs also displayed continuous electrical activity with firing of action potentials (AP) at a frequency of 0.6 to 1.6 Hz. These AP did not overshoot and were insensitive to 15 μM TTX. At high doses (100 nM) GnRH induced biphasic response in $[Ca^{2+}]_i$ with a spike phase, followed by a oscillatory phase. Addition of 100nM GnRH to the bath resulted in hyperpolarization from -35 mV to aprox. -70 mV and inhibition of AP, followed by a pattern of regular, slower oscillations (0.1-0.3 Hz) of Vm between -65 and -35 mV. AP were regularly fired on top of the slow waves. However, in some cells slow oscillations occurred without AP firing, suggesting that both phenomena are independently modulated. Slow oscillations were insensitive to 20 mM TEA and were broadened by 1 μM BK 8644. These observations suggest synchronization of $[Ca^{2+}]_i$ and electrical activity in pituitary cells. Modulation of a Ca^{2+}-activated current and plasma membrane Ca^{2+} channels may be involved in GnRH-evoked Vm oscillations.

543.4

COMPONENTS OF THE MULTIUNIT ACTIVITY (MUA) INCREMENTS ASSOCIATED WITH HYPOTHALAMIC GnRH PULSE GENERATOR ACTIVITY IN THE RHESUS MONKEY. H. Cardenas*, K. T. O'Byrne*, C. L. Williams* and E. Knobil. Lab for Neuroendocrinology, The University of Texas Medical School, Houston, TX 77225.

Secretory pulses of gonadotropic hormones occur synchronously with sharp increases in MUA (volleys) recorded from the mediobasal hypothalamus (Neuroendocrinology 39:256, 1984). Such recordings from 4 unanesthetized ovariectomized monkeys were digitized and analyzed for their component spikes using cluster analysis (Discovery Software - Brainwave System Corporation, Bloomfield, CO). The spikes were categorized by their various dimensions (principal components) and their firing frequencies compared to those of the MUA. Of the 22 individual spike clusters identified, 15 increased and 2 decreased their firing rates synchronously with the MUA volleys. The firing rate of the remainder was unchanged. No evidence of recruitment was found. It is concluded that the MUA volleys that reflect the activity of the GnRH pulse generator represent the synchronized increase in firing rate of single units in the mediobasal hypothalamus and the simultaneous decrease in the firing rate of a smaller population of others. (Supported by NIH grants HD 17438 and HD 08610 and by The Clayton Foundation for Research)

543.5

HYPOTHALAMIC ACTIONS OF C-TYPE NATRIURETIC PEPTIDE (CNP) TO ALTER PITUITARY HORMONE SECRETION. W.K. Samson and F.-L.S. Huang*. Anatomy&Neurobiology, Univ. MO Sch.of Med. Columbia, MO 65212.

C-type natriuretic peptide (CNP) shares a high degree of sequence homology with ANP and BNP. CNP is present in brain in higher levels than ANP or BNP and binds preferentially to the guanylyl cyclase-B (ANPR-B) receptor. We compared the effects of CNP and ANP on the hypothalamic control of LH and PRL secretion. Like ANP, CNP failed to significantly alter LH, PRL or GH release from cultured, dispersed anterior pituitary cells. Both CNP and ANP significantly inhibited LH secretion when administered centrally; however, the minimum effective dose of CNP was 0.1 nmole, while 1.0 nmole of ANP is required. The inhibitory effect of CNP and ANP was abolished by prior, central injection of the delta opioid antagonist naltrindole. While central injection of ANP inhibited PRL secretion, 1.0 nmole CNP stimulated ($p<0.001$) PRL secretion in conscious rats. Plasma GH levels were not significantly altered. The data suggest that CNP might be the preferential neuroactive natriuretic peptide in brain and that endogenous CNP might exert physiologic effects on pituitary function.

543.6

DOPAMINE CONTENT IN THE BRAIN AND PITUITARY OF TILAPIA *(Oreochromis mossambicus)*. S.Ishwar[1,2] and M.K.Sim*[3]. [1]Lab. of Neurobiology, Rockefeller University, New York, NY 10021 and [2]Dept. of Zoology and [3]Pharmacology, National University Singapore, Singapore.

Hypothalamic dopamine (DA) has been shown to participate in neuroendocrine regulation of gonadotropin release in fish (Peter et al., Rec Prog Horm Res 42:513, 1986), and DA turnover in hypothalamus is decreased by sex steroids. In the present study we used HPLC with electrochemical detection to examine the relationship between sex steroids and ex vivo brain and pituitary DA and DOPAC levels in Tilapia. Tissue was taken from intact, gonadectomized (14d) and gonadectomized (18d) with testosterone (T) or estradiol (E2) replacement on days 14 and 17 (n=4). In intact fish, DA and the DA/DOPAC ratio, tended to be highest in the pituitary and the tuberal area, and lowest in the optic tectum and thalamus. Although not statistically significant, there was a tendency for T to decrease, and E2 to increase, DA levels in all brain regions (telencephalon, tuberal area, lateral lobes, optic tectum and thalamus) and the pituitary.

In a subsequent experiment, electrolytic lesions were made stereotaxically (Ishwar and Munro, unpub.) in the hypothalamus of Tilapia (4 or 14d survival period) to understand the origin of DA projections to the pituitary. Relative to controls, animals with preoptic lesions (14d) had significantly higher DA levels in the pituitary. Lesions in other areas of the hypothalamus had no effect. Thus this experiment provides evidence for the existence of a preoptico-hypophysial neural system that inhibits pituitary dopamine in tilapia.

543.7

COMPLEX REGULATORY PATHWAY OF GnRH GENE TRANSCRIPTS BY FORSKOLIN, IONOMYCIN AND PHORBOL ESTERS IN THE GT1 CELL LINE. T.T.Yeo*,K.W.Dong*,M.Jakubowski*,M.Blum,J.L.Roberts. Fishberg Center for Neurobiology, Mt.Sinai School of Medicine, New York, NY10029.

The GT1 cell line is derived from a GnRH-expressing hypothalamic tumor made by genetically targeted expression of a 2kb rat GnRH promoter/SV40-T antigen DNA construct in transgenic mice. We have used this cell line as an *in vitro* model system for the endogenous mouse GnRH gene regulation. Using a sensitive solution hybridization/RNase protection assay we have reported that, in a static incubation system, prolonged (24hr) treatment of GT1 cells with the GnRH secretagous forskolin (10mM), ionomycin (1mM) or phorbol ester (100nM) produces a decrease in GnRH mRNA level while short term (1hr) treatment shows no significant change (Abs Endo Soc 91). Whether this result is due to cytotoxicity of prolonged treatment with these agents causing an overall decrease in RNA Pol II transcription remains to be elucidated. Using cytoplasmic cyclophilin mRNA levels as an internal reference, we now report that treatment with the above secretagougs decreases GnRH mRNA levels, rendering cyclophilin mRNA levels unchanged. We have also studied the hnRNA levels under the same conditions and find that short term incubation with ionomycin and phorbol ester decreases GnRH hnRNA levels while forskolin causes no significant change; prolonged incubation caused a significant decrease with all three pharmacological agents. Surprisingly, using a 2kb rat GnRH promoter/CAT reporter construct transfected GT1 cells, we see that forskolin causes a 4-6 fold stimulation in CAT activity, despite its inhibitory effects on the endogenous mouse gene. To further understand the effect of forskolin on GnRH gene expression, we have done a time course study using cyclophilin as an internal reference and find that GnRH mRNA levels show no significant change up to 3 hours of incubation and decrease afterwards in a time-dependent manner. The varying response of GnRH gene transcripts and promoter activity to different second messengers indicates a complicated regulatory pathway for this gene.

543.8

TREATMENT WITH 8-OH-DPAT (DPAT) ALTERS THE TIMING OF THE ESTRADIOL (E2)-INDUCED LH AND PROLACTIN (PRL) SURGE IN OVARIECTOMIZED (OVX) RATS. J.R. Cohen and M.R. Sterner. Lilly Research Laboratories, Eli Lilly & Co., Greenfield, IN 46140.

The induction of cyclic release of LH and PRL is dependent on an E2 stimulus. The timing of these surges, however, requires the integrity of one or more circadian oscillators. Serotonin (5HT) activity within the hypothalamus shows a diurnal rhythm which is altered by E2. In the present study, we examined the effects of a 5HT1a agonist, DPAT, on the timing of the E2-induced surges of LH and PRL.

Young adult Fischer 344 rats maintained on a 12L:12D photoperiod (lights on 0600h) were OVX (Day 0). All animals were implanted with a Silastic capsule containing E2 (250 µg/ml sesame oil) on Day 7 and the right atrium was cannulated on Day 8. On Day 9, rats were placed into one of 3 groups: control (saline, sc); DPAT (0.1mg, sc) at 1100h; DPAT (0.1mg, sc) at 1200h. Rats were bled at 1100 (prior to dosing), 1130, 1200 (prior to dosing), 1230 and hourly from 1300-2200h. LH and PRL were determined by RIA.

The onset of the E2-induced LH surge in control rats was clearly detectable by 1400h. Treatment with DPAT at 1100h delayed the onset of the LH surge by 2 hours with the first detectable rise in LH at 1600h. Delaying treatment with DPAT until 1200h produced a further shift in the onset of the LH surge, which was first detectable at 1700h. Delayed onsets were also seen in the timing of the PRL surge; The first detectable rise in PRL occurred at 1300h in controls, but was delayed until 1500h in both DPAT treatment groups.

These data suggest that 5HT, possibly acting via the 5HT1a receptor, may play a role in timing the onset of cyclic release of LH and PRL.

543.9

DUAL MECHANISMS INVOLVED IN THE INHIBITORY INFLUENCE OF SUCKLING ON LH RELEASE IN THE OVARIECTOMIZED RAT. T.J. Wu, N.H. McArthur and P.G. Harms. Departments of Animal Science and Veterinary Anatomy and Public Health, Texas A&M University, College Station, TX 77843.

A study was conducted to further understand the involvement of the endogenous opioid peptides (EOPs) in suckling-induced inhibition of LH release in the rat. The experiments utilized postpartum rats (day 7) that had been ovariectomized the day following parturition. The first experiment was designed to determine the effect of opioid antagonism by naloxone infusion (NAL; 1.0 mg/kg bw/h) on the increase in peripheral LH concentration after 18 h of pup removal and the decrease in LH concentration after 18 h of resuckling. NAL during 18 h of pup removal or during 18 h of pup removal followed by 18 h of resuckling neither accentuated nor attentuated ($p>0.10$) the LH concentration. The second experiment was designed to determine the effect of NAL on peripheral LH concentration in continuously suckled rats. Serum LH increased ($p<0.10$ and $p<0.005$, respectively) in response to 18- and 36-h of NAL. The third experiment was designed to determine the effect of pup removal during NAL on serum LH. Peripheral LH concentration was not different in the rats treated with 36 h of NAL whether they were suckled for the duration of the infusion or nonsuckled for the latter 18 h of infusion. These results suggest that suckling may inhibit LH release through two mechanisms. The first may be an opioid-independent or enhanced opioid tone, important for the initiation of the inhibitory effect of suckling, and the second, an opioid-dependent mechanism for the sustained inhibitory effect of suckling on LH release.

543.10

THE EFFECT OF AGING ON OPIATE RECEPTOR DENSITY IN THE MEDIAL PREOPTIC NUCLEUS OF OVARIECTOMIZED (OVX) AND ESTRADIOL (E2)-TREATED RATS. J.M. Lloyd, N.G. Weiland and P.M. Wise. Department of Physiology, University of Maryland, Baltimore, MD 21201

Endogenous opioid peptides (EOPs) are thought to modulate reproductive cyclicity in the female rat by exerting a tonic inhibitory effect on LH secretion. The LH secretory response depends on gonadal steroids. Previously, we have shown that in young animals the steroidal milieu influences opiate receptor densities in hypothalamic nuclei involved in reproductive function. The purpose of the present study was to determine whether age-related changes occur in the density or diurnal pattern of naloxone binding sites and to correlate these with steroid-induced LH surges. Young (3-4 mo) and middle-aged (9-11 mo) cycling and old (17-19 mo) repeatedly pseudopregnant (RPP) rats were OVX (day 0). Seven days later half of each group received Silastic capsules containing E2, and the animals were killed on day 8 at 2300 h or on day 9 at 0300, 1000, 1300, 1500, 1800 or 2300 h. Brain sections (20 µm) were incubated with 2.0 nM [^{3}H] naloxone ± 1 µm unlabelled ligand and slides were apposed to LKB Ultrofilm for 4 weeks. Receptor densities were assessed by quantitating the relative optical density within a fixed window placed over the medial preoptic nuclei. E2 decreased the densities of naloxone binding sites compared to OVX in all three age groups ($p<0.0001$). Naloxone binding was lower in old E2-treated compared to young and middle-aged rats ($p<0.01$), and the steroid-induced decline in receptor binding was least pronounced in middle-aged and most pronounced in old rats. Interestingly, the relative ability of E2 to influence the density of naloxone binding sites correlates with its ability to induce LH surges. Old rats exhibited a more robust E2-induced LH surge compared to middle-aged animals. We postulate that rats become less sensitive to estradiol during middle-age due to chronic elevated levels of estrogen and that responsiveness returns in older rats due to the prolonged diminished level of estrogen in these previously RPP animals.

543.11

NEURONAL HYPERTROPHY IN THE HYPOTHALAMI OF OLDER MEN. N.E. Rance and N.T. McMullen. Departments of Pathology and Anatomy, University of Arizona College of Medicine, Tucson, AZ 85724.

A striking neuronal hypertrophy occurs in the infundibular nucleus of postmenopausal women (Rance et al. J.Clin.Endo.Metab., 1990; Rance and Young, Endocrinology, 1991). To determine the gender specificity of this response, we measured neuronal cross-sectional areas in the infundibular nucleus of young (24,32 and 41 yrs of age) and older (60,61 and 68 yrs) men and compared them to data reported previously from the hypothalami of pre-(28,32 and 40 yrs) and postmenopausal women (58,62 and 74 yrs). Formalin fixed hypothalami were paraffin embedded, serially sectioned and Nissl-stained. Cross-sectional areas of 2427 neurons were traced using an image-combining computer microscope. The mean cross-sectional area (± SEM) of neurons in the infundibular nucleus of young men (147.0 ± 1.3) was identical to that of young women (147.0 ± 2.2). The mean area of infundibular neurons from postmenopausal women (190.4 ± 2.1) was 30% greater than those from young men and women. We were intrigued to find that infundibular neurons from older men (171.9 ± 1.7) were significantly larger than those from young men, but still smaller than infundibular neurons from postmenopausal women. We have previously provided evidence that postmenopausal neuronal hypertrophy is secondary to the loss of ovarian steroids (Rance et al, 1990, Rance and Young, 1991). The moderate hypertrophy of infundibular neurons in older men may be due to the reduced circulating testosterone levels which have been reported in this age group. (Arizona Disease Research Control Commission #0-029)

543.12

DIVERGENT EFFECTS OF SHORT-TERM GLUCOCORTICOID EXCESS ON THE GONADOTROPIC AND SOMATOTROPIC AXES IN NORMAL MEN. J.D. Veldhuis, G. Lizarralde and A. Iranmanesh, Dept. Internal Medicine, NSF Science Center in Biological Timing, Univ. Virginia Health Sciences Center, Charlottesville, VA 22908, and Salem Veterans Hospital, Salem, VA 24153.

To investigate the effects of short-term glucocorticoid on the neuroendocrine activity of the gonadotropic and somatotropic axes, 6 men underwent 10-min blood sampling for 24 h after placebo or 7 days of dexamethasone (DEX) administration (1.5 mg orally twice daily). Compared to placebo, DEX caused significant decreases in serum concentrations of estradiol, viz., from 144 ± 18 to 99 ± 18 pmol/L, free testosterone from 105 ± 10 to 87 ± 10 pmol/L, and DHEA-S from 6.0 ± 1.6 to 1.7 ± 0.3 umol/L ($P < 0.05$), but mean serum LH concentrations did not change: viz., 5.3 ± 1.2 IU/L (control) vs. 4.2 ± 0.61 IU/L (DEX). The mean plasma somatomedin C concentration rose from 0.74 ± 0.08 to 2.0 ± 0.35 U/mL ($P < 0.05$) and the mean serum GH concentration, from 1.2 ± 0.90 ug/L (basal) to 4.2 ± 1.5 ug/L (DEX) ($P < 0.01$). Deconvolution analysis revealed that the half-life of endogenous GH, the duration and the amplitude (maximal rate of secretion) of computer-resolved GH secretory bursts, and the mass of GH secreted per burst were not influenced significantly by DEX. On the other hand, DEX significantly increased GH secretory burst frequency from 12 ± 1.6 to 18 ± 1.6 episodes/24 h, decreased the GH interburst interval from 127 ± 23 to 79 ± 5 min, and increased the daily GH secretion rate (ug/L/day) from 41 ± 11 to 101 ± 11 ($P < 0.05$). No changes occurred in the half-life of LH, or LH secretory burst frequency, amplitude, mass, or duration, or the total daily LH production rate. We conclude that short-term glucocorticoid excess augments pulsatile GH secretion without influencing the episodic release of LH in normal men.

543.13

GLUCOCORTICOID AND ESTROGEN INHIBITION OF CRF INDUCTION OF POMC GENE EXPRESSION S. J. Dermer, D. Lorang, and J. L. Roberts
Fishberg Center for Neurobiology, Mount Sinai School of Medicine, New York, NY 10029

Glucocorticoids and estrogens inhibit expression of the POMC gene via steroid receptor-mediated events. In pituitary corticotrophs, glucocorticoids inhibit POMC transcription directly and by interfering with transcriptional induction by CRF. In the hypothalamus, estrogens have direct inhibitory effects on POMC transcription. We have focused on comparing the mechanisms by which the two steroid hormones and their respective receptors influence expression of the POMC gene. Using a variant (ER-1) of the AtT20 D16-16 mouse anterior pituitary tumor-derived cell line, which expresses estrogen receptor, we find that short term estrogen (E2) treatment (20 or 30 min) (100nM) inhibits the stimulatory effects of CRF treatment (10nM) on POMC primary transcript and hnRNA levels. This effect is found using pretreatment with either CRF or E2 followed by short term cotreatment, and when the two hormones are added simultaneously. In AtT20 cells, using a similar experimental paradigm for glucocorticoid and CRF treatment we find that pretreatment with DEX inhibits CRF induction of hnRNA levels, whereas pretreatment with CRF followed by cotreatment does not affect CRF induction. When we transiently transfect a POMC promoter/enhancer (-704 to +38)-CAT fusion reporter gene into ER-1 cells and treat with E2 (16 hr), we find that CRF induction of the POMC promoter is diminished versus cells which are treated with CRF alone. Similarly, DEX treatment (100nM) of ER-1 cells also leads to a reduction in CRF induction of the POMC-CAT reporter. These results, along with our previous findings that estrogens and glucocorticoids do not elicit the same response in POMC primary transcript for acute treatment times suggest that the two hormones may use different mechanisms of regulation of POMC gene expression initially, but their long term overall effects are inhibitory.

543.14

ENDOGENOUS OVARIAN STEROID(S) MAINTAIN IMMUNOCYTOCHEMICAL NUCLEAR LOCALIZATION OF GLUCOCORTICOID RECEPTOR IN ADRENALECTOMIZED FEMALE RAT BRAIN. W. C. McGimsey, J. A. Cidlowski*, W. E. Stumpf, M. Sar*. Departments of Cell Biology and Anatomy, and Physiology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599.

We have shown with the anti-glucocorticoid receptor antibody GR-57 nuclear localization of glucocorticoid receptor immunoreactivity (GR-ir) in brains of intact male rats, and adrenalectomized (ADX) male rats treated with corticosterone, dexamethasone, or RU486, and cytoplasmic GR-ir in brains of adrenalectomized male rats (McGimsey et al., Endocrinology 126S:484 1990; FASEB J 5:2835 1991). Since progesterone is known to bind GR this study tests the hypothesis that endogenous ovarian steroid(s) cause nuclear localization of GR-ir in ADX adult female rats. Vibratome sections of paraformaldehyde fixed brains from intact, adrenalectomized (ADX), and adrenalectomized-ovariectomized (ADX-OVX) adult female rats were incubated in GR-57 (1:10,000) for immunocytochemistry. GR-ir was nuclear in intact and ADX female rats' brains, and cytoplasmic in ADX-OVX rats' brains. Supported by USPHS Grant NS-17479.

543.15

OXYTOCIN RELEASES NOREPINEPHRINE IN VENTRO-MEDIAL & PERIVENTRICULAR HYPOTHALAMUS OF FEMALE RATS. P.A. Vincent and A.M. Etgen. Depts. Psychiatry & Neuroscience, Albert Einstein Coll. Medicine, Bronx, NY 10461

Oxytocin (OT) and norepinephrine (NE) have been shown to enhance steroid-dependent reproductive behavior in female rats. We are employing intracranial microdialysis to determine whether OT regulates hypothalamic NE release. Preliminary data show that systemic administration of OT (1 I.U., sc) to ovariectomized rats treated with estrogen plus progesterone results in release of NE when microdialysis probes are placed within 0.3 mm of the lateral border of the ventromedial hypothalamic nucleus (VMN). This brain region is known to be important in hormonal facilitation of reproductive behavior. Interestingly, the most robust release of NE was seen when dialysis probes were placed near the lateral border of the periventricular nucleus (PVN). In contrast, animals in which microdialysis probes were placed anterior, lateral or dorsal (> 0.3 mm) to the VMN or PVN did not exhibit the same pattern of NE release as animals with probes placed at the border of the VMN or PVN. Animals given OT in the absence of hormone did not show a release of NE regards of probe placement. These results suggest that OT-induced NE release in the hypothalamus may be related to female sexual behavior in rats.

HYPOTHALAMIC-PITUITARY-GONADAL REGULATION: LH AND LHRH II

544.1

EXOGENOUS PROLACTIN DOES NOT REVERSE THE BROMOCRYPTINE-INDUCED BLOCKADE OF PUBERTY IN THE FEMALE FERRET. K.D.Ryan*, E.A.Volk* and O.M.Reinmuth. Depts Physiology, Ob/Gyn and Neurology, Magee-Womens Hospital and Univ. Pittsburgh School Medicine, Pittsburgh PA 15213

Sexual maturation of female ferrets can be accelerated by changing the ambient photoperiod from a short day (8L:16D) to a stimulatory, long day (16L:8D) after they are 15 wks of age. Previous work has shown that an important endocrine correlate of puberty in female ferrets is a marked decrease in the ability of estradiol to inhibit LH secretion. However, the pubertal rise in LH was shown to be accompanied by a coincident increase in plasma prolactin (PRL) levels and when the pubertal rise in PRL was prevented by treatment of females with the dopamine agonist, bromocryptine, sexual maturation in response to long days was also prevented. The purpose of this study was to examine whether administration of exogenous PRL to ferrets maturing in response to a long day stimulus could reverse the ability of bromocryptine to block puberty. Immature female ferrets housed in short days were ovariectomized and treated with implants designed to deliver very low levels of estradiol (<10 pg/ml). At 17 wks of age, some animals were moved to stimulatory long days while the rest remained in short days. Bromocryptine or vehicle injections were begun 3 wks after onset of long days; 2 wks thereafter, Alzet mini-pumps containing ovine PRL were implanted in both bromocryptine- and vehicle-treated females. Blood samples were obtained from jugular venous cannulae 3x weekly to monitor plasma LH levels. As expected, vehicle-treated females exhibited a rise in LH secretion about 3 wks after onset of long days which did not occur in females which were treated with the DA agonist. This bromocryptine-induced blockade of puberty was not prevented by infusion of oPRL. The failure of exogenous PRL to reverse the blocking effect of bromocryptine on puberty suggests the action of the drug to block PRL is secondary to the effect of elevated DA activity on the maturing hypothalamus (or brain). We conclude that a decrease in central (perhaps hypothalamic) DA may mediate the pubertal loss of responsiveness to the negative feedback of estradiol on LH secretion in the female ferret. Supported by HD14489 and Magee-Womens Hospital Research Fund.

544.2

SHORT-TERM FEED RESTRICTION DECREASES IN VIVO RELEASE OF BETA-ENDORPHIN (BUT NOT OF LHRH AND NPY) FROM MEDIAN EMINENCE AND INCREASES NPY RELEASE FROM PARAVENTRICULAR NUCLEUS IN EWES. B.M. Prasad*, C.D. Conover, D.K. Sarkar, J. Rabii and J.P. Advis. Department of Animal Sciences, Rutgers Univ, New Brunswick, NJ 08903, VCAPP, Washington State Univ, Pullman, WA 99164, and Department of Physiology, Rutgers Univ, New Brunswick, NJ 08903.

We have previously suggested that the median eminence (ME) is a control site for LHRH release in ewes. During the follicular phase ME infusion of β-endorphin (β-end) inhibits, and of neuropeptide Y (NPY) increases, in vivo ME-LHRH release. Infusion of NPY in paraventricular nucleus (PVN) increases feed intake. Long term feed restriction (FR) decreases serum LH in ovx-ewes. However, feeding a diet providing 30% of NRC requirements of energy and protein for two months, does not affect estrous cyclicity and ovulation. We assessed if in vivo release changes of these neuropeptides underlies compensatory events after 14 days of FR. In vivo release from ME and PVN was measured during the follicular phase (day 14), by push-pull cannula sampling (flow rate: 100 µl/10 min/4h), in full fed and feed restricted ewes. All ewes were maintained under short photoperiod conditions. ME in vivo LHRH and NPY release, and plasma LH, were similar in feed restricted and control ewes. This was associated with a decreased ME-β-end (59.0±3.8 vs 7.2±2.5 pg/ 100 µl PPC perfusate, control vs FR, n=6, P<.01) and increased PVN-NPY release (13.4±1.9 vs 31.2±2.7, n=8, P<.01). Thus, feed restriction might induce a decrease in β-end inhibitory tone on median eminence LHRH neuronal terminals, temporally compensating for unknown inhibitory components which might ultimately constrain tonic and phasic LH control. The specificity of such compensatory event is suggested by the differential paraventricular and median eminence NPY release, observed in feed restricted ewes (supported by NJES-Hatch 06108 and USDA 89-37240-4587 to JP Advis).

544.3

Dopaminergic Inhibition of LH (GnRH) Release in the Growth-restricted Hypogonadotropic Lamb. H. I'Anson*, J.M. Manning and D.L. Foster*, Reproductive Sciences Program & Depts. of Obstetrics & Gynecology & Biology, University of Michigan, Ann Arbor, MI 48109-0404.

Growth-retardation induced by dietary restriction results in hypogonadotropism in the lamb, even in the absence of the ovaries, and is the result of central nervous system inhibition of GnRH release (I'Anson *et al., Endocrinology*, 126 (Suppl.):1473, 1990). The present studies examined if inhibitory dopaminergic neurons are activated during growth restriction. Our approach was to investigate the effects of the dopamine antagonist, fluphenazine, on LH secretion in ovariectomized (ovx), growth-restricted lambs. In the first study, lambs (n=6), maintained at weaning weight (ca. 20 kg) for 42 weeks, were injected iv with fluphenazine (Flu, 0.5 mg/kg) after 4 h of an 8 h frequent sampling period. An increase in LH in response to Flu was observed in 2 of the lambs. The second study compared the response to Flu in ovx, *ad libitum*-fed lambs (n=8, *ad lib*) and growth-restricted lambs which were maintained at 28kg BW (n=8). These latter lambs exhibited an LH pulse frequency (0.9 ± 0.3 pulses/4 h) intermediate between that observed in 20 kg growth-restricted lambs (0.0 ± 0.0 pulses/4 h) and *ad lib* lambs (3.4 ± 0.2 pulses/4 h), suggesting that the degree of dietary-induced hypogonadotropism was less pronounced. When the lambs were 53 weeks old (during seasonal anestrus), Flu was injected iv after 4 h of an 8 h frequent sampling period. An LH response to Flu was observed in all but 1 of the *ad lib* lambs and in 4 of 8 growth-restricted lambs. LH pulse frequency increased in both the *ad lib* lambs (2.7 ± 0.6 to 4.1 ± 0.8 pulses/4 h) and the growth-restricted lambs (0.0 ± 0.0 to 1.3 ± 0.3 pulses/4 h). Mean LH also increased in both groups (18.0 ± 3.4 to 25.3 ± 3.5 ng/ml and 0.5 ± 0.0 to 4.4 ± 1.5 ng/ml, respectively). These data suggest that (a) dopaminergic neurons inhibit GnRH secretion in the normally developing lamb; and (b) additional inhibitory pathways may also be active in the growth-restricted lamb. (Supported by NIH HD-07048, -18258,-18394, 07433, & USDA 87-CRCR-1-2550).

544.4

LUTEINIZING HORMONE DURING THE ESTROUS CYCLE OF THE FOOD DEPRIVED HAMSTER. L.P. Morin, R.S. Donham and M.H. Stetson. Dept. Psychiatry, SUNY, Stony Brook, NY 11794.

Acute food deprivation during estrous cycle Days 1-2 will block hamster ovulation (Morin, AJP, 251:R663, 1986). This is precipitated by retarded follicular development, low preovulatory estradiol level, absent ovulatory LH surge and loss of ovulatory response to exogenous estradiol or LH. The present three studies further elucidate the endocrine deficits in the phasically starved hamster.

A) Starved animals were injected with 5 μg estradiol benzoate or oil 8 hr before lights off on cycle Day 3; fed animals received oil. Fed controls ovulated; starved controls did not. Starved animals given estradiol failed to ovulate, but had a normal LH surge. B) LH was measured at 6 hr intervals across the estrous cycle. Starved animals had low LH levels throughout the cycle, including Day 4 when the ovulatory LH surge seen in fed animals was absent. Variability in LH measured during Days 2 and 3 tended to be greater for starved animals. C) LH was measured for 2 hr periods at 8 min intervals during the afternoon of Days 1 and 2 or during the morning of Day 2. Pulsatile LH was not abolished in animals that failed to ovulate in response to acute starvation.

The results confirm the absence of an ovulatory LH surge in phasically starved hamsters and show that estradiol priming will reinstate the surge without restoring ovulation. This is probably explained by the retarded follicular development in the food deprived animals. The precipitating endocrine events preventing normal follicle growth remain obscure.

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544.5

FOOD DEPRIVATION DECREASES *c-fos* EXPRESSION IN GONADOTROPIN-RELEASING HORMONE (GnRH) NEURONS IN SYRIAN HAMSTERS. S.J. Berriman and G.N. Wade. Neuroscience and Behavior Program and Dept. of Psychology, University of Massachusetts at Amherst, MA 01003.

The Syrian hamster estrous cycle is highly sensitive to the availability of metabolic fuels. Two days of food deprivation immediately following ovulation is sufficient to prevent the next estrus and ovulation. We tested the hypothesis that food deprivation early in the cycle affects the GnRH neuronal system. This would lead to a decrease in pulsatile luteinizing hormone (LH) secretion and disrupt events leading to follicular maturation, estradiol release, the preovulatory LH surge, and estrus. The proto-oncogene, *c-fos*, is a marker of activity in the GnRH neuronal system in rats, sheep, and hamsters. In hamsters, 60% of the GnRH neurons express Fos immediately following the preovulatory LH surge, a time when GnRH neurosecretory activity is increased. On days 1, 2, and 3 of the cycle, approximately 20% of the GnRH neurons express the Fos protein. Since GnRH cells express Fos early in the cycle, these cells may represent neurons responsible for pulsatile LH release. If pulsatile LH secretion is affected by metabolic signals, then GnRH neurons should express little or no Fos after hamsters have been food deprived. In this experiment, hamsters were food deprived or fed *ad libitum* on days 1 and 2 of the estrous cycle. On the evening of day 2, brains were fixed with 4% paraformaldehyde and double label immunocyto-chemistry was used to localized Fos and GnRH in 50 μ sections beginning at the diagonal band of Broca and extending to the mammilary bodies. Fos-expression in GnRH neurons was substantially reduced after 2 days of food deprivation. These data suggest that food deprivation affects LH secretion early in the cycle by acting via the GnRH neuronal system.

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544.6

NALOXONE INFUSION DOES NOT REVERSE THE HYPOGLYCEMIA-INDUCED REDUCTION IN LUTEINIZING HORMONE LEVELS OBSERVED IN CHAIR RESTRAINED OVARIECTOMIZED RHESUS MONKEYS L.E. Heisler, R.L. Reid* and D.A. Van Vugt. Dept. of Physiology and Dept. of Obstetrics and Gynecology, Queen's University, Kingston, Ontario, Canada, K7L 3N6

Administration of corticotropin releasing hormone (CRH) to monkeys, rats and sheep inhibits luteinizing hormone (LH) release. This inhibition is prevented by naloxone suggesting that endogenous opioid peptides mediate the effects of CRH on LH. Since CRH activity and the activity of the pituitary-adrenal axis are increased by stressful stimuli, we examined the effect of insulin induced hypoglycemic stress on LH release in ovariectomized rhesus monkeys and the ability of naloxone to reverse the effects of this stress. Ovariectomized monkeys were restrained in primate chairs and administered insulin (1.5 U/kg) after a four hour control period followed by an infusion of saline or naloxone (2mg/hour) for an additional four hours. Naloxone infusions were also given in the absence of insulin administration. Blood was collected at 15 minute intervals and assayed for LH, cortisol and glucose. All LH measurements are expressed as a percentage of the baseline established in the first hour of the experiment. LH levels decreased significantly within 2 hours after insulin administration (70.3±2.81 %) but did not fall in the non-insulin treated group (94.4±2.98 %). Naloxone infusion failed to reverse the insulin-induced suppression of LH (66.2±3.46 %). As expected, glucose levels decreased within 1 hour after insulin injection (from 4.9±0.44 mmol/L to 1.8±0.21 mmol/L) and cortisol levels rose within 2 hours (from 38.0±4.58 μg/dL to 71.8±5.53 μg/dL). These results indicate that endogenous opioid peptides do not mediate the hypoglycemic stress-induced decrease in LH levels observed during these conditions in ovariectomized rhesus monkeys. (Supported by MRC of Canada)

544.7

DEFINITION OF METABOLIC STATES ASSOCIATED WITH THE SUPPRESSION AND RESTORATION OF LH SECRETION CAUSED BY FASTING AND REFEEDING IN RHESUS MONKEYS. D.L. Helmreich, D.B. Parfitt*, and J.L. Cameron, Depts of Behavioral Neuroscience, Psychiatry, Physiology and the Center for Neuroscience, University of Pittsburgh, Pittsburgh PA 15260.

We have previously demonstrated in adult male rhesus monkeys that one day of fasting can cause a slowing of pulsatile LH secretion within the first 4 h after the missed meal. Additionally, we have shown that this slowing can be rapidly reversed by refeeding, and the degree of restoration of LH secretion is correlated with the size of the refeed meal. To define the metabolic states associated with these changes in LH secretion, we measured levels of metabolic hormones and substrates during fasting and refeeding. To characterize changes during fasting, blood samples were collected at 2 h intervals from 1100 h on a day of normal feeding (daily meal at 1100 h) until 1800 h on the subsequent day during which animals were fasted (n=5). Plasma glucose concentrations were not different in the afternoon on the day of fasting (51.6±1.41 mg/dl) compared to a day of feeding (56.53±0.87 mg/dl), although insulin levels were significantly reduced on a day of fasting. There were also no significant differences in ß-hydroxybutyrate (ß-HBA) levels on the afternoon of fasting (0.29±0.4 mmol/L) compared to the day of feeding (0.41±0.8 mmol/L). To examine changes that occur with refeeding, monkeys were fasted for one day and the next day they were refed different amounts of food (0, 200, or 600 Kcal) at 1100 h; blood samples were taken at hourly intervals from 0800-2400 h (n=6). Monkeys refed 0 Kcal maintained low glucose (46.35±1.21 mg/dl), low insulin (12.35±0.76 μIU/ml), and high ß-HBA (0.62±0.03 mmol/L) throughout the refeed day. Monkeys refed 200 and 600 Kcal had a rapid increase in glucose and insulin levels and an immediate decrease in ß-HBA levels after refeeding. These levels remained constant in animals refed 600 Kcal. However, in animals refed 200 Kcal, glucose and insulin levels began to decline by midafternoon, while ß-HBA levels gradually increased. These data indicate that suppression of LH secretion begins to occur on a day of fasting while monkeys remain euglycemic and are not yet ketogenic, and that the degree of restoration of LH secretion correlates with the extent of the metabolic changes occurring during refeeding. It is hoped that the results of these studies will help to identify possible "metabolic signals" that modulate the neuronal systems governing LH secretion during periods of altered nutritional intake.

544.8

EFFECTS OF OVERFEEDING ON SUBSEQUENT FASTING-INDUCED SUPPRESSION OF PULSATILE LH SECRETION IN MALE RHESUS MONKEYS (*Macaca mulatta*). D.A. Schreihofer, D.B. Parfitt*, and J.L. Cameron. Depts. of Behavioral Neuroscience, Psychiatry, Physiology, and the Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.

We have previously shown that one day of fasting significantly suppresses the frequency of pulsatile LH secretion in adult male rhesus monkeys, and that this suppression is evident within the first four hours following a missed meal. We have hypothesized that restricting food intake may suppress the central drive to the reproductive axis via "metabolic signals" occurring during the transition from an energy storing (fed) state to an energy mobilizing (fasted) state. To further examine this hypothesis, we overfed adult male rhesus monkeys with the anticipation that animals would remain in a fed state longer, and that the decrease in LH secretion normally seen with fasting would be delayed. For these studies, monkeys were maintained with chronic indwelling venous catheters on a jacket/tether/swivel system. On day 1 of the study, 5 monkeys were allowed to overeat (i.e. fed 100 pellets of Purina monkey chow vs. the standard meal of 25-35 pellets at 1100 h), and on day 2 monkeys were fasted. Animals ate 1672±180 Kcal on the day of overeating, compared to 620± 38 Kcal on a day of normal feeding. Blood samples were collected from 1200 h to 2400 h at 20 min intervals on the day of fasting and on a separate day of normal feeding for measurement of pulsatile LH secretion. In contrast to our previous findings, when a day of fasting was preceded by overfeeding there was no suppression of mean plasma LH concentrations (normal feeding: 15.01±1.37 ng/ml; fasting: 14.33±0.62 ng/ml) or LH pulse frequency (normal feeding: 1.2±0.37 pulses/4 h; fasting: 1.2±0.2 pulses/4 h) during the first 4 h following the missed meal. However, there was a significant suppression ($P<0.05$) of both mean LH and LH pulse frequency measured from 1600 h to 2400 h on the fasting day following overfeeding. These findings suggest that the delay in the suppression of LH secretion is of short duration even though animals overate significantly. To begin assessing the metabolic state of animals who have overeaten, insulin was measured in four animals. On a day of normal feeding, the postprandial rise in insulin levels following the meal returns to baseline levels (<20μIU/ml) by 2400 h. In contrast, after overfeeding insulin levels remain elevated (>50μIU/ml) into the afternoon of the day of fasting (1200 h), suggesting that these animals are still in an energy storing state during the time when LH suppression is delayed. These data lend further support to the hypothesis that the suppression of pulsatile LH secretion during short-term fasting occurs during the transition from an energy storing state to an energy mobilizing state.

544.9

STIMULATION OF LUTEINIZING HORMONE-RELEASING HORMONE (LHRH) RELEASE FROM HYPOTHALAMIC EXPLANTS BY gamma-AMINOBUTYRIC ACID (GABA) IS BLOCKED BY beta-ENDORPHIN. Richard W. Clough, Dept. of Anatomy, Southern Illinois Univ. School of Medicine-Carbondale, Carbondale, IL. 62901

GABA has stimulatory and inhibitory effects on luteinizing hormone (LH) secretion in male and female animals, and, the direction of the effect presumably reflects a site specificity in the brain (Ondo, Science 186,1976). In vitro studies demonstrate that GABA stimulates LHRH release from arcuate nucleus-median eminence fragments (Masotto and Negro-Vilar, GABA and Endocrine Function, Raven Press, NY, 1986). We have confirmed that naloxone (NAL) stimulates LHRH release from preoptic area-mediobasal hypothalamic (POA-MBH) explants in perifusion tissue culture (Clough et al., Neuroendo. 51, 1990). The similarity between the effects of GABA and NAL led us to investigate whether the stimulatory GABA-effect may involve the opioid system. POA-MBH explants were removed and placed in culture as previously described (Clough, et al., Brain Res. 446,1988). Following equilibration, POA-MBH explants were exposed to culture media containing either: GABA ($2X10^{-4}M$); beta-endorphin ($1.4 \times 10^{-7}M$), or a combination of the two drugs. Subsequent to drug challenge, explants were exposed to KCl (57mM) for viability assessment. Fractions of effluent were collected every 15 minutes, frozen and subsequently assayed for LHRH by radioimmunoassay. Exposure of explants to GABA resulted in a significantly increased LHRH release. Beta endorphin had no effect on basal release. However, exposure of explants to combined GABA and beta-endorphin completely abolished the stimulatory effect of GABA on LHRH release. In all experiments, a final KCl challenge effectively stimulated LHRH release. This study suggests that the stimulatory effect of GABA on hypothalamic LHRH release, and presumably stimulation of pituitary gland LH, is mediated by inhibition of hypothalamic beta-endorphin neurons and subsequent release of tonic inhibition (as seen with NAL). Supported by NIH HD24426.

544.10

OPIATE REGULATION OF LHRH NEURONAL RESPONSIVENESS TO NOREPINEPHRINE (NE). **J-J. He and C.A. Barraclough.** Dept. Physiology, Sch. Med. Univ. Maryland, Baltimore, MD 21201.

These studies examined the effects of naloxone (NX) or morphine (MOR) on LHRH neuronal responsiveness to ICV norepinephrine (NE, 45 ug) alone or following electrochemical stimulation (ECS) of the medial preoptic area (MPOA) in ovariectomized, estrogen-treated rats.. Pretreatment with NX (5 mg/kg at -15 min) neither suppressed nor enhanced the release of LH evoked by ICV NE (45 ug at 0 min) or combined MPOA-ECS + NE. Thus, the opiate synapses which exist on LHRH neurons, do not to seem modulate LHRH neuronal responsiveness to NE but opiates suppress the presynaptic release of NE. In contrast, when MOR (20 mg/kg sc) was injected 45 min prior to ICV NE, LH release more than doubled (200-450 ng/ml). When MOR (-15 min) was combined with MPOA-ECS (0 min) + NE (30 min) peak LH values increased from 200 (NE only) to 1600 ng/ml. MOR blocks spontaneous LH surges and hypothalamic NE turnover and markedly increases prolactin secretion, an observation confirmed in these studies. This latter effect has been attributed to increased serotonin (5-HT) secretion which, in turn, may suppress dopamine (DA) release. To test this possibility, in other rats, MOR (10 ug/0.7 ul) was infused into the dorsal raphe nucleus (DRN) (-15 min) and ICV NE was given at 30 min. Amplified LH responses were equivalent to those obtained after sc MOR. Neither DRN-MOR nor saline alone altered basal LH secretion. Combined DRN-MOR + MPOA-ECS + NE resulted in an 8 fold rise in plasma LH (peak surge values). Thus, the combined priming of LHRH neurons with 5-HT (or suppression of DA) coupled with the removal of inhibitory GABA influences (by MPOA-ECS) and stimulation by NE may be important endogenous events which lead to LH surges. None of these treatments alone can produce this heightened LHRH neuronal response. Supported by HD-02138.

544.11

NEUROPEPTIDE Y (NPY) POTENTIATION OF LHRH-INDUCED LH SECRETION IS STEROID-DEPENDENT. J.E. Levine and A.C. Bauer-Dantoin. Department of Neurobiology & Physiology, Northwestern University, Evanston, IL 60208.

We recently demonstrated that NPY potentiates the ability of pulsatile LHRH infusions to restore LH surges in pentobarbital(PB)-blocked, proestrous rats. The present study tested the hypothesis that facilitory actions of NPY occur only during ovarian steroid-induced LH surges and not in unprimed, ovariectomized (OVX) animals. At 0900h on day 6 after OVX, female rats received 30 μg estradiol benzoate (EB) or oil sc, and 2d later received 5 mg progesterone (P) or oil sc. Hourly blood samples were obtained between 1100-2100h on the day of P/oil injections. At 1330h, rats received injections of PB to block endogenous LHRH release, or saline. Every 30min from 1400-1800h, PB-treated rats received iv pulses of LHRH (15ng/pulse) or saline, along with concurrent pulses of NPY (1 or 5μg/ pulse) or saline. Pulsatile LHRH infusions restored LH surges in EB+P treated, PB-blocked rats. Concurrent administration of NPY (5μg/pulse) significantly ($p<.01$) potentiated LHRH-induced LH surges in EB+P treated rats. The same dose of NPY was only marginally effective in rats treated with EB alone and completely ineffective in altering LHRH-induced LH secretion in oil-treated rats. These results demonstrate that the potentiation of LHRH-induced LH secretion in vivo is steroid-dependent and provide further support for the idea that the pituitary actions of NPY are specifically important for the generation of LH surges. (NIH HD20677)

544.12

STEROID ACTIVATION OF C-FOS EXPRESSION IN GnRH NEURONS IS BLOCKED BY PHENOXYBENZAMINE. J.C. Pearson, L. Jennes, E. DuPré*, and J. Hester*. Dept. of Anatomy, Wright State Univ. Sch. of Med., Dayton, OH 45435.

Progesterone (P) enhances the activation of GnRH neurons during estradiol (E)-induced LH surge, and together with E stimulates the expression of the proto-oncogene product fos in the GnRH cells. Since GnRH neurons lack P receptors it is suggested that the steroids influence GnRH cells via neural intermediaries. The present study tests the hypothesis that the influence of P on GnRH neurons is mediated at least in part by noradrenergic receptor mechanisms. OVX rats were primed with E implants followed by sc injection of P on day 2. After four hours, rats received an injection of the α-adrenergic receptor blocker, phenoxybenzamine (POB, 2mg/kg, i.v.), and were perfusion-fixed 2 hours later. Treatment with POB resulted in significantly lower peak LH levels as measured with RIA and in a dramatic reduction in the number of GnRH cells which showed fos immunoreactivity when compared to control rats. These data indicate that the enhancement of GnRH neuron activity by P was blocked by POB, and suggest that α-adrenergic receptor mechanisms are involved in the LH surge. (Support: WSU BMS Res. Grant and NIH HD24697).

544.13

ALPHA-1 ADRENERGIC REGULATION OF ESTROGEN-INDUCED INCREASES IN LUTEINIZING HORMONE-RELEASING HORMONE (LHRH) mRNA AND RELEASE. G.D. Weesner*, L.C. Krey* and D.W. Pfaff, Laboratory of Neurobiology and Behavior, The Rockefeller University, New York, NY 10021.

Prazosin, an α-1 adrenergic antagonist, was used to examine the relationship between adrenergic inputs and the stimulatory effects of estrogen on LHRH mRNA and release. Bilateral cannulae were implanted just dorsal to the preoptic area (POA). Estrous cycles were monitored daily by vaginal smears. On the morning of proestrous, each rat was ovariectomized and assigned to one of three treatment groups: Control - injected with sesame oil; Surge - injected with estradiol benzoate (EB, 10 μg) to produce an LH surge; or, Surge+Prazosin - injected with EB and a prazosin-filled inner cannula was put into the POA. Between 4-6 pm of the following day, rats were anesthetized, decapitated, trunk blood collected, and brains were stored in liquid nitrogen. *In situ* hybridization was performed using a ^{32}P end-labelled 59-mer complementary to LHRH mRNA. Reduced silver grains, proportional to LHRH mRNA content, were quantified. Treatment with estrogen alone resulted in a 50% increase ($P<.05$) in numbers of cells expressing LHRH. This estrogen-induced increase was completely blocked ($P<.01$) by prazosin. Prazosin also decreased ($P<.01$) the median number of grains per cell from 81 (Surge) to 65 grains per cell (Surge+Prazosin). When the number of grains in LHRH-expressing neurons were totalled, EB increased ($P<.05$) LHRH gene expression by 53%. Prazosin completely blocked ($P<.01$) the estrogen-induced increase. Finally, the estrogen-induced LH surge was blocked by the local administration of prazosin. Thus, coincident with the LH surge, estrogen increased the number of LHRH-expressing neurons as well as total potential LHRH synthetic capacity. Administration of prazosin into POA blocked the ability of estrogen to increase LHRH mRNA and LH release. Therefore, it appears that an endogenous α-1 ligand, probably norepinephrine, helps mediate the stimulatory effects of estrogen on LHRH biosynthesis and release.

545.1

INTESTINAL DISTENTION ACTIVATES A VAGO-VAGAL REFLEX TO ALTER WATER ABSORPTION BY RAT JEJUNUM IN VIVO. X. Zhang*, X. Chu*, W. Renehan, R. Fogel, Henry Ford Hospital, Detroit MI.

Little is known about the central pathways that transmit information about intestinal stimuli or how intestinal afferents regulate absorption. The aim of this study was to determine whether 1) stimulation of vagal mechanoreceptors by intestinal distention alters water absorption along the rat jejunum and 2) the role of the dorsal motor nucleus of the vagus (DMNV) in the mediation of this effect. Intestinal water absorption was measuured in two segments of intestine. One loop was distended, whereas the adjacent noncontiguous loop of jejunum served as control. Intestinal distention did not increase water absorption in the control group when sympathetic and parasympathetic nerves were intact. However, distention increased absorption in the control loop if the sympathetic nerves were cut. Truncal vagotomy or selective afferent vagotomy prevented the distention effect. Our data suggested that vagal afferents, terminating in the brainstem initiated a reflex involving vagal efferents. To further explore this phenomenon we used glass micropipettes filled with 3.0 M KCl to record the activity of DMNV neurons while distending a segment of the jejunum. We found that 37 of 44 DMNV neurons were inhibited by distention. These results indicate that intestinal distention decreases the activity of neurons in the DMNV, and the inhibition of DMNV activity is responsible for increased absorption in the small intestine.

545.2

FLUOXETINE (Fl) PRETREATMENT POTENTIATES INTRA-CISTERNAL (ic) TRH ANALOGUE-STIMULATED GASTRIC ACID SECRETION IN RATS. R.A.Shockley* and R.L.Stephens, Jr. Department of Physiology, The Ohio State Univ., Columbus, Ohio 43210.

Ic injection of the TRH analogue RX77368 (RX) has been demonstrated to produce a vagal-dependent stimulation in gastric acid secretion. Accumulating evidence exists regarding the interaction of serotonin (5HT) with TRH containing neuronal systems. This study was performed to assess the effect of 30 min pretreatment with the 5HT uptake inhibitor Fl (10mg/kg,iv; or 60μg,ic) on the TRH analogue-induced gastric acid response. Systemic Fl produced a 43 - 85% increase in ic RX (30-300ng)-induced gastric acid output in urethane-anesthetized, acute gastric fistula rats while not altering the response to ic RX (10ng). In addition, ic Fl produced a 71 % augmentation of the acid secretory response of ic RX (100ng). The results show that Fl pretreatment potentiates the effect of ic RX on acid secretion. The effect appears to be impulse dependent, and central sites appear to be involved, although peripheral sites of the interaction cannot be excluded. The data suggests an interaction of synaptic 5HT with a RX-elicited event (activation of TRH receptors and/or firing of the motor vagus), resulting in potentiation of the RX-induced gastric response. Supported by DK 42880.

545.3

IMMOBILIZATION-INDUCED HYPOCALCEMIA IS MEDIATED BY GASTRIC VAGAL ACTIVATION: THE STOMACH AS THE MAIN CALCIUM REGULATOR DURING STRESS CONDITION. S. Aou, J-Y. Ma*, H. Matsui* and T. Hori. Dept. of Physiology, Fac. of Med., Kyushu Univ., Fukuoka 812 Japan.

The mechanism of immobilization (IMB)-induced hypocalcemia was investigated in female Wistar and Wistar King A rats (120-160 g body weight). The rats were randomly divided into two groups: one was IMB group in which rats were tied their four limbs on the board bars in supine position for two hrs and the other was a control group which accepted the same treatment except IMB. Calcium ion levels were measured by the calcium ion meter (Ciba-Corning, type 634) using blood samples taken just after IBM by cardiac puncture under light ether anesthesia. The peripheral muscarinic antagonist, atropine methyl bromide, applied 20 min before the IMB suppressed IMB-induced hypocalcemia in a dose-dependent manner. The vagotomy of the cervical trunk, but not that of the thyroid/parathyroid branches, abolished the stress-induced hypocalcemia. In the subdiaphragma level, the vagotomy of the gastric branch as well as gastrectomy suppressed calcium reducing effect of IMB, while the vagotomy of the coeliac branch or partial resection of the intestine did not prevent stress-induced hypocalcemia. The inhibitors of gastrin release, secretin and galanin as well as the histamine H_2 blocker, cimetidine, were effective to suppress calcium lowering effect of IMB.

It is concluded that decreased calcium level caused by IMB is due to the activation of the vagus innervating to the stomach. Gastrin and histamine are also involved as the consequent factors through the activation of the gastric vagus.

545.4

MORPHOLOGY AND DISTRIBUTION OF VAGAL AFFERENT INNERVATION OF RAT GASTROINTESTINAL TRACT. H.-R. Berthoud, and T.L. Powley. Lab. of Regulatory Psychobiology, Purdue University, W. Lafayette, IN 47907.

In order to selectively label vagal afferents to the gastrointestinal tract, the fluorescent carbocyanine dye DiI (0.2-1.0 μL of 20% DiI-oil in methanol) was injected into the left nodose ganglion of rats that had undergone an ipsilateral supranodose vagotomy 12 days earlier. After survival times of 20-50 days the animals were perfused and specimens of mucosa, submucosa and muscularis externa layers from throughout the gut were either whole-mounted or sectioned in a cryostat and analyzed by conventional as well as laser scanning confocal epifluorescence microscopy.

DiI labeled afferent vagal fibers and terminals were found in all layers of the GI wall. For the first time vagal afferents in the circular and longitudinal smooth muscle layers of the stomach, presumably gastric stretch receptors, could be morphologically characterized. Projecting through the myenteric plexus, where they gave off collaterals, these mechanoreceptive fibers climbed to either layer, where they ran in close contact with the interstitial cells of Cajal, parallel to the muscle bundles for long distances and often giving off collaterals. In the myenteric plexus throughout the GI tract, profusely arborizing terminal trees were indicated by patches of fine DiI granules covering entire ganglia or individual ganglion cells and, together with the muscle collaterals, possibly providing the basis for local reflex activity. Numerous, putative chemoreceptive, vagal afferent fibers were found coursing through the submucous plexus and to richly innervate the mucosal villi.

With this technique it should be possible to characterize vagal afferents to the liver, pylorus and duodenum that serve important physiological mechanisms involved in energy metabolism and feeding. NIH grants DK-27627 and NS-26632.

545.5

FUNCTIONAL ZONATION OF RESPONSES TO ANTRAL MECHANORECEPTOR ACTIVATION IN THE DORSAL VAGAL COMPLEX (DVC) OF RATS. Monica J. McCann and Richard C. Rogers. Ohio State University, Dept. of Physiology, Columbus, OH 43210.

Activation of gastric vagal afferents (by antral distention or electrical stimulation of the vagus) either excites (ON) or inhibits (OFF) the firing rate of cells in the DVC. In our extracellular recordings, ON cells were located dorsal to the OFF cells. The average depth from the dorsal surface of the brainstem was 536μ ±15 and 628μ ±14 for ON and OFF cells, respectively ($p < 0.001$). This, together with histological evidence, suggested that ON and OFF cells have different functions, i.e., ON cells may be NTS neurons, whereas OFF cells could be DMN neurons.

We studied this hypothesis directly by using electrophysiological criteria to identify distention-related cells as NTS or DMN neurons. Our results support the hypothesis that ON and OFF cells can be functionally distinct populations of neurons. Almost all ON cells (94%) are NTS cells, whereas many OFF cells (64%) are DMN neurons. These studies provide insight into the central organization of gastric vago-vagal inhibitory reflexes. Supported by NS08690 (MJM) and NS24530 (RCR).

545.6

TOPOGRAPHY AND DENDROARCHITECTURE OF HEPATIC PREGANGLIONIC NEURONS WITHIN THE DORSAL MOTOR NUCLEUS OF THE VAGUS. F.B. Wang and T.L. Powley. Lab. of Regulatory Psychobiology, Purdue University, W. Lafayette, IN 47907.

The hepatic branch of the abdominal vagus, a branch implicated in the control of diverse physiological functions, contains roughly 200 efferent axons which originate from the dorsal motor nucleus of the vagus (dmnX). Our recent results with a digital atlas of the dmnX generated from a composite distribution of several animals (Neurosci. Abst. 15: 264, 1989) suggested that the hepatic preganglionics might be organized as two coherent cell groups. To further evaluate this organization and patterns revealed by the dendroarchitecture, the cells were identified and then filled with Lucifer Yellow: Sprague-Dawley rats received selective vagotomies sparing only the hepatic branch; the hepatic preganglionics were retrogradely labeled with Fast Blue; the animals were perfused; 200-300 μm horizontal sections of the brain stem were vibratomed; and the labeled cells were stained with intracellular iontophoresis of Lucifer Yellow.

The hepatic preganglionics were organized into two relatively compact ensembles. One group of cells lies just rostrolateral to the area postrema, within the medial gastric column. The second group lies somewhat more caudally and laterally, within the lateral column of the dmnX which gives rise to the accessory celiac branch. In each of the two aggregates, the dendrites of the neurons distribute predominantly horizontally and most remain within their respective ensemble. This dual compartmentation of the hepatic preganglionics suggests the hypothesis that the two ensembles might represent respectively the two major secondary branches which separate from the hepatic branch to innervate different visceral targets, namely one which projects to the liver or hepatic field and another to the small intestine and pancreas. NIH grants DK-27627 and NS-26632.

545.7

CHEMICAL CODING IN THE DORSAL MOTOR NUCLEUS OF THE VAGUS FOR GASTROINTESTINAL FUNCTIONS ? N.R. Carlson, H.-R. Berthoud, and T.L. Powley. Lab. of Regulatory Psychobiology, Purdue University, W. Lafayette, IN 47907.

The variety of receptors in the dorsal motor nucleus of the vagus (dmnX) suggests the possibility that selective control of different patterns of visceral responses might be achieved at least in part by chemical coding or addressing. Two such identified receptors, those for thyrotropin-releasing hormone (TRH) and angiotensin II (ANG II) are among the binding sites found in high density in the dmnX and could be involved in such a chemical coding scheme. To test this hypothesis, multiple-barrelled pipettes were used to pressure inject, in random order, large injections (300nl, designed to encompass a large portion the dmnX) of TRH (1mM), ANG II (1mM), and saline into the dmnX of 16 rats (the same site/animal) while gastric acid secretion and motility throughout the GI tract were simultaneously monitored.

As has been previously observed by others, TRH significantly increased acid output and gastric and duodenal motility. Further, TRH increased jejunal--but neither cecal nor colonic--activity. In contrast, ANG II did not activate either gastric secretory or motility, while it did significantly increase duodenal and motility. Electrical stimulation of the cervical vagus elicited responses from all locations. The different patterns of GI activation produced by the peptides suggest but do not prove that, in the dmnX, different endogenously released peptides may generate different visceral response patterns. Additionally the peptide-specific patterns suggest that at least some of the TRH responses are mediated by the gastric subnuclei of the dmnX and their corresponding peripheral branches of the vagus, whereas, conversely, some if not all of the ANG II responses are mediated by the celiac subnuclei of the dmnX and their corresponding peripheral branches.
NIH grants DK-27627 and NS-26632.

545.8

PANCREATIC NEURONAL CIRCUITS DEFINED BY TRANSNEURONAL TRANSPORT OF PSEUDORABIES VIRUS. C. Sternini, R. De Giorgio, K. Anderson, L.W. Enquist*, J.P. Card and L. Rinaman. CURE and Dept. of Medicine, UCLA School of Medicine, Los Angeles, CA 90073, Medical College of Pennsylvania, Philadelphia, PA 19129 and DuPont Merck Pharmaceutical Co., Wilmington, DE 19880.

Transneuronal transport of pseudorabies virus (PRV) was used to define the organization of neuronal circuits influencing pancreatic functions. Injection of PRV into the duodenal or splenic region of the rat pancreas identified a neuronal circuit including spinal afferent neurons, autonomic motoneurons of the brainstem and other central neurons throughout the neuraxis. Specifically, injection of PRV into the pancreatic parenchyma 1) revealed that spinal afferent neurons innervating the pancreas contain calcitonin gene-related peptide, 2) visualized a synaptically linked circuit of central neurons within the neuraxis involved in visceral function regulation and 3) identified a population of sympathetic preganglionic neurons in the intermediolateral cell column of the thoracic cord that project to postganglionic neurons in abdominal ganglia innervating the pancreas. Simultaneous injection of PRV and the β subunit of cholera toxin confirmed the brainstem-pancreatic connections revealed by pseudorabies virus transport. These findings confirm the specific uptake and transneuronal passage of α-herpesvirus in the nervous system and define the neuronal circuits that contribute to the control of pancreatic functions.
Supported by NIH grant DK38752 and DuPont Merck Pharmaceutical Co.

545.9

SEROTONERGIC MODULATION OF REPETITIVE FIRING ACTIVITY IN DORSAL MOTOR NUCLEUS NEURONS STUDIED *IN VITRO*. C.L. McNair and M.S. Dekin, School of Biological Sciences, University of Kentucky, Lexington, KY 40506-0225.

A brainstem slice preparation from adult guinea pigs was used to study both the location of serotonin (5-HT) containing nerve terminals and 5-HT's mechanism of action on dorsal motor nucleus (DMX) neurons. DMX neurons were filled with Lucifer yellow and stained with 5-HT antibodies. Labeled DMX neurons had large, ovoid cell bodies (30-40 um) and possessed several primary dendrites. Numerous serotonergic varicosities were observed in close apposition to cell bodies, while few appeared in the dendritic fields. During depolarization, DMX neurons exhibited a high frequency burst of action potentials which rapidly adapted with time, i.e., spike frequency adaptation (SFA). A large after-hyperpolarization (AHP) was observed at the end of the depolarization. In the presence of bath applied 5-HT, SFA was reduced in a dose-dependent manner (maximum response at 50 uM). 5-HT had no significant effects on input resistance (70 to 80 megohm), resting membrane potential (-55 to -65 mV), or the AHP. Both SFA and the AHP were blocked by the addition of 1 mM Cd^{++} to the bath suggesting that a calcium-activated potassium conductance (I_{KC}) mediated these properties. Apamin (100 nM), a selective antagonist for low conductance (LK) type I_{KC} channels, completely blocked SFA and the majority of the AHP. Charybdotoxin (50 nM), a selective blocker for intermediate and large conductance type I_{KC} channels had no effect on either SFA or the AHP. In the presence of both apamin and 5-HT, DMX neurons exhibited voltage-dependent conditional bursting activity. These data suggest that (1) 5-HT modulated a voltage-dependent inward current localized to the soma of DMX neurons and (2) this inward current could effectively compete with the LK type I_{KC} channels which normally limit the repetitive firing activity of these neurons. (Supported by NIH grants HL40369, HL02314, and RR07114).

545.10

NUCLEUS RAPHE OBSCURUS IN CONTROL OF GASTROINTESTINAL FUNCTION: AFFERENT PATHWAYS. Z.K. Krowicki* and P.J. Hornby. Dept. of Pharmacology and Exper. Ther., Louisiana State University Medical School, New Orleans, LA .

The caudal nucleus raphe obscurus (NRO) has emerged as an important nucleus in control of gastrointestinal function. The purpose of the present study was to determine the afferent pathways to the region of the NRO controlling GI function by using WGA-apoHRP-Au in Sprague-Dawley rats. Double-barelled micropipettes were filled with WGA-apoHRP-Au (1% solution) and L-glutamate (500mM) and inserted into the NRO in anesthetized rats with strain gauges attached to the stomach. Results. In two rats, L-glutamate microinjection increased the Minute Motility Index (+2.0 and +6.5) prior to WGA-apoHRP-Au (10 and 20nl) microinjection. Four additional rats received 10 nl WGA-apoHRP-Au into the same region of the NRO. Numerous retrogradely labelled cells were located in the rostral medullary raphe nuclei, of which some were also serotonin-immunoreactive (ir) cells, and in the A1 noradrenergic group, of which some were also tyrosine hydroxylase-ir cells. Retrogradely labelled cells were located in the dorsal hypothalamic nucleus, the region above the ventral tegmental area, and the dorsal pons. Few retrogradely labelled cells were noted in the paraventricular nucleus of the hypothalamus, bed nucleus of the stria terminalis and central amygdala. Conclusions: Medullary projections to the NRO are similar to those projecting to the dorsal vagal complex, whereas supra-medullary projections to the NRO differ substantially from those known to project to the dorsal vagal complex. These latter are potentially new pathways mediating control of GI function in anesthetized rats. Supported by the PMAF and an Institutional BRSG.

545.11

C-FOS IS EXPRESSED IN ANATOMICALLY DISTINCT BRAIN NUCLEI FOLLOWING INHIBITION OR POTENTIATION OF FOOD INTAKE AND GASTRIC MOTILITY IN RATS. B.R. Olson, G.E. Hoffman, A. Sved, E.M. Stricker, J.G. Verbalis. Departments of Medicine, Physiology and Behavioral Neuroscience, University of Pittsburgh, Pittsburgh, PA 15261

Most treatments that inhibit food intake (FI) and gastric motility (GM) in the rat result in activation of central oxytocinergic (OT) pathways, one of which projects to the dorsal motor nucleus of the vagus (DMN) and the nucleus tractus solitarius (NTS). These experiments mapped the sites of c-fos expression within the dorsal medulla, hypothalamic paraventricular nucleus (PVN), bed nucleus of the stria terminalis (BNST), and central amygdaloid nucleus (Ce) in male rats 1h after ip administration of chemical agents that either inhibit (CCK, 100 μg/kg) or stimulate (insulin, 5 U/rat) FI and GM. We also studied animals treated with 2-deoxy-D-glucose (2-DG, 500 mg/kg), which inhibits GM and stimulates OT release, but paradoxically increases food intake. The patterns of c-fos staining are summarized in table below:

	Medulla		PVN	BNST	Ce
Treatment	DMN	NTS	(OT)		lateral subdivision
CCK	-	+	+	-	+
INSULIN	+	-	-	+	-
2-DG	+	+	+	+	+

The c-fos expression following insulin-induced hypoglycemia and CCK suggests that functionally distinct neural pathways become activated with stimulation or inhibition of FI and GM. Activation of OT-neurons, the lateral subdivision of the Ce, and the NTS occurs with inhibition, but not with stimulation, of GM. However, OT-pathway activation is absent with insulin-stimulated FI but is present with 2-DG-stimulated FI, suggesting that glucoprivation-induced excitatory inputs to FI can override OT-related inhibitory ones under certain conditions in rats.

545.12

PARAVENTRICULAR NUCLEUS STIMULATION-INDUCED GASTRIC DAMAGE IN HYPOPHYSECTOMIZED RATS. L.E. Hierlihy, J.L. Wallace*[†] and A.V. Ferguson. Dept. of Physiology, Queen's University, Kingston, ONT K7L 3N6 and [†]Dept. of Medical Physiology, University of Calgary, Calgary, ALTA T2N 4N1.

Electrical stimulation in the paraventricular nucleus (PVN) of the hypothalamus has been shown to induce gastric mucosal lesions in the rat (Ferguson et al., 1988). Such damage is dependent upon descending projections to vagal motoneurons although vagal efferent stimulation in isolation does not induce any significant gastric damage. Considering the well established role of the PVN in the control of pituitary secretion, the present study was designed to determine whether pituitary hormones are required for the development of PVN-induced damage.

Hypophysectomized male Sprague Dawley rats were obtained from Charles River Inc. Fasted animals were anaesthetised with urethane and fitted with indwelling femoral arterial catheters and tracheal tubes. The animals were placed in a stereotaxic frame and a monopolar electrode advanced toward the region of PVN. Electrical stimulation was applied for 1 hour (200μA, 60Hz, 100μs). The stomach was then removed, examined macroscopically and assigned a damage score ranging from 0-3. The brain was perfused, removed and prepared for histological determination of stimulation sites.

PVN stimulation in hypophysectomized animals resulted in a gastric damage score of 2.0 (median) whereas electrical stimulation in anatomical regions immediately surrounding PVN did not induce significant damage (1.0). PVN-induced damage observed in hypophysectomized animals was not significantly different from that observed in animals with an intact pituitary (1.5). In conclusion, these studies indicate pituitary hormones do not play a major role in the formation of PVN-induced gastric damage.
Acknowledgments: Supported by MRC of Canada.

545.13

CHARACTERIZATION OF THE VISCERAL NEURAXIS BY TRANSNEURONAL PASSAGE OF PSEUDORABIES VIRUS.

R.R. Miselis, B.H. Lee*, L.W.Enquist* and J.P. Card, University of Pennsylvania, Phila., PA 19104 and DuPont Merck Pharmaceutical Co. Wilmingington, DE 1988.

We have used transneuronal passage of an attenuated strain of pseudorabies virus (Bartha) to define the visceral neuraxis innervating the esophagus, stomach and cecum. From each viscus the forebrain-spinal cord extent of the central visceral neuraxis was labeled in a projection specific fashion. The labeling in the vagal complex at short survival times occurred in a motor viscerotopic pattern as seen with conventional tracers. At longer survival times the virus passed transneuronally to label putative 2nd order neurons in the 1)intermediolateral cell column, nucleus of solitary tract and area postrema 2)A_1, A_5 and caudal raphe neurons, 3)juxtacapsular area of hypothalamus, 4)paraventricular hypothalamic nucleus, 5)central nucleus of amygdala, 6)bed nucleus of stria terminalis, 7)insular cortex, 8)2nd or 3rd order passage to SFO, OVLT and nucleus medianus, preoptic area, anterior and lateral hypothalamus, septum 9)3rd order passage to parabrachial nucleus and locus coeruleus. The pattern of viral replication in these regions was also projection specific as seen with conventional tracers. This provides highly defined visceral neuraxis directly related to specific visceral organs and ties the circumventricular organs into it.

545.14

DIAPHORASE POSITIVE NEURONS IN THE RAT OESOPHAGUS. D. Vyas, D. Bieger and C.R. Triggle[1]. Fac. of Med., Memorial Univ. of Nfld., St. John's, Nfld. Canada A1B 3V6 and [1]Dept. of Pharmacol. and Ther., Univ. of Calgary, Calgary, Al. Canada T2N 4N1

Previous work in our laboratory has suggested the involvement of NO (nitric oxide) in non-adrenergic non-cholinergic inhibition at the level of the tunica muscularis mucosae (TMM) of the rat oesophagus. Since diaphorase reportedly is a marker for NO-synthase containing neurons in both the CNS and PNS, we have examined its occurrence and distribution in this organ. Oesophagi of Sprague-Dawley rats were stretched, dissected into two layers viz, TMM and tunica muscularis externa (TME) and fixed in paraformaldehyde prior to staining. Diaphorase was reacted with either NADH or NADPH as electron donor and nitro blue tetrazolium as an electron acceptor. In adult animals, stained nerve cells were found throughout the myenteric plexus (MP) as solitary neurons or in groups of up to 15 per ganglion. Stained fibers and terminals were present in both TME and TMM. Staining was identical with NADPH or NADH as substrate. In larger ganglia, the staining was of uneven intensity and barely detectable in some cells. In contrast, in 5d old animals enzyme activity of MP ganglia was of uniform intensity. No neurons were seen in the submucous plexus of either young or old animals. We conclude : 1. Use of NADPH as substrate does not reveal a unique neuron population in the oesophageal MP. 2. The histochemical activity observed is probably due to DT-diaphorase. 3. In oesophageal MP neurons this enzyme is probably colocalized with markers other than those implicated in CNS neurons. 4. An association of DT-diaphorase with NO-synthase containing neurons cannot be ruled out at present. (Supported by MRC, Canada.)

SOMATIC AND VISCERAL AFFERENTS: NOCICEPTION

546.1

TEMPOROMANDIBULAR JOINT NOCICEPTORS RESPONDING TO HORIZONTAL AND VERTICAL DISPLACEMENTS OF THE MANDIBLE OF THE GOAT. B. Loughner* and B. Cooper.
Depts. of Oral and Maxillofacial Surgery, Oral Biology and Neuroscience, University of Florida, Gainesville, FL., 32610.

Experiments were undertaken to characterize nociceptors of the temporomandibular joint (TMJ). TMJ afferents were isolated from the trigeminal ganglion with tungsten microelectrodes. Force-movement curves indicated that capsular nociceptors responded exclusively to intense forces that produced extreme displacements of the mandible. Thirty-four units with receptive fields on the TMJ capsule were activated by jaw movement. Nineteen afferents responded preferentially to movement in the vertical plane (VP or opening) and 15 responded best to movement in the horizontal plane (HP or lateral displacements). Once preferred movements were determined, tests of dynamic and static reactivity were conducted. Simple linear regression was used to construct power functions relating either instantaneous (inst.) dynamic force, inst. force velocity, inst. movement, static force, and inst. position to inst. interspike interval. Comparisons between best fitted curves suggested that both VP and HP reactivity was best characterized by dynamically applied stimuli (16/19 VP; 10/12 HP). Very few units transduced static force or position (4/19 VP and 3/15 HP), and 19 additional units (12 VP and 7 HP) could not be fit to functions. Conduction velocities were observed to be in the A delta and C fiber range, but due to inaccessibility of many of the receptive fields, could only be obtained in a portion of the population. (supported by NIDR, DEO8701 and DEO7200.

546.2

THE ACTION OF NGF ON SENSORY NEURONS MATURING IN VIVO IS SPECIFIC FOR HIGH THRESHOLD MECHANORECEPTORS (HTMRs). A. M. Ritter and L. M. Mendell
SUNY at Stony Brook, NY 11790

Dorsal root ganglion (DRG) cells subserve a multitude of physiological functions, and some of this heterogeneity is reflected in somal spike shape. HTMRs, which convey information about noxious mechanical stimuli, have long duration, large amplitude spikes with an inflection or "hump" on the falling phase. Low threshold mechanoreceptors (LTMRs) which respond to innocuous stimulation of the skin, have smaller amplitude, shorter duration spikes lacking the hump. NGF is known to be transported by a subpopulation of DRG cells in the adult animal, and here we ask what effect chronic treatment has on somal spike shape. Rats were treated with NGF (1 μg/g s.c.) from birth to 5 weeks of age. Intracellular recordings were made under α-chloralose anaesthesia from physiologically identified DRG cells with conduction velocities above 2 m/s. The somal spikes of LTMRs were completely unaffected by this treatment. However, somal spikes of HTMRs were longer in duration than spikes of HTMRs from untreated control animals. No accompanying change was seen in spike amplitude, AHP duration, input resistance, or resting membrane potential. Thus, the effect of NGF treatment seems to be specific for HTMRs, and opposite in direction to the effects of anti-NGF (Ritter and Mendell, 1990, Soc. Neurosci. Abstr. Vol 16(1):477). It has been reported that NGF shortens AP duration in cultured embryonic DRG cells (Chalazonitis et al, 1987, PNAS 84:289). It appears that NGF acts differently on intact neurons than on ones which have been axotomized and cultured. Supported by MH 18018 and NS PO1 14899

546.3

REGULATION OF NOCICEPTOR FUNCTION BY NGF IN THE NEONATAL AND ADULT RAT. By G.R. Lewin, A.M. Ritter and L.M. Mendell Dept. Neurobiology & Behavior, SUNY at Stony Brook, NY11794.

Treatment of rats with anti-sera to NGF has consequences which are specific for nociceptive afferents in the sural nerve (SN) (Ritter et al., Nature 350: 500, 1991). We now ask what are the physiological consequences for these afferents if they are provided with excess NGF *in vivo*. To examine this question we treated rats with NGF (1-2 μg/g, s.c.) every day for the first week then every other day until the end of the treatment. Two treatment regimes were used, neonatal NGF (from birth to 2 weeks) and adult NGF (from 2-5 weeks). When these treatments ceased animals were left to recover for at least 3 weeks, they were then anaesthetized with urethane (1.25g/Kg) and recordings were made in dorsal root filaments from Aδ afferents (2-17 ms^{-1}) projecting through the SN. NGF treatment did not alter the make-up of the Aδ population (unlike anti-NGF); about 40% were High Threshold Mechanoreceptors (HTMRs). Seventy-six percent (28/37) of control HTMRs were slowly-adapting (SA) and 24% (9/37) were rapidly adapting (RA); the latter tended to have higher mechanical thresholds (Burgess & Perl, J Physiol (Lond) 190: 541, 1967). A mechanoreceptive field could not be found for five fibers, ie. silent fibers. Adult NGF treatment left the Aδ population unchanged; 61% (11/18) of HTMRs were SA, 39% (7/18) were RA and two silent fibers were found. The mechanical thresholds (Von Frey hairs) of control and adult NGF treated SA HTMRs were not significantly different, being 9.8 $\pm$ 4.8g and 4.2 $\pm$ 0.6g, respectively. In contrast, HTMRs in neonatally NGF treated animals were all of the SA type (33 units). Their mean mechanical thresholds were significantly lower than control SA HTMRs (mean 2.5 $\pm$ 0.2g), $p<0.05$. Furthermore, no silent fibres were encountered in these animals. These experiments show that excess NGF can have profound and long lasting effects on Aδ nociceptors, but only if given in the first 2 weeks of life. Supported by NIH-NS 14899, NS 16996, and NIMH 18018.

546.4

THE POST-NATAL DEPENDENCE OF MYELINATED NOCICEPTORS ON NGF IS NOT MEDIATED BY CELL DEATH. By L.M. Mendell, G.R. Lewin and A.M. Ritter Dept. Neurobiology & Behavior, SUNY at Stony Brook, NY11794.

By chronically treating animals with anti-sera to 2.5S NGF (anti-NGF treatment) it is possible to produce an almost total depletion of Aδ (2-13 ms^{-1}) high threshold mechanoreceptors (HTMRs) with a concurrent increase in D-hair afferents in the cutaneous sural nerve (SN) (Ritter et al. Nature 350: 500, 1991). Here we have asked if sensory neuron cell death induced by anti-NGF treatment is necessary for this loss of HTMRs to occur. Rats were treated with anti-NGF (5μl/g, s.c.) from post-natal day (PND) 0-14 and from PND 2-14. When these animals reached adulthood (5 weeks) they were anaesthetized with urethane (1.25g/kg) and recordings were made in dorsal root filaments from Aδ afferents projecting through the SN. After anti-NGF treatment from PND 0-14 and 2-14 HTMRs made up only 16% and 17% of the total Aδ afferents respectively, compared to 45% in control animals. In a parallel series of experiments sensory neurons in the L4 and L5 dorsal root ganglia of anti-NGF treated animals were counted using the method of Rose & Rohrlich (J Comp Neurol 263: 365, 1987). Anti-NGF treatment from PND 0-14 produced a significant ($p<0.05$) sensory cell loss averaging 20%. However, only small C cells with mean diameters of approximately 25 μm or less were susceptible to this effect; medium size Aδ cells (Harper & Lawson J Physiol (Lond) 359: 47, 1985) were probably not affected. Furthermore, anti-NGF treatment from PND 2-14 produced no significant cell loss despite producing the same depletion of HTMRs. Thus the loss of HTMRs observed physiologically after anti-NGF treatment can occur in the absence of sensory neuron death. This supports the idea that in the absence of NGF putative HTMRs take on the phenotype of D-hair afferents which increase in number after anti-NGF treatment. Supported by NIH-NS 14899, NS 16996, and NIMH 18018.

546.5

MUSCLE NOCICEPTORS IDENTIFIED IN HUMANS: INTRANEURAL RECORDINGS, MICROSTIMULATION AND PAIN. D.A. Simone, G. Caputi, P. Marchettini and J. Ochoa. Good Samaritan Hosp. and Med. Ctr., Portland, OR 97210, and Oregon Health Sciences University.

Although muscle pain is a common feature in athletics and in various diseases, little is known about underlying somatosensory mechanisms (Marchettini et al., Neurology, March 1991, page 164). We have used intraneural microstimulation and recording in human nerve fascicles supplying skeletal muscle to identify muscle nociceptors. During intraneural stimulation of common peroneal nerve fascicles, seven subjects identified and mapped a field of deep cramp-like pain projected to muscle. Through natural stimulation with mechanical pressure on projected fields, receptive fields of mechanoreceptor units with moderate to high threshold could be located within or adjacent to the areas of projected pain. Conduction velocities ranged 3.1-13.5 m/s for Group III (n=7) and 0.6-1.9 m/s for Group IV (n=6) fibers. None of the Group III or Group IV units were spontaneously active. Mean receptive field areas, mapped with a 3 mm^2 blunt probe, were 3.2 ±0.84 and 4.7 ±1.03 cm^2 for Group III and Group IV afferents, respectively. The initial area of projected pain experienced immediately upon intraneural stimulation at threshold for pain sensation ranged from 0.78-20.4 cm^2. Continuous intraneural stimulation at steady intensity resulted in gradual enlargement of the painful area.

Injection of 0.01% capsaicin into one receptive field caused broad cramp-like pain: the recorded Group III nociceptor became spontaneously active.

This constitutes the first electrophysiological characterization of both Group III and IV human muscle nociceptors, and a direct endorsement that their activity evokes cramp-like pain. Supported by NIH NS 24766 and 28747.

546.6

DISSOCIATION OF CAPSAICIN DESENSITIZATION AND INHIBITION OF PLASMA EXTRAVASATION EVOKED BY ANTIDROMIC C-FIBER STIMULATION. Richard B. Carter, Joan M. Meyer, Georgiana C. Salyers*, and Lisa M. Leming*, Miami Valley Laboratories, The Procter & Gamble Company, Cincinnati, OH 45239.

Capsaicin evokes an initial neurogenic response that decreases upon repeated administration. Plasma extravasation (PE) was used to measure neurogenic response and was determined by measuring the increase in Evans blue dye content of skin from the dorsal hindpaw evoked by topical capsaicin application or antidromic stimulation of the saphenous nerve. Capsaicin (0.125%-1.0%) was applied topically to the dorsal surface of one hindpaw and vehicle to the other. PE was determined 24 hrs later. Whereas a single application of capsaicin produced a marked reduction in capsaicin-evoked PE, it failed to affect antidromically-stimulated PE. To determine whether desensitization represents a specific loss of capsaicin sensitivity or a generalized loss of chemical responsivity, either xylene (100%) or mustard oil (1.6%) was used to evoke PE. A single application of capsaicin produced a marked reduction in both xylene- and mustard oil-evoked PE. The effect of frequency of capsaicin dosing on block of neurogenic inflammation was also examined. Capsaicin (0.125%-1.0%) was applied topically either 1 time/day for 1 day, 1 time/day for 4 days, or 3 times/day for 1 day. Whereas a single desensitizing application of capsaicin failed to inhibit antidromically-stimulated PE 24 hrs after dosing, capsaicin applied 3 times daily for 1 day did. These effects were dose-dependent. These data indicate that desensitization and block of neurogenic inflammation are distinct and separable actions of capsaicin. Desensitization to capsaicin is accompanied by a generalized loss of chemical sensitivity in unmyelinated sensory afferent fibers.

546.7

RUTHENIUM RED ANTAGONISM OF CAPSAICIN'S INHIBITORY EFFECT ON C-FIBERS IN THE ELECTRICALLY-INDUCED PLASMA EXTRAVASATION MODEL. M.M. Rolfes* and J.M. Meyer. Procter & Gamble Company, Cincinnati, OH 45239-8707

Ruthenium Red (RR) is an inorganic dye which has been used in studies to antagonize the actions of capsaicin on sensory neurons and their terminals. In these studies the effect of RR on capsaicin inhibition of electrically-induced cutaneous plasma extravasation (P.E.) was investigated. Ruthenium Red or vehicle was injected near the saphenous nerves of male, Sprague-Dawley rats, followed 15 minutes later by capsaicin or vehicle injection. Two hours later, Evans blue dye was injected in the jugular vein and the saphenous nerves were electrically stimulated to produce P.E.

RR control studies showed no significant difference in P.E. between RR- or saline-treated limbs. In capsaicin control studies, 0.327 mM capsaicin inhibited P.E. by 47.6%, while 3.27 and 16.35 mM capsaicin inhibited P.E. by 72% and 87.8%, respectively. In experimental studies, the inhibitory effect of 0.327 mM capsaicin was antagonized by all concentrations of RR tested, while 3.27 mM capsaicin was antagonized by 10 mM and 1.0 mM RR, but not by 0.1 mM RR, and 16.35 mM capsaicin was antagonized by 10 mM RR, but not by 0.1 or 1.0 mM. This dose-responsive effect of RR on capsaicin inhibition which shifts the capsaicin dose-response curve to the right and down suggests that RR may be a competitive antagonist of capsaicin.

546.8

CONDUCTION VELOCITY (CV) OF RAT PRIMARY AFFERENT NEURONES WITH PERIPHERIN-LIKE IMMUNOREACTIVITY (PER-LI) OR CARBONIC ANHYDRASE ACTIVITY (CA). E. Prabhakar* and S.N. Lawson*, (SPON: Brain Research Association.). Dept. of Physiology, Medical School, Bristol BS8 1TD.

PER-LI is in all neurofilament (NF) negative and a few NF positive rat dorsal root ganglion (DRG) neurones all with small somata (Ferri et al., Brain Res. 515:331 1990). Since NF negative rat DRG neurones have C-fibres, it seemed possible that PER-LI was in all C- and some or all Aδ-neurones. If, as previously suggested (Szabolcs et al., Brain Res. 492:129 1989), CA is restricted to type I and II proprioceptive afferents it should be expressed only in fast conducting A-neurones.

We have made intracellular recordings from L3, L4 and L5 DRGs *in vitro* at 36.5±1°C, taken from pentobarbitone anaesthetised 6-8 week female Wistar rats. The neurones were classified according to their peripheral nerve CV into Aα/ß- (>12m/sec); Aδ- (1.5-12m/sec) and C-neurones (<1.5m/sec). After recordings were complete, a fluorescent dye (ethidium bromide or Lucifer yellow) was injected into the soma. The DRGs were then fixed with Zamboni's fixative and frozen serial 7µm sections were cut. PER-LI was examined using a polyclonal antibody, and Hanssons' method (Hansson, Histochemie 11: 112-128, 1967) was used to detect CA in the dye-filled neurones.

Of the labelled cells tested, all A-fibre neurones with CVs>10m/sec were positive for CA, while the slower conducting C-neurones were negative. PER-LI was found in most C-neurones but only in 8% of A-neurones and these had the slower conducting Aδ fibres.

These data indicate that PER-LI is indeed only in C- or slowly conducting Aδ-neurones, while CA is only in neurones with faster conducting A-fibres.

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546.9

AXON REFLEX VASODILATATION IN CAT TOOTH-PULP. B.Matthews and N.Vongsavan* Dept. of Physiology, University of Bristol, Bristol BS8 1TD, England.

In recent experiments we have shown that electrical stimulation of the peripheral cut end of the inferior alveolar nerve in cats causes both pulpal vasodilatation and an increase in the rate of outward fluid flow through exposed dentine in the lower canine tooth. These effects required the recruitment of small Aδ and C fibres; stimulating the Aβ fibres alone had no effect. In the present experiments we have investigated whether the vasodilatation is produced by stimulation of afferent fibres with receptors in the same tooth.

Young adult cats were anaesthetized with sodium pentobarbitone (42mg/kg i.p. then 3mg/kg i.v. as required). Observations were made on their lower canine teeth. Approximately 1mm was removed from the tip of each tooth with a diamond disc under Ringer's and the exposed dentine was etched for 30s with 30% orthophosphoric acid. The exposed dentine was enclosed by a cap filled with Ringer's. A laser Doppler flow meter (model MBF3D; Moor Instruments, Axminster, England) was used to monitor pulpal blood flow. Its probe was fixed to the tooth at right angles to the enamel surface and 2mm from the labial gingival margin. Both the ipsilateral cervical sympathetic trunk and inferior alveolar nerve (IAN) were cut to prevent reflex effects.

Stimulation of the exposed dentine for 30s by gentle mechanical probing, application of osmotic stimuli (sat. $CaCl_2$), drying, and sub-atmospheric pressures (-250 to -500 mmHg), which are known to excite intradental nerves, all caused an increase in pulpal blood flow. The increase was between 24.0 and 73.7% of the resting flow. The blood flow took 6-10 min. to return to resting levels, similar to that observed after IAN stimulation. The results provide strong evidence for a axon reflex vasodilatation in tooth-pulp. Similar evidence has been obtained by Olgart et al., (*Acta Physiol Scand* 1988, 133:399) but they did not exclude reflex effects.

546.10

MECHANOSENSITIVE AND CHEMOSENSITIVE UNITS OF THE LUMBAR SPINE: AN IN VITRO STUDY. J Cavanaugh, A Avramov*, AC Ozaktay. Bioengineering Center, Wayne State Univ., Detroit, MI 48202.

An in vitro model was developed to characterize the response of somatosensory units of the lumbar spine to mechanical loading and chemical mediators of nociception. **METHODS:** 24 male New Zealand White rabbits were anesthetized with Na pentobarbital, iv. The lumbar spine, perfused with Kreb's solution through the abdominal aorta, was excised with dorsal roots intact and spinal cord removed and clamped in a chamber filled with Kreb's solution. While recording from dorsal roots, the spine was loaded in tension and compression via a threaded rod attached to a 45 kg load cell. Units were also characterized by their response to local mechanical and electrical stimulation and by injection of algesic substances interarterially or locally. **RESULTS:** Low threshold mechanoreceptors, high threshold mechanoreceptors and phasic receptors were characterized in the lumbar facet joints and paravertebral muscles. Conduction velocities ranged from 0.4 to 22 m/s. Some units responded to spine loads less than 1 kg while others did not respond until loads exceeded 5 kg. Other units were activated by injection of bradykinin and serotonin into the intervertebral disc and adjacent tissue. **ACKNOWLEDGEMENTS:** Supported by NIH Grant NS-28994 and a Whitaker Foundation Grant.

546.11

FINE CUTANEOUS AFFERENT FIBERS IN THE RAT. J.W. Leem, K. Sheen, W.D. Willis and J.M. Chung. Marine Biomed. Inst., Depts. of Anat. & Neurosci. and of Physiol. & Biophys., Univ. Texas Med. Br., Galveston, TX, 77550.

The aim of this study was to make a systematic examination of the properties of fine afferent fibers in the sural nerve innervating the skin of the rat hind foot.

Single unit recordings were made from fine filaments dissected from the sural nerve in 41 anesthetized (sodium pentobarbital, 50 mg/kg) rats. The properties of units were examined using various mechanical and thermal stimuli applied to the cutaneous receptive fields with intensities ranging from innocuous to noxious.

Of a total of 95 fine afferent units identified, 29 were Aδ (3.28-29.41 m/s) and 66 C fibers (0.49-1.70 m/s). Aδ fiber samples included nociceptors (76%), cold receptors (7%), and D-hair receptors (17%). C fibers included nociceptors (47%), cold receptors (20%), and mechanoreceptors (33%). No warm receptors were identified. Basically, the properties of both Aδ and C fibers are similar to those described in other mammalian species. The proportions of units of each type, however, are different.

The results indicate that mechanoreceptors and cold receptors are common among the C fiber population in rat skin, whereas the predominant type of Aδ fibers is nociceptors. The proportion of different types of fine fibers in the sural nerve is more comparable to the cat than the primate. (Supported by NIH grants NS21266, NS11255 and NS09743 and a grant from Bristol-Myers Squibb Co.)

PAIN MODULATION: SPINAL AND TRIGEMINAL

547.1

NOXIOUS CHEMICAL STIMULATION OF THE NASAL MUCOSA WITH CO_2 PULSES: CORRELATION OF HUMAN PSYCHOPHYSICAL DATA WITH RESPONSES OF RAT MEDULLARY DORSAL HORN NEURONS. I. Euchner*, P. Peppel*, F. Anton* (SPON: ENA). Institute of Physiology I, University of Erlangen-Nürnberg, 8520 FRG.

For noxious stimulation of the nasal mucosa an olfactometer presenting precisely controlled CO_2 pulses was used. These stimuli were embedded in a constant flow of warm and humidified air (flow rate in rat: 6 ml/s; in human: 85 ml/s).

Twelve volunteer subjects rated their pain intensities with a VAS. Randomized CO_2 concentrations ranging from 50-85% (pulse duration 2s) were used to plot the respective stimulus-response functions (SRF). The effect of stimulus duration (CO_2 pulses ranging from 0.125 - 4s, constant concentration) was examined in addition. Electrophysiological experiments with comparable stimulation protocols were performed in halothane anesthetized rats. The interstimulus interval was 30s in all the tests.

In both psychophysical and electrophysiological experiments we found consistent and reliable linear SRFs to different concentrations of CO_2. In humans, pain intensity increased with stimulus durations lasting up to 1s and then reached a plateau level. This finding was paralleled in a subpopulation (11 of 20) of rat MDH neurons which displayed a phasic discharge behaviour to whatever stimulus duration being presented. The other neurons showed phasic and tonic responses.

Our results prove that the psychophysical response behavior to controlled CO_2 pulses and the respective discharges of MDH neurons are closely correlated. The method described here thus provides a precise tool for the quantitative physiological and pharmacological investigation of chemically induced pain. (supported by the DFG)

547.2

EFFECTS OF TWO PERIPHERAL NERVE MANIPULATIONS THAT AFFECT NOCICEPTION ON NERVE FIBER CALIBER AND ON GLUTAMATE AND SUBSTANCE P (SP) IMMUNOREACTIVITY IN THE DORSAL HORN OF THE RAT. W.M. Al-Ghoul and A. Rustioni. Dept. Cell Biol. and Anat., Univ. of North Carolina, Chapel Hill, NC 27599.

Two peripheral nerve manipulations that cause long term effects on nociception are (1) loose ligature (a manipulation that decreases nociceptive thresholds and is a possible chronic pain model) and (2) capsaicin (a C-fiber neurotoxin that increases nociceptive thresholds) administration. We examined the effects of these manipulations on nerve fiber caliber and on glutamate and SP (two neurochemicals implicated in mediation of nociception) immunoreactivity in the superficial laminae of the dorsal horn of adult rats 2-4 weeks after loosely ligating the left sciatic nerve or 2-3 months after applying capsaicin to the right saphenous nerve. Fiber caliber and density of immunoreactivity were quantified using video microscopy.

Ligature resulted in an 18 to 54% drop in mean cross sectional area of sciatic nerve myelinated fibers on the ligature side relative to the control side (previous studies have shown that capsaicin impairs unmyelinated fibers). Furthermore, ligature reduced SP immunoreactivity by 18 to 36% compared to the control side but it did not affect the density of glutamate immunoreactivity. Conversely, capsaicin application did not affect the density of SP immunoreactivity but it resulted in an increase of 10% in the density of glutamate immunoreactivity as compared to control. Thus, differences in the effects of loose ligature and capsaicin on behavior are paralleled by differences in their effects on nerve structure and on immunoreactivity in the dorsal horn. Additional work is underway to examine the effects of the two manipulations on NMDA receptors in the superficial dorsal horn.

547.3

NEURONAL RESPONSES TO STIMULATION OF THE CERVIX, UTERUS AND COLON IN THE SPINAL CORD OF FEMALE RATS. C.H. Hubscher, K.J. Berkley and P.D. Wall*. Dept. Psychology, Florida St. Univ., Tallahassee, FL 32306.

Afferents from the cervix arrive in the rat spinal cord in two distinct areas, S1 and L1. Sixty-three cells which responded to pressure on the cervix were examined in these two areas in 7 nulliparous rats in estrus. The rats were decerebrated and had a T10 transection under brief anesthesia. In addition to the cervix, mechanical stimuli were applied to the uterus, colon and skin. Only minimal stimuli were used to avoid long-term sensitization. In the S1 segment, 33 cervix-responding cells were found in all dorsal horn laminae. All these cells had extensive cutaneous receptive fields and 45% also responded to the colon (39% excited, 6% inhibited). In addition, 42% responded to the uterus (18% inhibited by one uterine horn and excited by the other, 18% inhibited by the contralateral or both horns, 6% excited by either horn). In the L1 segment, 30 cervix-responding cells were found in the deep dorsal horn. All had large cutaneous receptive fields, 57% were excited by the colon, while 76% were excited and 8% inhibited by uterine distension of either horn. Thus, in both areas, all cervix-responding cells had in addition cutaneous receptive fields but they were differentiated by their location and by the degree of convergence from other pelvic structures. (Supported by NIH grant RO1 NS11892.)

547.4

TONIC DESCENDING MODULATION OF SPINAL NEURONS WITH RENAL INPUT. A. Standish, M. A. Vizzard, and W. S. Ammons. Department of Physiology, Thomas Jefferson University, Philadelphia, Pennsylvania

Experiments were conducted to determine the effect of tonically active descending pathways on thoracolumbar spinal neurons that respond to renal nerve stimulation in anesthetized cats. The influences of tonic descending controls on seventy-two spinal neurons were assessed by examining changes in cell discharge rate associated with reversible block of spinal cord conduction by local cooling. We determined the effect of spinal cold block on spontaneous activity, neuronal responses evoked by renal afferent nerve stimulation and neuronal responses to somatic stimulation. The interruption of descending pathways by cold resulted in either enhanced (tonically inhibited neurons), reduced (tonically excited neurons), or unchanged neuronal responses. The spontaneous activity of 61% of the neurons increased from 7.4 ± 2.1 imp/s before cooling to 24.1 ± 5.0 imp/s during cooling. A decrease in activity was observed in 10% of the neurons and 29% of the neurons had no change in activity. Cooling increased 75% of cell responses to renal nerve stimulation. Approximately a two fold increase in the spikes/stim for both A-delta and C-fiber mediated responses were observed. In addition, four neurons had C-fiber input which was revealed by cold block. Decreased responses to A-delta and C-fiber input occurred in 13% of the neurons during cooling. Twelve percent of the cell responses to renal nerve stimulation were not affected by cold block. All but one of the unchanged responses were mediated by A-delta input. All neurons had somatic receptive fields and most exhibited increased responses to somatic stimulation during cooling. In addition, receptive field sizes of 23 neurons increased during cold block. These data show that the majority of spinal neurons with renal input had increased spontaneous activity, increased cell responses to renal afferent nerve stimulation, increased cell responses to somatic stimulation and therefore were predominantly modulated by tonic descending inhibitory influences. Supported by NIH Grant HL363678.

547.5

MATURATION OF APV- AND SPANTIDE-SENSITIVE SLOW VENTRAL ROOT POTENTIALS IN RAT SPINAL CORD. L. M. Gibbs, J. J. Kendig. Department of Anesthesia, Stanford University School of Medicine, Stanford, CA 94305-5123

The neonatal (1-6 day old) rat spinal cord exhibits a slow ventral root potential (slow VRP) of very long duration (10-40 s). Dorsal root-evoked motor neuron responses in older animals are reported to be of much shorter duration (1-3 s). We have investigated the maturation of the slow VRP in isolated superfused (27-28°C) spinal cord preparations from rats of ages 1-21 days, intact, hemisected or prepared as thick transverse slices. In cords from animals 1-9 days old, a single stimulus to a lumbar dorsal root evoked a monosynaptic reflex followed by a 20-40 s slow VRP at the corresponding ipsilateral ventral root; maximum amplitude of the slow VRP was at 3-4 s. Both the substance P antagonist spantide (10 μM) and the NMDA receptor antagonist APV (20 μM) depressed the slow VRP. Cords from 12-21 day animals displayed a VRP of 3-4 s duration, with maximum amplitude <1 s after the stimulus. APV, but not spantide, depressed this response; low Mg++ increased the amplitude but not the decay time constant. These results suggest that a very slow substance P-mediated component of the VRP is selectively lost or inhibited at 10-11 days postnatal development in rat spinal cord.

547.6

TONIC DESCENDING INHIBITION OF SLOW VENTRAL ROOT POTENTIALS IN ISOLATED NEONATAL RAT BRAINSTEM-SPINAL CORD. A. Tarasiuk*, L. M. Gibbs and J. J. Kendig. Department of Anesthesia, Stanford University School of Medicine, Stanford, CA 94305-5123

Descending inhibition from brainstem nuclei modulates responses to noxious stimuli; its presence in the neonatal rat is controversial. We examined the extent of tonic descending inhibition in brainstem-spinal cords isolated from 1-6 day old Sprague-Dawley rat pups. Brainstem and lumbar regions were superfused separately. Slow ventral root potentials (slow VRP's) (10-40 s), which are mediated by NMDA receptors and by substance P and are linked to nociception, were evoked by electrical stimulation of a lumbar dorsal root and recorded at the corresponding ipsilateral ventral root. Integrity of brainstem-spinal cord connections was verified by evoking a medullospinal reflex from the trigeminal nerve root to the lumbar ventral root. Three different manipulations were used to assess descending modulation: severing the brainstem from the cord, cooling the brainstem from 28 to 15°C, and perfusing the brainstem with the local anesthetic lidocaine (1-5 mM). In each case the amplitude of the lumbar slow VRP increased, by as much as 25%. The effects of cooling and of lidocaine were reversible. Descending projections from brainstem nuclei are anatomically present in the neonate; these results show that they functionally inhibit slow spinal reflexes.

547.7

HYBRIDIZATION HISTOCHEMICAL ANALYSIS OF SPINAL NEURONS THAT EXPRESS THE α_2 ADRENERGIC RECEPTOR IN A RAT MODEL OF PERIPHERAL MONONEUROPATHY Frank Williams, Angela Birnbaum, George Wilcox, and Alvin Beitz. Departments of Veterinary Biology and Pharmacology, University of Minnesota,St. Paul, 55108

Several recent reports have implicated brainstem noradrenergic projections to the spinal cord in the modulation of antinociception. The present studies sought to determine whether chronic pain is associated with a physiologic response to increased adrenergic activity in the spinal cord dorsal horn. Specifically, we sought to test whether the biosynthesis of α2 adrenergic receptor mRNA is altered. Unilateral sciatic ligatures were placed in male Sprague-Dawley rats according the the method of Bennett and Xie (Pain 33:87). Hyperalgesia on the ligatured side was confirmed by comparing the latency of paw withdrawal following the plantar application of radiant heat. Difference scores averaged -5.2 sec. Sixteen to 18 days following ligation, lumbar spinal cords were removed under Nembutal anesthesia, cryosectioned (15μm), and the sections prepared for hybridization histochemistry. The sequences of the oligo-DNA probes (48 mers) were complimentary to unique portions of the mRNA encoding the large cytoplasmic loop of the human α_2-C10 or the α_2-C4 subtypes. Control probes either matched the mRNA or had random sequences. The hybridization patterns in the dorsal horn on the ligatured side differed from the control side in two ways. First, in the lateral portion of lamina I, significant increases in the α_2 mRNA hybridization signal were noted on the ligatured side. Depending on the individual probe, calibrated gray levels from video micrographs were 111% to 129% of those on the unligatured side, while background gray levels lowered to an average of 64% of the unligatured side ($p < .05$, ANOVA & Fisher PLSD). Second, frequencies of α_2-mRNA hybridization-positive neurons in the medial half of laminae II & III was increased on the ligatured side. Combining frequency data for all of the antisense probes, the average was 125% of the control dorsal horn ($p < .05$, ANOVA & Fisher PLSD). Hybridization-positive neurons were largely found in lamina II. The data suggest that chronic pain is associated with increases in α_2 adrenergic receptor biosynthesis, and imply that dorsal horn adrenergic input is selectively activated as a result of the neuropathic lesions. Supported by NS 28016 (FW), DA06687 & DE06682 (AB), DA01933 & DA04274 (GW), DA07234 (AB).

547.8

DESCENDING FACILITATION AND INHIBITION OF SPINAL MECHANICAL TRANSMISSION. M. Zhuo and G.F. Gebhart. Dept. Pharmacology, Univ. Iowa, Iowa City, Iowa 52242.

The present work investigated descending modulation from nuclei reticularis gigantocellularis and gigantocellularis pars alpha (NGC/NGCα) on spinal mechanical (noxious pressure and non-noxious brush) transmission of class 2 (WDR) cells. Electrical stimulation at 26/46 sites in the NGC/NGCα produced biphasic, only facilitatory (n=5) or only inhibitory (n=15) effects on responses of spinal units to noxious mechanical pressure. Electrical stimulation produced biphasic (n=9), only facilitation (n=2) or only inhibition (n=5) of unit responses to non-noxious mechanical brush. Descending influences on noxious and non-noxious mechanical transmission of the same spinal units, however, was the same in only 3/9 cases. Glutamate microinjection into the NGC/NGCα facilitated at lesser and inhibited at greater concentrations unit responses to noxious mechanical pressure as well as non-noxious brush. Local anesthesia of the ventrolateral funiculi (VLF) selectively abolished descending facilitation while dorsolateral funiculi (DLF) transections selectively abolished inhibition. These results suggest that: (1) descending biphasic modulation from the NGC/NGCα is qualitatively the same on non-noxious and noxious mechanical responses; (2) descending facilitation and inhibition are conveyed in the VLFs and DLFs, respectively.

547.9

LIDOCAINE INHIBITION OF HYPERACTIVE DORSAL HORN NEURONS IS NOT MEDIATED BY SUPRASPINAL SYSTEMS. P Marchettini*, M Lacerenza*, ML Sotgiu*[1], S Smirne*, F Lacquaniti. Istituto Scientifico H San Raffaele Dept. of Neuroalgology and C.N.R.[1] Milano, Italy.

Previous studies have shown an inhibitory action of systemic lidocaine on deafferented dorsal horn neurons. To study further the mechanism of this action a model of neuropathic pain was prepared in 20 adult male Wistar rats. All animals received 4 ligatures of the sciatic nerve of one limb. Signs of pain behavior were checked daily. Acute experiments were performed in all animals under pentobarbital anesthesia, 30-45 days after surgery or immediately in the presence of autotomy. Dorsal horn neurons responding both to non-noxious and noxious mechanical stimuli were isolated and recorded at lumbar level on the side ipsilateral to the lesion (24 units) and on the contralateral side (22 units). Units mean spontaneous activity (21.2 +- 3.2 s/sec) of the ipsilateral side was significantly greater ($p < 0.001$ t test) than that of the contralateral side (4.3 +- 1.6 s/sec). Section of the spinal cord at thoracolumbar level, performed during recording, increased the spontaneous activity of both the ipsilateral and contralateral side neurons. Following iv injections of 3-4 mg/kg lidocaine 2% diluted, significant ($p < 0.001$ t test) selective reduction of ipsilateral neurons spontaneous activity was observed. The present findings indicate that lidocaine action on hyperactive dorsal horn neurons is not mediated by supraspinal systems.

547.10

ANALGESIA PRODUCED BY A SYNTHETIC PEPTIDE CONTAINING THE AMINO ACID SEQUENCE 11-22 OF VASOACTIVE INTESTINAL PEPTIDE (VIP). J. Steinman[1], L. Rosario[1], D. Askin[1]*, J. Rigaud[1]*, P. Cash[2]*, F. Jordan[2]* and B. Komisaruk[1], Inst. Animal Behavior[1] & Dept. Chemistry[2], Rutgers-The State Univ., Newark, NJ 07102

Evidence is presented that analgesia is produced in female rats (n's=7-10/group) by injection directly to the spinal cord (intrathecally, IT) of a 12 amino acid-containing fragment of VIP that consists of the sequence of amino acids that are normally found at positions 11-22 of the 28 amino acid-containing parent molecule. The peptide fragment was synthesized in this laboratory. Analgesia was indicated by a significant elevation in threshold to elicit vocalization in response to electrical shock of the tail (VOCT). A dosage of 52 μg of the peptide (30 nmoles) in 5 μl Ringer's Solution (RS) significantly elevated VOCT over RS control injections at 20, 30, 40, 60 and 90 min post-injection, reaching a peak elevation of 98% over baseline at 60 min, whereas the RS group was -5% of baseline at the same time. A smaller, but significant elevation also was observed at 60 min following IT administration of 26 μg of VIP_{11-22} (30% over baseline; $p < .03$). No significant effect was observed at a dose of 13 μg. A significant elevation of tail flick latency after IT injection of 52 μg was observed at 60 min post-injection, but not at other times.

Our previous studies have shown that VIP_{11-22} and VIP_{11-28} are produced when VIP_{1-28} is incubated with spinal cord homogenate. Significant analgesia occurs when each of these compounds is administered IT. Thus, VIP_{1-28} may be a pro-peptide for these smaller, analgesically active fragments. [Supported by NIH RR08223, NIH R22948 (BK) and the Busch Foundation (FJ,BK)].

547.11

Electroacupuncture modifies the expression of c-*fos* in the spinal cord induced by noxious stimulation. J. H. Lee*, D. R. Brown and A. J. Beitz. Dept. of Vet. Biology, Univ. of Minnesota, St. Paul, MN 55108.

Acupuncture is thought to be mediated in part by descending pain modulation pathways that impinge on nociceptive neurons in the spinal cord dorsal horn. Many investigators have suggested that low frequency electroacupuncture (EA) is mediated by endogenous opioid mechanisms for its analgesic effect, whereas non-opioid systems have important roles for the analgesic effect of high frequency EA. The present study was designed to investigate the effect of EA on c-*fos* expression in the spinal cord induced by noxious stimulation and to evaluate the opiate sensitivity of 4 Hz versus 100 Hz EA applied bilaterally for 60 min duration to the point Zusanli (S36) located in the hindlimb. Mechanical NS (10 sec/min for 10 min) was applied to the right hindpaw following 30 minutes of EA treatment and the resulting c-*fos* expression in the spinal cord dorsal horn was compared to that obtained in rats only exposed to the noxious stimulation. The opiate sensitivity of the 4 Hz versus 100 Hz stimulation frequency was evaluated by giving naloxone (ip,5mg/kg) treatment 10 minutes before initiating EA. Both 4Hz and 100Hz EA reduced the number of c-*fos*-immunoreactive neurons in the dorsal horn induced by noxious stimulation by 58% and 50%, respectively. The suppression of c-*fos* expression induced by 4 Hz EA was completely reversed by prior treatment with naloxone. On the other hand, the suppression of c-*fos* induced by 100Hz EA was only partially blocked by this opiate antagonist. These data indicate that both high and low frequency EA inhibit the expression of c-*fos* in the dorsal horn induced by noxious stimulation and further suggest that low frequency (4Hz) EA is primarily mediated by endogenous opioid systems, while both opioid and non-opioid mechanisms appear to be involved in mediating the analgesic effect of 100Hz EA. Supported by NIH grants DA 06687, DE 06682 and DC 01086.

547.12

Evaluation of the Release of i-CGRP in Rat Dorsal Horn following Carrageenan induced Inflammation.

Mary G. Garry and Kenneth M. Hargreaves, Univ. of Minnesota, Dept. of Restorative Sciences, Minneapolis, MN.

The physiology of dorsal horn neuropeptides in inflammation is largely unknown. Several studies have examined peptide content in the dorsal horn using various models of inflammation, however, these studies leave the release profiles for neuropeptides undefined. The purpose of this study was to examine the release of calcitonin gene-related peptide (i-CGRP) in vitro from the dorsal horn of the spinal cord following carrageenan (CARRA) induced inflammation in the hindpaw. Male Sprague-Dawley rats received an intraplantar injection of CARRA (6mg) into the right hindpaw. Prior to sacrifice, contralateral (CL) and CARRA paws were tested for hyperalgesia (radiant heat), edema, and hyperthermia. Spinal cords were collected 6, 24, and 48 hrs. following the CARRA injection. The lumbar enlargement was divided into dorsal quadrants, chopped into 300μ cubes, and superfused with oxygenated Krebs (pH 7.4). Quadrants from the CL and CARRA sides were superfused with capsaicin (CAP; 10μM) to evoke the release of i-CGRP from primary afferent neurons. I-CGRP was assessed by RIA (antiserum kindly provided by M. Iadorola). After injection of CARRA (6hr), the inflamed paws exhibited a decreased withdrawal latency (2.9±0.4 sec vs. CL: 11.6±0.9 sec; $p<0.01$), edema (10.9±0.3 mm vs. CL: 5.3±0.1 mm; $p<0.01$), and hyperthermia (34.0±0.2°C vs. CL: 31.9±0.2°C; $p<0.01$). Preliminary results indicate that basal release of i-CGRP was 161 fmol/gm tissue (CL) and 389 fmol/gm tissue (CARRA). CAP evoked release was 1829 fmol/gm tissue (CL) and 4535 fmol/gm tissue (CARRA). This marked difference in the release of i-CGRP between the CARRA and CL side appeared to be resolved at 24 and 48 hrs. Similarly, the hyperalgesia in the inflamed paw was resolved at these later time points while edema and hyperthermia persisted. These data suggest that, during inflammation, there is a temporally related increase in the basal and evoked release of i-CGRP from superfused dorsal quadrants. These data also suggest that there is a correlation between i-CGRP release from primary afferent neurons and hyperalgesia.

547.13

Evaluation of the Release of Immunoreactive CGRP from Peripheral Tissue Using an *In Vitro* Superfusion Method.

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Superfusion of spinal cord slices is useful for evaluating the regulation of neuropeptide release from central terminals of primary afferent fibers. To date, however, there is little information on the regulation of release from terminals located in peripheral tissue. Bovine dental pulp was selected for these experiments since the tissue is readily available and has a relatively high concentration of immunoreactive CGRP.

Mandibular incisors were extracted at the slaughterhouse and transported on ice to the laboratory. Pulps were harvested, sliced and chopped (300 μ cubes) with a McIlwain tissue chopper. Tissue (200-250 mg) was superfused with Kreb's buffer (400 μL/min; 3 min fractions) for a 120 min baseline recovery period; samples were collected in 100 μL glacial acetic acid and lyophilized. Potassium (60 mM)-evoked release of immunoreactive CGRP was measured by a RIA (antiserum kindly provided by Dr. M. Iadarola).

Initial studies demonstrated that bovine iCGRP diluted in parallel to rat CGRP in the RIA and was present in dental pulp at a concentration of nearly 8 fmol/mg wet weight. Basal levels of release averaged 15.3 ± 1.7 fmol/G/9 min (n=4) over the observation period. Stimulation of dental pulp with 60 mM potassium increased superfusate levels ($p<0.01$) of iCGRP to 155 ± 21 fmol/G/9 min (n=7).

These studies indicate that superfusion of dental pulp permits evaluation of the regulation of neuropeptide release from peripheral tissue stores.

547.14

ADRENALECTOMY ENHANCES C-FOS-LIKE IMMUNOREACTIVITY IN TRIGEMINAL SUBNUCLEUS CAUDALIS IN RESPONSE TO NOXIOUS CORNEAL HEAT STIMULATION. J. Lu and D.A. Bereiter. Section of Neurobiology & Dept of Surgery, Brown Univ/RI Hospital, Providence, RI 02903

The role of glucocorticoids in somatic sensory function is not well defined, however, receptors for glucocorticoids are found in substantia gelatinosa of the spinal cord and certain forms of stress-induced analgesia may involve adrenal steroids. Expression of the proto-oncogene, *c-fos*, by noxious sensory stimuli induces fos-like immunoreactivity (FLI) in spinal laminae consistent with the location of nociceptor specific neurons. To assess the influence of glucocorticoids on FLI evoked by a well-defined noxious stimulus, the distribution of FLI-positive neurons within trigeminal subnucleus caudalis (Vc) was quantified in barbituate-anesthetized rats in response to thermal stimulation of the corneal surface. Noxious (52°C) or innocuous (42°C) thermal stimuli were applied in 20s pulses for 15min to adrenal intact, adrenalectomized (ADX), and adrenalectomized rats given corticosterone replacement (ADX+B). Rats were perfused and sections were processed for FLI at 2h after stimulation. In intact rats, noxious corneal heat evoked a reliable increase in FLI somatotopically localized to the ipsilateral superficial laminae of the caudal Vc (460±104 cells). In ADX rats, noxious corneal heat evoked a dramatic ($P<0.001$) increase in the number of FLI-positive neurons (2552±166 cells) in Vc, whereas in ADX+B rats the number of FLI-positive cells (827±75) was reduced compared to that of ADX rats. Sparse FLI was seen also in Vc at obex levels ipsilaterally, in the deep laminae of ipsilateral caudal Vc, and in contralateral caudal Vc in all groups. ADX alone or innocuous corneal heat had little effect on FLI in Vc. FLI was seen also in NTS and ventrolateral medulla bilaterally after noxious corneal heat and was increased in ADX compared to intact rats. The results are consistent with the hypothesis that glucocorticoids modify the function of superficial medullary dorsal horn neurons in response to noxious trigeminal input. Supported by NIH grant NS26137.

547.15

TIME COURSE OF AMINO ACID RELEASE INTO THE LUMBAR DORSAL HORN OF THE PRIMATE DURING DEVELOPMENT OF AN ACUTE ARTHRITIS. L.S. Sorkin, K.N. Westlund, K.A. Sluka, P.M. Dougherty and W.D. Willis. Marine Biomedical Institute, Univ. of Texas Med. Branch Galveston, Tx. 77550.

Extracellular levels of several amino acids were measured in the dorsal horn of anesthetized primates. Half hour continuous samples were obtained via a microdialysis probe before, during and for 4.5 hours after injection of kaolin/carrageenan into the articular capsule of one knee and assayed using HPLC with fluorescence detection.

Glutamate, aspartate, glycine and serine increased ipsilateral to the inflammation following injection of irritative agents; glutamine levels decreased. Within two and one half hours after the injection, these amino acid levels returned to near baseline. Movement of the knee after, but not before inflammation lead to release of these same amino acids.

In animals that later were found to have depletion of immunoreactive-substance P (SP) in the dorsal horn on the side of the inflammation, taurine (Tau) levels rose starting two to three hours after the injection. In animals with less than 10% SP depletion, Tau remained at baseline levels throughout the experiment.

Thus, during the first stages of joint inflammation, EAAs are released into the dorsal horn. This is followed by increased levels of IAAs, presumably representing activation of descending endogenous analgesia systems. This phase is followed by a semiacute response consisting in part of increased extracellular levels of SP as well as Tau. While SP is presumably part of a nociceptive transmission system, Tau via its ability to reduce Ca^{++} influx, could be part of a second analgesia mechanism aimed at semiacute and/or chronic pain. Supported by NS11255, NS09743 and Bristol Myers-Squibb.

547.16

CHANGE IN GLUTAMATE IMMUNOREACTIVITY IN THE MEDIAL ARTICULAR NERVE OF THE PRIMATE DURING ACUTE EXPERIMENTAL ARTHRITIS. K.N. Westlund, Y.C. Sun, K.A. Sluka, P.M. Dougherty, L.S. Sorkin and W.D. Willis. Marine Biomedical Institute, University of Texas Medical Branch, Galveston, Texas 77550.

Glutamate (GLU) and other excitatory amino acids have been shown to play a key role in nociception and the hyperalgesia associated with the acute inflammatory response. In an effort to more fully understand the role of GLU in this process, the percentage of axonal fibers immunoreactive for GLU was determined in the medial articular nerve, a source of preterminal afferent fibers innervating the knee joint of the monkey. After induction of the experimental knee joint inflammation with a kaolin/carrageenan mixture, comparison was made of the percentage of GLU positive axons from the control side versus the side of the inflammation. A doubling in the percentage of GLU axons was observed on the side of the experimental arthritis as compared to the medial articular nerve of the other side suggesting that GLU content is greatly increased in the preterminal afferents. These increases were observed only when injection of kaolin/carrageenan was combined with joint flexion and mechanical stimulation in the anesthetized preparation. GLU positive axons were of small diameter and unmyelinated or were included in the thinly myelinated group in the control nerve. Following induction of the inflammation, axonal diameter determination revealed an increase in the percentage of thinly myelinated GLU axons which would correspond to the A delta fiber type. An increase in GLU could explain the enhanced mechanosensitivity and increased resting discharges observed in afferents in this model of inflammation. These findings implicate increased GLU as a causal factor in the lacinating pain of movement and hyperalgesia experienced in the inflammatory state. (Supported by grant #NS11255, NS09743, NS08860, RCDA NS01445-02 and Bristol-Myers Squibb.)

547.17

SUBSTANCE P, CALCITONIN GENE-RELATED PEPTIDE AND GLUTAMATE ALTERATIONS IN THE SPINAL CORD OF THE PRIMATE DURING ACUTE ARTHRITIS. K.A. Sluka, P.M. Dougherty, L.S. Sorkin, W.D. Willis and K.N. Westlund. Marine Biomedical Institute, University of Texas Medical Branch, Galveston, Texas 77550.

The effects of an experimentally induced arthritis on immunoreactivity of putative primary afferent neurotransmitter/neuromodulators were examined. Immunoreactive staining for substance P (SP), calcitonin gene-related peptide (CGRP), and glutamate (GLU) in the monkey dorsal horn was examined following inflammation of one knee joint induced by injection of kaolin and carrageenan (5% and 5%). Spinal cords were examined at different time periods after induction of arthritis (2.5, 4, 6, and 8 hours). Side to side differences in the density of immunoreactive staining were determined by a computer-assisted quantitation system. A significant decrease in immunoreactivity of the ipsilateral dorsal horn was found for SP at 4, 6, and 8 hours. The decrease for SP was 68.3% at 4 hours, 54.7% at 6 hours, and 65.5% at 8 hours. Immunoreactivity for CGRP was significantly decreased 31.5% after 8 hours. Immunoreactivity for GLU, on the other hand, showed an increase ipsilaterally of 31.4% at 4 hours, 33.7% at 6 hours, 39.9% at 8 hours. The frequency of peripheral stimulation of the joint was shown to be important for changes in GLU and SP immunoreactivity. Without frequent joint stimulation in the early stages of the development of arthritis, increases in GLU immunoreactivity were not as prominent. In these same animals SP and CGRP showed no quantitative side to side differences without the joint stimulation. These studies suggest that GLU may be involved in arthritic pain at rest, whereas SP, CGRP and GLU may mediate pain induced by joint movement. (Supported by grants NS11255, NS09743, NS08860, RCDA NS01445 and Bristol-Myers Squibb.)

547.18

ENHANCEMENT OF RESPONSES OF MONKEY SPINOTHALAMIC CELLS TO EXCITATORY AMINO ACIDS ACCOMPANIES THE DEVELOPMENT OF ACUTE ARTHRITIS. P.M. Dougherty, K.A. Sluka, L.S. Sorkin, K.N. Westlund and W.D. Willis. Marine Biomedical Institute, 200 University Blvd, UTMB, Galveston, Texas 77550-2772.

The experiments described here show a potentiation of the responses of spinothalamic tract (STT) neurons to both chemical and mechanical stimuli in monkeys during the development of an acute arthritis. A total of 12 young adult monkeys (Macaca fascicularis) were anesthetized and a laminectomy was performed to expose the lumbar enlargement. STT neurons in the L4-L6 segments were recorded with carbon filament containing multibarrel electrodes. STT cells were isolated and identified by use of antidromic pulses passed through an electrode placed in the ventral posterior lateral nucleus of the thalamus. The responses of STT cells to mechanical stimulation of the receptive fields and to chemical stimulation by iontophoretically released glutamic, aspartic, quisqualic and *N*-methyl-D-aspartic acids were recorded before and following induction of arthritis. Arthritis was produced by injection of 0.5 ml of a 5% kaolin-5% carrageenan mixture into the knee joint capsule. The results of this study show an increase of the responses of 8 of 12 cells to non-noxious flexion of the knee joint following this injection. The responses of these cells to noxious pinch of the skin were also enhanced. The enhancement of both these responses followed a similar time course, with onset of changes one to two hours following injection of the knee joint. The results also demonstrate that the increase in responses of STT neurons to mechanical stimuli is accompanied by an increase in response to iontophoretically applied excitatory amino acids (EAA's), particularly those acting at non-*N*-methyl-D-aspartate (non-NMDA) receptors (e.g., glutamate, aspartate and quisqualate). (Supported by NS08860, NS09743 and NS11255.)

547.19

POTENTIATION OF RESPONSES OF MONKEY SPINOTHALAMIC (STT) CELLS TO EXCITATORY AMINO ACIDS ACCOMPANIES THE DEVELOPMENT OF CAPSAICIN-INDUCED HYPERALGESIA. W.D. Willis and P.M. Dougherty. Marine Biomedical Institute, 200 University Blvd, UTMB, Galveston, Tx. 77550-2772.

The present study addresses the role that excitatory amino acids (EAA's) may play in the sensitization of STT neurons by intradermal (i.d.) capsaicin (CAP). A total of 10 young adult monkeys (Macaca fascicularis) were anesthetized and a laminectomy was performed to expose the lumbar enlargement. STT neurons located in segments L4-L6 were recorded with carbon filament containing multibarrel electrodes. STT cells were isolated and identified by use of antidromic pulses passed through an electrode placed within the ventral posterior lateral nucleus of the thalamus. The responses of the STT cells to mechanical stimulation of the receptive fields and to chemical stimulation by iontophoretically released glutamic, aspartic, quisqualic and *N*-methyl-D-aspartic acids were recorded before and following injection of capsaicin. Capsaicin was injected at a concentration of 3% in 0.1 ml of a mixture of ethanol/tween 80 and saline into the center of the receptive field of each cell. Testing of responses to mechanical and chemical stimulation began at 30 minutes after injection and was continued for up to two hours afterward. Our results show that the responses of STT cells to *N*-methyl-D-aspartate, glutamate, aspartate, and quisqualate become increased in those cells which also show increased mechanical responses following i.d. CAP. Finally, in each of the cells showing an increase in response to mechanical stimulation the responses to at least one EAA (and often more than one EAA) became increased with a time course identical to that for the mechanical responses. (Supported by NS08860, NS09743 and NS11255.)

547.20

NEUROPLASTIC EFFECTS INDUCED BY LINGUAL INFLAMMATORY IRRITANT ON CUTANEOUS AND DEEP RECEPTIVE FIELD PROPERTIES OF NEURONS IN TRIGEMINAL (V) SUBNUCLEUS CAUDALIS.

X.-M. Yu, J.W. Hu and B.J. Sessle. Faculty of Dentistry, Univ. of Toronto, Ont. M5G 1G6 Canada.

We have documented that the inflammatory irritant mustard oil injected into the masseter muscle can change the cutaneous receptive field (RF) properties of caudalis nociceptive neurons. This study's aim was to characterize the properties of caudalis neurons receiving deep as well as cutaneous RF inputs and to test the effect on them of mustard oil. Of 196 single neurons recorded in V subnucleus caudalis in 15 anaesthetized rats, 74% received cutaneous input only, 14% deep input only and 12% were neurons (CD) receiving nociceptve cutaneous and deep inputs. Reversible effects of mustard oil (5μl) injected into the tongue on cutaneous and deep RFs were observed in 8 of 9 CD neurons tested . There was a significant increase of both cutaneous and deep RFs and, compared with the effect of vehicle injection, the expansion of deep RFs was significantly greater (45%, $p<0.01$) and longer in duration (10-15 min); also, deep RFs expanded more than cutaneous RFs ($p<0.05$). These results provide further support for injury-induced neuroplasticity in V nociceptive neurons and suggest that deep inputs may particulary manifest this effect. Supported by NIH grants DE04786 and DE09559.

PAIN MODULATION: PHARMACOLOGY II

548.1

CAPSAZEPINE: A COMPETITIVE ANTAGONIST OF THE SENSORY NEURON EXCITANTS, CAPSAICIN AND RESINIFERATOXIN. S. Bevan*, S. Hothi*, G.A. Hughes*, I.F.James, H.P. Rang*, K. Shah*, C.S.J. Walpole*and J.C. Yeats*,Sandoz Institute for Medical Research, 5 Gower Place, London WC1, UK.

Capsaicin depolarizes and excites a subset of primary afferent, sensory neurons by opening a membrane ion channel that is permeable to monovalent and divalent cations. This action is shared by resiniferatoxin (RTX), another plant product that is 100-1000 times more potent than capsaicin. Here we report the discovery of a compound, capsazepine, that acts as a specific, competitive antagonist of capsaicin and RTX. The competitive antagonism provides evidence that capsaicin and RTX act on a specific membrane receptor. Whole cell currents were recorded under voltage clamp from dorsal root ganglion (DRG) neurons isolated from neonatal rats. Capsazepine (0.5-1μM) reversibly reduced or abolished the response to 0.1-0.5μM capsaicin, but had no effect on the response to GABA. In ion flux studies, capsazepine antagonized the capsaicin induced uptake of $^{45}Ca^{2+}$ in neonatal rat cultured DRG neurons, with an IC50 of 0.5μM. Capsaicin dose-response curves for both $^{86}Rb^{+}$efflux from cultured DRG neurons and ^{14}[C]-guanidinium efflux from adult rat vagus nerves provided evidence that the antagonism was competitive. Parallel shifts in the log concentration-effect curves were seen with increasing concentrations of capsazepine. Schild plots of these data were linear with a slope very close to 1, and yielded K_d estimates of 100nM (DRG) and 690nM (vagus) . A similar competitive antagonism between capsazepine and RTX was noted in ^{86}Rb efflux and $^{45}Ca^{2+}$ uptake studies on DRG neurons.

548.2

SELECTIVE ANTAGONISM OF CAPSAICIN-INDUCED EXCITATION OF C-FIBERS BY CAPSAZEPINE, A NOVEL CAPSAICIN ANTAGONIST. L. Urban N. Seno' and A. Dray. Sandoz Institute for Med. Res., London, 5 Gower Pl., WC1E 6BN, U.K.;Sandoz Pharm. Ltd., Tokyo 106, Japan

There are several lines of evidence indicating that the selective excitatory action of capsaicin on primary sensory neurons is produced by activation of a specific receptor (Bevan & Szolcsanyi, 1990). This suggestion has been recently supported by using a competitive capsaicin antagonist, capsazepine (Bevan et al., 1991). We now show selective antagonism of the capsaicin-induced activation of sensory C-fibers by capsazepine, using two *in vitro* preparations: the mouse spinal cord - DRG, and the rat hindpaw skin - saphenus nerve.

Capsaicin (0.2-1.0 *u*M, 30 sec) applied to the receptive field of single primary afferent fibers selectively excited C- and A-delta mechano-heat receptors. Capsazepine (0.7 *u*M, 5 min) perfused to the receptive field prior to capsaicin significantly reduced the firing evoked in single fibers. In the spinal cord a majority of dorsal horn cells were depolarised when primary afferents were activated by capsaicin (0.4-0.7 *u*M) applied to the DRG. Superfusion of the DRG (but not the spinal cord) with capsazepine blocked the capsaicin-induced depolarization of dorsal horn cells. Depolarization evoked in the same cells by application of substance P (0.8 *u*M) to the cord was not affected by capsazepine. The antagonistic effects of capsazepine were reversible, and similar concentrations were ineffective when administered alone in both preparations.

These data show that capsazepine acts as a selective capsaicin antagonist and support the concept that capsaicin activates C-fibers via a specific receptor site.

Bevan, S. and Szolcsanyi, J., TINS, 11 (1990) 330-333.
Bevan, S., et al., Br. J. Pharmacol. 102 (1991) 77P.

548.3

CAPSAICIN ANTAGONISED BY CAPSAZEPINE; IN VIVO STUDIES. M.N.Perkins, A.Dray, and E.A.Campbell.* Sandoz Institute for Medical Research, London, WC1E 6BN, UK.

Capsazepine, has been shown to be a selective, competitive antagonist of capsaicin-induced activation of sensory neurones *in vitro* (Bevan et al 1991, Dray et al 1991). We now show that capsazepine antagonises both capsaicin-induced agonist responses and antinociceptive effects *in vivo*. The capsaicin- evoked reflex blood pressure fall in anaesthetised rats and the bronchoconstrictor responses in anaesthetised guinea pigs were both antagonised by capsazepine infused at 0.2-0.5 µmol/min for 5 min. In carrageenin- induced paw pressure hyperalgesia in rats the antinociceptive action of capsaicin (20µmol/kg sc) was reversed by 100µmol/kg sc capsazepine. In the tail -flick model in rats and mice capsazepine (100µmol/kg sc) prevented the antinociceptive action of capsaicin (20µmol/kg sc). Lower doses of capsazepine were generally ineffective. In a model of persistent hyperalgesia, produced by the injection of uric acid into a rat knee joint ,the antinociceptive action of capsaicin (20µmol/kg sc) was partially reversed by capsazepine at 100 µmol/kg and completely reversed at 200µmol/kg. In each of these models, systemic capsazepine alone was without effect. These data show that capsazepine acts as a capsaicin antagonist *in vivo* and supports the concept that both the agonistic and antinociceptive actions of capsaicin are mediated by the same specific membrane receptor.

Bevan et al, 1991 Br.J.Pharmacol. 102, 77P
Dray et al, 1991 Br.J.Pharmacol. 102, 78P

548.4

CAPSAZEPINE; CAPSAICIN ANTAGONISM AND EVIDENCE FOR A SPINAL MECHANISM IN CAPSAICIN-INDUCED ANTINOCICEPTION. A. Dray and A. Dickenson* Sandoz Institute for Medical Research, and *Department of Pharmacology University College, London, England.

Systemic capsaicin produces antinociception by an action on spinal afferent nerve terminals (Dickenson et al 1990, Eur J. Pharmacol. 187,225). The effects of capsaicin are likely to be mediated by a specific receptor. This is supported by recent studies using the competitive antagonist capsazepine (Bevan et al 1991, Br J. Pharmacol, 1991, 102, 77P; this meeting). Presently we have used capsazepine to show antagonism of capsaicin-induced activation of nociceptors and that antinociception produced by systemic capsaicin is mediated via a capsaicin receptor. Spinal ventral root responses were recorded in vitro from the neonatal rat cord/tail preparation following brief application of capsaicin and other noxious stimuli. Responses evoked by capsaicin administered to the tail or spinal cord were selectively and reversibly reduced by capsazepine (IC50= 200nM). In anesthetized adult rats, C- but not Aβ-fiber evoked discharges of WDR dorsal horn neurones, were reversibly reduced by a low dose of capsaicin (20 µmol/kg s.c.). This was antagonised by capsazepine administered either s.c. (100µmol/kg) or directly to the spinal cord (5nmol in 50µl). Capsazepine alone did not affect neuronal responses to peripheral stimuli. Local injection of capsaicin into the peripheral receptive field first activated C-fibres and then reduced their electrical excitability. Co-injection of capsazepine reduced both these effects of capsaicin. *These results show that capsazapine antagonises a number of actions of capsaicin. Specifically the data support the hypothesis that antinociception, following systemic capsaicin, is due to an effect via capsaicin receptors on spinal afferent fibres .*

548.5

EFFECTS OF INTRATHECAL CAPSAICIN AND STRYCHNINE AND TOPICAL COLCHICINE ON THE THERMAL HYPERALGESIA FOLLOWING SCIATIC NERVE CONSTRICTION MODEL IN RATS. T. YAMAMOTO* and T.L. YAKSH. Depart. Anesth. UCSD, La Jolla, CA 92093.

A chronic sciatic nerve constriction injury produces ipsilateral hyperalgesia in rats and this hyperalgesia was proposed as an animal model of peripheral neuropathy. This syndrome is thought to reflect activation of small afferents and an altered processing of noxious input due to trans-synaptic change in the dorsal horn. To investigate possible mechanisms leading to the hyperalgesia, we examined the effects of intrathecal (IT) capsaicin (CAP; C-fiber neurotoxin), IT strychnine (STR; glycine antagonist) and topical (TOP) colchicine (COL; axon transport blocker) on the hyperalgesia following sciatic nerve constriction injury. Under halothane, both sciatic nerves were exposed. 4 ligatures were tied loosely around one (lesioned) but not the other (normal) nerve. For CAP treatment, 75µg of CAP or vehicle (VEH; 20% cyclodextrin) were injected IT 2 days after nerve lesion. For STR treatment, 15µg of STR or saline was injected IT at the surgery day and 1 day and 2 days after surgery under halothane anesthesia. For COL treatment, 5 mM and 50 mM of COL or saline were TOP applied adjacent to the lesioned nerve, proximal to the constriction. 7 days after nerve lesion, latency to withdrawal from a thermal stimulation was examined in the ipsilateral and contralateral hind paw of each rat. A difference score (DS) was calculated by subtracting the average latency of the control from the average of the lesioned side. CAP but not STR or COL treatment resulted in a significant increase in the latency of the normal paw.

	CAP	VEH	STR	saline	COL 5	COL50	saline
DS (sec)	-5.7	-3.7	-4.5	-2.8	-1.2	-0.1	-3.0
(±SEM)	±1.3	±0.4	±0.3	±0.5	±0.2	±0.3	±0.3

These results suggested that : 1) C fiber afferents did not play an important role in producing hyperalgesia; 2) glycine receptor activity inhibits developing hyperalgesia; 3) the hyperalgesic state depends upon axonal transport.

548.6

PROSTAGLANDINS STIMULATE THE RELEASE OF SUBSTANCE P FROM RAT SENSORY NEURONS GROWN IN CULTURE. K.J. Waite* and M.R. Vasko, Dept. Pharmacol. and Tox., Indiana U. Sch. Med., Indianapolis, IN 46202

When injected into peripheral tissues or directly onto the spinal cord, prostaglandins (PGs) lower the algesic threshold in animal models of nociception. One possible mechanism to explain this hyperalgesia is that PGs increase the release of neurotransmitters from nociceptive sensory neurons. To test this hypothesis, we studied the effects of PGs on the release of substance P (SP) from rat sensory neurons grown in culture.

Neurons were dissociated from dorsal root ganglia of 16-18 day old rat embryos and grown in culture for 14 days. Release studies were performed by exposing neuronal cultures to Krebs buffer containing 3.5mM KCl (basal release) or buffer containing 1uM capsaicin, 30mM KCl, and/or PGs (stimulated release). Buffer was then assayed for SP using RIA.

Exposure of neurons to 1uM capsaicin or 30mM KCl resulted in a 3-4 fold increase in SP release that was calcium dependent. Addition of 10uM PGE2 to the cells significantly increased SP release by 2 fold from 26.9 ± 1.5 pg/well/10 min to 48.9 ± 1.6 pg/well/10 min. In contrast, neither 10uM PGD2 nor 10uM PGF2α produced a significant increase in SP release. PGF2α did, however, enhance the potassium-stimulated release of SP. These results demonstrate that PGs can stimulate release of neurotransmitter from rat sensory neurons and suggest that this effect may contribute to PG-induced hyperalgesia. (Supported by the Arthritis Foundation and NS21697).

548.7

SYSTEMIC MORPHINE COMPLETELY BLOCKS ACUTE C-FIBER STIMULATION-INDUCED DEPLETION OF CGRP AND REDUCES DEPLETION OF GALANIN. C.M. Klein, C.M. Pover, R.E. Coggeshall, and L.S. Sorkin. The Marine Biomedical Institute, Dept. of Anatomy and Neuroscience and Dept. of Physiology & Biophysics., The University of Texas Medical Branch, Galveston, Texas 77550.

In past studies, we observed decreases in immunoreactive staining of calcitonin gene-related peptide (CGRP) and galanin (GAL) in the L4-L_5 dorsal horn following acute stimulation of the sciatic nerve at C- but not A-fiber strength. In the present study, we determined if depletion of these substances is blocked by morphine.

Adult Sprague-Dawley rats were anesthetized (50 mg/kg pentobarbital i.p.) and the sciatic and tibial nerves isolated. After being placed on a respirator, they were paralyzed with gallamine (15 mg, i.v.) and administered morphine (9 mg/kg i.v.). Fifteen mins after morphine administration, the sciatic nerve was stimulated. (1 Hz, 0.3 ms) for 20 mins, fiber volleys were monitored from the tibial nerve. Immediately after stimulation, rats were perfused with 4% paraformaldehyde, 0.1% picric acid in 0.1 M phosphate buffer. The L_4-L_5 spinal cord was removed and processed for immunocytochemistry by the PAP method. Systemic morphine administration blocked C-fiber induced depletion of CGRP and attenuated but did not eliminate the decreased staining for GAL.

These findings may indicate that 1) CGRP immunoreactive C-fibers are inhibited by opiates and 2) some galanin immunoreactive primary afferent fibers are unaffected by opiates or galanin immunoreactive cells in the dorsal horn are activated by opiate insensitive afferent input. Supported by grants NS10161, NS07185, NS11255 and Bristol Myers-Squibb.

548.8

SUBSTANCE P(1-7) SPINAL ANTINOCICEPTION AND INTERACTION WITH THE β-FNA-SENSITIVE OPIATE RECEPTOR SUBTYPE. V.M. Goettl and A.A. Larson. Dept. of Veterinary Biology and Program in Neuroscience, Univ. of Minnesota, St. Paul, MN 55108.

Substance P (SP), a putative neurotransmitter of pain, is cleaved by peptidases in the synaptic cleft resulting in several metabolites including the N-terminal peptide SP(1-7). SP(1-7) has been shown to have specific CNS membrane binding sites which interact with the mu-opiate ligand DAMGO as well as biological activity including antinociception when given intracerebroventricularly in mice. To investigate SP(1-7) antinociception at the spinal level, doses of 0.5, 5, 50 or 1000 pmoles were given intrathecally (i.t.) to male Swiss-Webster mice. Tail flick latency, hot plate paw lick latency or the number of abdominal stretches 5 min after an intraperitoneal injection of 0.3 ml of 1% acetic acid was determined 3, 5, 10 or 30 min after i.t. SP(1-7). SP(1-7) was not significantly antinociceptive ($p<0.05$) with the tail flick latency. A dose of 0.5 pmole of SP(1-7) significantly increased the hot plate rear paw lick latency ($p<0.05$) at 3 and 30 min. post i.t. injection. Doses of 5, 50 and 1000 pmole significantly decreased ($p<0.05$) the number of abdominal stretches 30 min post i.t. injection. To determine if opiate receptor subtypes were involved, mice were injected i.t. with 1µg of β-FNA, a mu_2 selective antagonist, or 0.5µg naloxonazine, a mu_1 selective antagonist, 24 hrs before the abdominal stretch assay; 1 µg naltrindole, a delta selective antagonist, was coadministered with the i.t. SP(1-7). Only the β-FNA blocked the antinociceptive effect of 50 pmoles SP(1-7) 30 min. post i.t. injection. These experiments demonstrate that SP(1-7) may attenuate pain transmission at the spinal level through the β-FNA-sensitive opiate receptor subtype. (NIDA 04090,04190,00124)

548.9

SPINAL MUSCARINIC TYPE 2 RECEPTORS MEDIATE THE ANTINOCICEPTION INDUCED BY N-METHYLCARBACHOL IN THE PEDUNCULOPONTINE TEGMENTAL NUCLEUS (PPTg). E.T. Iwamoto, L. Marion*, and J.R. Holtman. Pharmacology Dept., Univ. of Kentucky Col. of Med., Lexington, KY 40536

N-methylcarbachol (NMC) microinjection into the PPTg produces antinociception as assessed by the hot-plate and tail-flick tests. Adult male Sprague-Dawley rats were implanted with both 25 ga guide cannulas aimed at the PPTg and intrathecal PE10 catheters terminating in the lumbar enlargement. One wk after surgery, animals received an intrathecal injection of 0.1 μmole of drug in 10 μl phosphate buffer 12.5 min before the injection of 40 nmole NMC in 0.5 μl buffer into the PPTg. NMC produced hot-plate and tail-flick antinociception for 15 to 25 min which was not accompanied by losses of corneal or righting reflexes, a placing reaction, or a negative geotaxic response. Intrathecal (-)-scopolamine or methoctramine antagonized the antinociception produced by NMC injection into the PPTg; mecamylamine or pirenzepine had little effect. Mecamylamine, (-)-scopolamine, pirenzepine, or methoctramine did not alter baseline nociception when administered alone intrathecally. The data indicate that either a local or a descending spinal muscarinic M2 system mediates the antinociception produced by nicotinic stimulation of the PPTg. (Supported by NS 28847 and the KTRB.)

548.10

IMPACT OF SCOPOLAMINE AND YOHIMBINE ON PAIN REACTIVITY AND SHOCK-INDUCED ANTINOCICEPTION IN SPINALIZED RATS. P.S. Chen*, P.A. Illich, M.W. Meagher, & J.W. Grau. Dept. of Psychology, Texas A&M Univ., College Station, TX 77843.

We have previously shown that exposure to three brief (2 sec) 3.0 mA tail-shocks elicits an opioid antinociception, and that longer (75 sec) tail-shocks elicit a nonopioid antinociception in spinalized rats. The present experiments evaluate whether cholinergic or noradrenergic systems play a role in the production of these antinociceptive effects. In both experiments, subjects were spinalized at T2 and tested 8-10 hr later. Fifteen min after subjects received the test drug, or its vehicle, 3 tail-flick tests were performed to establish baseline levels of nociception. Subjects then received either no shock, 3 brief (2-s), or 3 long (75-s) 3.0 mA tail-shocks, after which nociception was assessed 5 more times with the tail-flick test. We found that the cholinergic antagonist scopolamine (1 mg/kg) decreased baseline tail-flick latencies. A similar effect has been observed in intact subjects (e.g. Grau et al., Behav. Neurosci., 105, 62, 1991). Scopolamine did not, however, affect the magnitude of either brief or long-shock induced antinociception. In contrast, the alpha-2-noradrenergic antagonist yohimbine (10 mg/kg) attenuated the antinociception observed after both shock schedules without affecting baseline tail-flick latencies. We are currently assessing the impact of intrathecal application of these drugs and the role of other neurochemical systems.

548.11

STIMULATION OF THE PEDUNCULOPONTINE TEGMENTAL NUCLEUS PRODUCES ANTINOCICEPTION THAT IS MEDIATED BY MUSCARINIC AND NICOTINIC RECEPTORS IN THE NUCLEUS RAPHE MAGNUS. L.F. Fitzgerald and H.K. Proudfit. Dept. of Pharmacology, Univ. of Illinois at Chicago, Chicago, IL 60680.

Previous studies have suggested that the pedunculopontine tegmental nucleus (PPTg) is involved in the descending control of nociception, probably through a connection to the nucleus raphe magnus (RMg). For example, electrical stimulation of the PPTg in the lightly-anesthesized rat produces antinociception in the tail flick test. The present experiments were designed to determine whether antinociception produced by stimulation of the PPTg is mediated by cholinergic receptors in the RMg. The results of these experiments demonstrate that microinjection of atropine, a muscarinic antagonist, or mecamylamine, a nicotinic antagonist, into the RMg blocked the antinociception produced by PPTg stimulation. In addition, injection of methacholine, a muscarinic agonist, or nicotine into the RMg produced antinociception. These results suggest that both muscarinic and nicotinic receptors within the RMg mediate the antinociception produced by PPTg stimulation.
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548.12

NICOTINE INDUCED ANTINOCICEPTION IS MEDIATED BY SPECIFIC CHOLINERGIC AND OPIOID RECEPTORS. M.E.Wewers, A.K.Rattan and G. A. Tejwani*. Ohio State University College of Medicine & College of Nursing, Columbus, OH 43210.

The objectives of this study were to investigate the role of nicotinic, muscarinic and opioid receptors in nicotine induced antinociception in adult male Sprague Dawley rats. Antinociception was measured by tail-flick and hot plate 52° C tests after subcutaneous or lateral intracerebroventricular nicotine injections. Nicotine (10-1000 μg/kg,subcutaneous) or (50-200 μg/kg,intracerebroventricular) induced a dose dependent increase in antinociception in both tests. Antinociception produced by subcutaneous nicotine was antagonized by mecamylamine, a nicotine receptor antagonist but not by scopolamine hydrobromide, scopolamine methyl nitrate or hexamethonium. Opioid receptors antagonist naloxone blocked subcutaneous nicotine effects completely and μ receptor antagonist ß-Funaltrexamine blocked subcutaneous nicotine effect only in hot plate while κ receptor antagonist WIN 44,441-13 (WIN) blocked subcutaneous nicotine effects in both tests. Antinociception produced by intracerebroventricular nicotine was antagonized by mecamylamine, hexamethonium, scopolamine hydrobromide and scopolamine methyl nitrate as well as naloxone, ß-Funaltrexamine, WIN and δ-receptor antagonist naltrindole in both tests. Nicotine inhibited binding of opioid ligands to receptors in the cortex with $(IC)_{50}$ of 2.3 mM (^{3}H-naloxone), 2.6 mM (^{3}H-DAGO), 3.4 mM (^{3}H-DSTLE) and 12.9 mM (^{3}H-EKC). It is concluded that nicotine induced antinociception is mediated by μ, δ and κ receptors in addition to nicotinic and muscarinic receptors.(Supported by USPHS 5K08NR00018).

RETINA AND PHOTORECEPTORS: GANGLION CELLS AND CENTRIFUGAL CONTROL

549.1

MORPHOLOGICAL CLASSIFICATION OF RETINAL GANGLION CELLS PROJECTING TO THE OPTIC TECTUM IN TURTLE. D. Zhang, M.J. Endl* and W.D. Eldred. Department of Biology, Boston University, Boston, MA 02215.

There is little detailed information about particular types of retinal ganglion cells which project to specific central targets. In this study we have examined retinal ganglion cells which project to the optic tectum in turtle. Rhodamine crystals were applied to the optic tectum and retrograde transport carried the rhodamine back to the ganglion cell bodies. Intracellular injection of Lucifer yellow was used to label the rhodamine containing ganglion cells. Following photoconversion using diamino-benzidine, detailed camera lucida drawings were made. More than 20 morphologically-distinct classes of ganglion cells were identified based on their stratification within the inner plexiform layer (IPL), the complexity of their dendritic arborizations and the presence or absence of dendritic beads. Ganglion cells were seen with mono-, bi-, tri-stratified or diffuse dendritic arborizations. Different subclasses of mono-, bi-, or tri-stratified cells were distinguished using the location of their arborizations within the IPL. Several different classes of diffuse type ganglion cells were also distinguished by having concentrations of processes within certain strata of the IPL. Other classes of ganglion cells with the same stratification within the IPL, were distinguished by the presence or absence of dendritic beads. Many of the cells with asymmetric dendritic arborizations had dendritic trees which were parallel or sometimes orthogonal to the visual streak. Overall, the ganglion cells projecting to the optic tectum in the turtle displayed a great degree of morphological diversity, which may reflect a similar heterogeneity in their functions. This research supported by EY04785 to W.D.E.

549.2

MORPHOLOGY OF HAMSTER RETINAL GANGLION CELLS. E. N. Yamasaki, C. R. Schätz and D. O. Frost. Dept. of Neurology, Massachusetts General Hospital, Charlestown, MA 02129.

Neonatal surgery in hamsters can produce novel, functional retinal projections to non-visual nuclei. The retinal ganglion cell (RGC) types that make these projections are unknown. Such data would reveal the relative plasticity of different RGC types and help interpret neurophysiological data on the novel pathways. To begin clarifying this issue, we have examined the morphology of RGCs in normal adult hamsters. In living retinae, RGCs previously labeled by injections of rhodamine labeled latex microspheres into the superior colliculus (to which virtually all RGCs project) were intracellularly injected with lucifer yellow (LY), revealing their soma/dendritic morphology. The retinae were then fixed and LY-filled RGCs were either photoconverted using DAB and drawn with a computer microscope (n=22) or drawn under epifluorescent illumination using a camera lucida (n=24).

RGC soma and dendritic field diameters and the number of dendritic branch points and dendritic spines were measured. RGCs were classified as types I, II or III following Perry's (1979) scheme for the rat. Type I RGCs have soma and dendritic field diameters of 21-28 and 233-391 µm, respectively. Type II RGCs have soma and dendritic field diameters of 14-24 and 173-272 µm, respectively. Type III RGCs have soma and dendritic field diameters of 17-22 and 192-270 µm, respectively. Dendritic field diameter increases with increasing eccentricity and decreasing RGC density. Type I and type III RGCs have few dendritic spines; type II RGCs have many spines. Type II RGC dendrites are highly branched, type III are sparsely branched and type I are intermediate. The dendritic fields of some RGCs of each type are stratified in the inner plexiform layer within presumptive ON or OFF sublaminae.

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549.3

PARASOL RETINAL GANGLION CELLS IN MACAQUES CONNECT INTRACELLULARLY TO OTHER PARASOL CELLS, AND TO AMACRINE CELLS FOUND IN THE GANGLION CELL LAYER.

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Dr. David Vaney (personal communication) has recently shown in cats and rabbits that intracellularly injected fixable biotin derivatives are able to pass into other retinal cells of the same type, and may also pass into certain other specific retinal cell types. We have applied the approach to cells in the ganglion cell layer of an *in vitro* wholemounted preparation of the macaque retina, using 3% Neurobiotin (Vector) in 0.1 M Tris, and a positive current of 1 to 3 nA for 1 to 3 min. After fixation the retina was placed in detergent to aid penetration, followed by avidin then biotinylated HRP, and reacted with DAB.

Around and outside the dendritic fields of parasol cells we observed a ring of 2 to 6 labeled somata having the same size as the injected cell (i.e. larger than all other common cell types in this layer). None of these large somata were found that overlapped or were within the dendritic field of the injected cell, suggesting that the other labeled parasol cells belong to the same type as the injected cell, and that the population of this type formed territorial domains. In addition, 2 to 7 cells with smaller somata (ca 7 µm diameter) were also labeled; these were found both within the dendritic field of the injected cell, and in the surrounding region. Some were sufficiently well labeled to trace their processes; they lacked an axon, had 2 to 3 main processes, and showed a sparse field of processes that seldom branched. They showed the morphology of certain amacrine cells; we could not determine the full extent of their dendritic fields, but they were considerably larger than that of the injected parasol cell.

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549.4

IDENTIFICATION OF ALL CELLS IN A SMALL PATCH OF FOVEA OF MACAQUE RETINA

Karl Klug*, Stanley J. Schein, Patricia Masarachia*, Peter Sterling & Yoshihiko Tsukamoto*. Dept of Psychology, UCLA, Los Angeles, CA, Dept of Anatomy, University of Pennsylvania, Philadelphia, PA, & Hyogo Coll Med, Hyogo, Japan.

We identified, from serial ultrathin sections, all cells in a small patch of retina containing 50 contiguous cone pedicles at 1.5^0 eccentricity. The corresponding region of the inner nuclear layer contained 98 midget bipolar cells, 52 diffuse bipolar cells, 49 horizontal cells, 63 amacrine cells, and 59 Müller cells. A comparable region of the ganglion cell layer contained 122 cells.

Midget bipolars had a thick dendrite that almost invariably approached a single pedicle closely before breaking up into a fine arborization. Fifty midget bipolars had pale somata. We traced 40 to their terminals: all were in the outer third of the inner plexiform layer (IPL), within sublamina *a*. Forty-eight midget bipolars had dark somata. We traced 23 to their terminals: all were in the inner third of the IPL, within sublamina *b*. Thus, virtually every pedicle contacted 1 *a*- and 1 *b*-midget bipolar. The only exceptions were 2 pedicles that contacted no *b*-midget bipolars. Moreover, with just 1 exception, every midget bipolar was contacted by only 1 pedicle.

Diffuse bipolar dendrites branched immediately upon entering the outer plexiform layer, extending processes horizontally under several pedicles. We traced 28 to their terminals, 18 in sublamina *a* and 10 in sublamina *b*. These partially overlapped with the midget terminals but were located more centrally.

Thus, the numbers of *a*-midget and *b*-midget bipolars, diffuse bipolars, and horizontal cells are virtually identical to the number of cone pedicles. This simple rule may apply throughout central retina (Schein & Klug 1990, Martin & Grünert 1990). There are, however, fewer cells in the ganglion cell layer, 122, than the total number of cone bipolar cells, 150.

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549.5

THE SPATIAL RESOLUTION OF COMBINED ON- AND OFF- GANGLION CELL ARRAYS IS NOT LIMITED BY POSITIONAL DISORDER AMONG THE SAMPLING ELEMENTS.

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The additional irregularity present in combined on- and off- ganglion cell arrays potentially results in a loss of spatial information, and this has been taken as evidence that the two subvarieties form independent sampling arrays. However, the hypothesis that the positional disorder of the combined arrays is sufficient to prevent any potential increase in spatial resolution from being realized has not been empirically tested. Positional disorder in the spacing of the ganglion cell array does not reduce resolution per se, but acts like a low pass spatial filter, and the degree of demodulation produced at any spatial frequency can be determined directly from the statistics of the nearest neighbor histogram (French, et al., 1977). The relative importance of this 'position' filter can be determined by comparing its spatial bandwidth to the Nyquist limit of the array and to the bandwidth of the receptive field centers which also act as low pass spatial filters. For both Alpha and Beta Cells in cat retinas, nearest neighbor histograms for on-, off- and combined arrays are accurately described by gaussian curves of approximately equal width. Fourier transform of these gaussian profiles indicates that the spatial bandwidth of these 'position' filters are 3-5 times higher than the Nyquist limit for combined on- and off- arrays, and 2-3 times higher than the bandwidth of the receptive field centers. When nearest neighbor histograms are convolved with receptive field center profiles, the effective bandwidth of the receptive field center is reduced by less than 10%, and is still significantly higher than the Nyquist limit of the combined array. Thus, the positional disorder within combined on- and off-center ganglion cell arrays is not sufficient to prevent them from cooperating to achieve higher spatial resolution.

549.6

GLUTAMATE AND SEROTONIN IMMUNOCYTOCHEMISTRY INDICATE CENTRIFUGAL FIBERS AND A VISUAL STREAK IN THE SKATE RETINA.

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We have performed both glutamate and serotonin immunocytochemistry in the retina of the skate, *Raja erinacea*. The glutamate immunoreaction labelled ordinary ganglion cells as well as giant superficial ganglion cells. In the inner nuclear layer, a regular pattern of labelled cell bodies was observed in retinal wholemounts. In the nerve fiber layer, single smooth fibers exhibited unusually strong immunoreactivity. A characteristic feature of these fibers was their antidromic branching, hence we tentatively classify them as centrifugal axons.

Serotonin-like immunoreactivity (SLI) in wholemounts revealed not only the class of bipolar cell previously reported but also a network of intensely staining amacrine cells. The density distribution of SLI bipolar and amacrine cells provides convincing evidence for the existence of a visual streak in the skate retina. The strongest SLI was observed in a small number of varicose fibers radiating from the optic disc. These fibers extended over the whole retinal surface and appeared to make *en passant* contacts with the perikarya of SLI amacrine cells. We suggest that they may also represent a class of centrifugal fibers as demonstrated in other species.

SLI bipolar cells have a morphology typical of "OFF" bipolar cells, branching as they do in layers 1 and 4 of the inner plexiform layer. Presently, we are quantitatively analyzing the topographical distribution of SLI and PKC-immunoreactive bipolar cells. The latter have a morphology typical of the "ON" rod bipolar cells identified in many duplex retinas.

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549.7

DECREASE IN THE NUMBER OF LARGE AND MEDIUM RETINAL GANGLION CELLS FOLLOWING TRANSECTION OF THE OPTIC NERVE IN ADULT FERRETS.

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As a prelude to the study of retrograde transneuronal degeneration in the retina of the adult ferret (Mustela putorius furo), an assessment was made of retrograde degeneration in retinal ganglion cells (RGC) in 3 ferrets following intracranial transection of the optic nerve. Survival times ranged from 5-7 mos. 5 micron sections of flat mounted, plastic embedded retinae were stained with 0.1% Thionin. Corresponding regions of the retinae were localized and the cells were counted utilizing a Camera Lucida drawing tube and a Bioquant LED cursor which were tied directly to the Sigma Scan (2.5, Jandel Scientific) computer program. Data analysis showed that there was a significant decrease in both the Alpha and Beta RGC size ranges among all the experimental animals when compared to controls. Compared to the cat, our findings are consistent with those of Tobimatsu, et al., (Electroenceph. and Clin. Neurophy., 73: 341-353, 1989) but are at variance with those reported by Hollander, et al., (Exp. Brain Res., 59: 633-635, 1985). The latter group of investigators maintained that there was long term survival (15.5 mos.) of large and small RGCs after optic nerve section. Based on our findings, it appears that both large and medium RGCs in the ferret undergo retrograde degeneration within as brief a period as 5 mos.

549.8

WAVELENGTH-DEPENDENT INHIBITORY INPUT TO ON-OFF AND OFF GANGLION CELLS IN THE TIGER SALAMANDER RETINA.

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Response-intensity and spectral sensitivity functions for ON, OFF and ON-OFF ganglion cells were obtained from extracellular recordings from the dark-adapted, flat-mounted, isolated retina of the larval tiger salamander. Two different types of ON-OFF ganglion cells were identified based on the form of the response-intensity function measured with different stimulus wavelengths. In the first type, both the ON and OFF response-intensity functions at all wavelengths were sigmoid-shaped with a relatively narrow dynamic range. These cells were similar to those described previously in the mudpuppy retina. In the second type, the shape of the OFF response-intensity function varied with stimulus wavelength. Between 450 to 600 nm, the OFF response-intensity function was not sigmoidal but had two distinct peaks with a period of suppressed OFF activity over a range of about 1.5 log units interposed between them. Between 650 to 700 nm, the OFF response-intensity function became more sigmoidal in form, with only a single peak. The shape of the ON response-intensity function of these cells did not display this wavelength dependence and appeared similar to that of the first type of ON-OFF ganglion cells. In all, 74% of the ON-OFF ganglion cells recorded from were of the second type. The OFF response-intensity function of some sustained OFF ganglion cells also exhibited this wavelength-dependent inhibition, while the ON response-intensity function of sustained ON ganglion cells did not. The period of suppressed OFF responses observed with short wavelength, medium intensity light may indicate that a subpopulation of ON-OFF and OFF ganglion cells receive inhibitory, possibly rod-dominant input from an interneuron over an interval within their dynamic range. Supported by EY07001, EY04446, Retina Research Foundation (Houston) and Research to Prevent Blindness, Inc.

549.9

DO NMDA CHANNELS CONTRIBUTE TO THE RESTING CONDUCTANCE OF RETINAL GANGLION CELLS? J. Gottesman & R.F. Miller Dept. Physiology & Graduate Program in Neuroscience, Univ. of Minnesota, Mpls. MN 55455.

The voltage-dependent block of the *N*-methyl-D-aspartate (NMDA) receptor by magnesium ions is thought to prevent this glutamate receptor subtype from being active when cells are at their normal resting potential. Voltage-clamped retinal ganglion cells in a larval tiger salamander slice preparation display inward currents to applied NMDA under Mg^{2+}-free conditions (V_{hold} -70 mV). NMDA-evoked responses are reduced by increasing external Mg^{2+}, but even 1 mM Mg^{2+} does not abolish responses at -70 mV (1 mM Mg^{2+} 30% of Mg^{2+}-free response; n=40). These data demonstrate that NMDA currents are generated at the resting potential.

We used fluctuation analysis of the current noise in voltage-clamped cells to determine if the NMDA channel is active under steady-state resting conditions. Under control conditions (V_{hold} -70 mV 1 mM Mg^{2+}), power spectra in the presence of 25-250 μM NMDA were sometimes, but not always, well fit by a single Lorentzian function. These data are consistent with prior results indicating that NMDA can evoke inward currents under control conditions.

In all cells tested (n=14), application of the selective NMDA antagonist D-AP7 reduced resting current noise amplitude and yielded power spectra with a 1/f form. Depolarizing the cell or reducing extracellular Mg^{2+} increased the noise power. This increase could be reversed by application of D-AP7. It remains to determined if the power spectra in Mg^{2+}-free Ringers with no added agonist conform to a single Lorentzian function, characteristic of an NMDA channel. However, the voltage- and D-AP7-sensitivity of the resting current noise suggest that the NMDA channel is a component of the resting conductance of all ganglion cells. This study emphasizes the importance of precise knowledge of levels of extracellular Mg^{2+} to more accurately predict the influence of the NMDA receptors at rest and near threshold.

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549.10

SYNAPTOLOGY OF A PHYSIOLOGICALLY IDENTIFIED Y-CELL AND W-CELL IN THE CAT RETINA. A.J. Weber and L.R. Stanford. Department of Comparative Biosciences and the Waisman Center, University of Wisconsin, Madison, WI 53706.

Following intracellular horseradish peroxidase injection in an in vivo preparation, the synaptology of a Y-cell and a W-cell in the cat retina are being studied by complete serial section reconstruction of their dendritic arbors. The Y-cell, injected at a retinal eccentricity of 2.1°, had an ON-center receptive field, nonlinear spatial summation, and responded phasically to a stimulus of standing contrast. This neuron has a soma diameter of 30μm and its dendritic tree has a mean diameter of 331μm. We have thus far identified 269 synaptic contacts onto the labeled dendrites of this Y-cell. Sixty five percent of this input is from amacrine cells and 35% originates from bipolar cells. The W-cell has a soma diameter of 9.5μm and was injected at a retinal eccentricity of 4.5°. This cell had an OFF-center receptive field, linear spatial summation, and responded tonically to a stimulus of standing contrast. The dendritic arbor of this neuron is extensive although very sparse. More than 50% of the dendritic tree has been reconstructed at this time, and we have found that the number of synaptic inputs received by its dendrites is extremely low. Only 25 bipolar cell inputs and 8 synapses from amacrine cells have been identified.

We have previously analyzed the pattern and distribution of synaptic input onto the dendritic arbors of two physiologically identified retinal X-cells. Similar data for the two cells currently being reconstructed will compare the distribution of bipolar and amacrine cell input among X-, Y-, and W-cells to determine whether the proportions of amacrine and bipolar cell input contribute to the functional differences among these retinal ganglion cell types. Supported by NIH grants EY 04977 and EY 05869.

549.11

DIRECTIONAL SELECTIVITY AND SYNAPTIC POTENTIALS OF IDENTIFIED TURTLE RETINAL GANGLION CELLS. R. D. DeVoe, P. L. Carras*. Indiana University, School of Optometry, Bloomington, IN 47405.

Intracellular recordings and Lucifer Yellow stainings reveal that ganglion cell types G3, G4, G6, G7, G9, G13, G15, G19 and G20 of Kolb (1982) are directionally selective (DS). Impulse discharges of any cell type are more narrowly tuned in polar plots than are the underlying synaptic potentials, which lack null directions. Thresholding makes the synaptic potentials narrowly tuned like the impulse discharges.

The masked drifting gratings used elicited periodic synaptic potentials. For G3, G15 and G19 ON-OFF cells, the dominant harmonic was the supra-threshold stimulus fundamental in preferred directions but the sub-threshold second harmonic in anti-preferrred directions (the frequency doubling expected from ON-OFF cells). In most cells tested, the amplitudes of periodic responses in preferred and anti-preferred directions were nearly the same (nonDS) for small mask apertures, became maximally different (DS) at some optimal intermediate aperture, and became the same again at still larger apertures. These optimal mask apertures were either ≤ (G6, G15, G19, G20) or ≥ (G3, G7, G9) dendritic field diameters. The latter cells thus had DS centers larger than their dendritic fields. G7 and G20 cells also had dynamic, DS, inhibitory surrounds with preferred directions opposite to their centers. In contrast, optimal diameters of flashed spots were always ≤ dendritic field diameters. Only G19 cells lacked static inhibitory surrounds.

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549.12

GABA AND FACILITATION IN TURTLE RETINAL DIRECTIONAL SELECTIVITY. Randall D. Smith*, Norberto M. Grzywacz, and Lyle J. Borg-Graham, Center for Biological Information Processing, MIT, E25-201, Cambridge, MA 02139 and the Smith-Kettlewell Institute.

Barlow and Levick (1965) proposed a model for retinal directional selectivity based on an asymmetric inhibitory process. Later experiments (Caldwell et al., 1975, Ariel and Adolph, 1985, Smith et al., 1991) indicated that GABA (an inhibitory neurotransmitter in the retina) is important in the production of directionality. The first question addressed in this work is: 1) Is GABA the output of an asymmetric process responsible for directional selectivity? Another mechanism that might contribute to the directionality is preferred-direction facilitation (Barlow and Levick, 1965; Grzywacz and Amthor, 1988). The second question we address is: Is there an asymmetric facilitatory process that contributes to directional selectivity?

We examined the effect of picrotoxin (a GABA_A antagonist) on directional selectivity for a range of contrasts and speeds. In 36% of the cells, we found a motion asymmetry despite saturating concentrations of picrotoxin. In 26% of the cells, the preferred and null directions were actually reversed. These results indicate that GABA is not the output of an asymmetric process, which mediates directional selectivity.

To answer the second question, we used apparent-motion protocols. The results show preferred-direction facilitation for the control condition and preferred- and null-direction facilitation for the picrotoxin condition. This strongly suggests that facilitation does not contribute to directional selectivity independently of GABA. We will present a model based on amacrine input/output asymmetry that accounts for these results.

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549.13

DISTINGUISHING MODELS OF DIRECTIONAL SELECTIVITY WITH PATCH CLAMP RECORDINGS FROM AN ISOLATED RETINA PREPARATION OF TURTLE. Lyle Borg-Graham and Norberto M. Grzywacz, Center for Biological Information Processing, MIT, E25-201, Cambridge, MA 02139, and the Smith-Kettlewell Institute.

Several lines of evidence strongly suggest that an inhibitory process underlies retinal directional selectivity (Barlow and Levick, 1965; Wyatt and Daw, 1975; Ariel and Adolph, 1985). A model for this computation postulates that this inhibitory process works at the dendritic tree of the directionally selective (DS) ganglion cell (Torre and Poggio, 1978). Alternatively, a recent model postulates that this inhibitory process acts on the dendritic tree of an amacrine cell and hence, that the input onto the ganglion cell is already DS. In this model, the dendrites receive distributed excitatory and inhibitory inputs and output the ganglion cell via the dendritic endings. The dynamics of the inputs and the cable properties of the dendrites make the output of these dendrites DS.

To distinguish these alternatives, we recorded the responses of directionally selective ganglion cells of turtle to moving stimuli under conditions in which the inhibitory input onto these cells has been dramatically reduced. The recordings used a whole-cell patch clamp technique in an isolated retina preparation (Borg-Graham and Grzywacz, 1991). With this technique, we reduced the inhibitory input onto the ganglion cell in two ways: 1) by voltage clamping the cells to the reversal potential of the inhibitory currents and 2) by removing ATP-Mg++ from the patch electrode. Under dramatically reduced inhibition, directional selectivity was essentially unaffected. Therefore, we conclude that the input to the ganglion cell is DS.

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549.14

ON AND OFF RESPONSES FROM OPTIC NERVE COMPOUND ACTION POTENTIAL RECORDINGS IN TWO TELEOST FISHES. C.W. Hawryshyn, L. Beaudet, and H.I. Browman, Dept. of Biology, University of Victoria, P.O. Box 1700, Victoria, B.C., Canada V8W 2Y2.

We characterized the photopic spectral sensitivity (340 to 720 nm) of adult African cichlids (Haplochromis burtoni) (HB), and large (> 55 g) rainbow trout (Oncorhynchus mykiss) (OM), using optic nerve compound action potential recordings. The ON responses in both species exhibited sensitivity peaks corresponding to S, M, and L wavelength photoreceptor mechanisms. In HB, these peaks occurred at 450-460 nm, 540-550 nm, and 590-610 nm, whereas in OM, the peaks were recorded at approximately 440 nm, 540 nm, and 620 nm. The OFF response in both species was characterized by two broad peaks in the S and L wavelength parts of the spectrum. Under a M and L wavelength adapting background, the relative sensitivity of the OFF response (from 500-700 nm) in HB was consistently higher than that for the ON. However, under the same conditions, the relative sensitivity of the OFF response in OM was consistently lower than that for the ON. L.B. was supported by a NSERC Canada Postgraduate Scholarship, H.I.B. by a MRC Canada Postdoctoral Fellowship, and C.W.H. by a NSERC Canada URF and Operating Grant (# URF 0043984). We thank R.D. Fernald for supplying the cichlids.

549.15

CIRCADIAN RHYTHMS IN THE JAPANESE QUAIL RETINA

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Retinal sensitivity of the Japanese quail exhibits a circadian rhythm. When the animal is held in constant darkness, retinal sensitivity as measured by the ERG b-wave, increases at night and decreases during the day (Uchiyama et al., Neurosci, Abstr., 16, 1333, 1990). Spectral sensitivity also shifts with time of day. Maximum spectral sensitivity is about 500 nm at night reflecting rod-dominance and about 600 nm during the day reflecting cone-dominance. thus the change in rod-cone dominance of the retina (Purkinje shift) is controlled in part by an endogenous circadian clock.

What is the origin of the circadian changes in the retina? To investigate this question we analyzed both the photoreceptor (a-wave) and postphotoreceptor (b-wave) components of the ERG and found that only the b-wave exhibits a circadian rhythm in amplitude and spectral sensitivity. The spectral sensitivity of the a-wave is broad and does not change with time of day. We tentatively conclude that the circadian clock does not modulate photoreceptor sensitivity directly but rather influences the pathways that carry those signals to the inner retina.

Retinal levels of melatonin and dopamine have been found by others to undergo circadian changes in galliform birds. We are currently investigation whether those neuromodulators have a role in the circadian rhythm of the quail retina.

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549.16

CENTRIFUGAL INPUTS ENHANCE VISUAL RESPONSES OF RETINAL GANGLION CELLS WITHOUT CHANGING THEIR SPATIAL CODING PROPERTIES IN THE JAPANESE QUAIL.

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Stimulation of the centrifugal fibers enhances visual responses of the retinal ganglion cells (RGC) of birds. The effect has been attributed to disinhibition, that is reduction of the inhibitory surround of the receptive field of the RGC (Miles,'72). The inhibitory surround is thought to contribute significantly to the contrast sensitivity of the RGC, and thus disinhibition of the surround may decrease contrast sensitivity. However, a behavioral study showed that transection of the centrifugal fibers projecting to the retina does not cause any major change of visual acuity of the pigeon (Knipling,'78). To further investigate this problem, we studied the effects of the centrifugal inputs on the spatiotemporal properties of the RGC receptive fields of the Japanese quail. Urethane-anesthetized, curarized animals were used. Spatial frequency analysis showed that stimulation of the centrifugal fibers enhanced the responses of > 60% of single optic nerve fibers without changing the shape of their spatial frequency turning curve. Centrifugal inputs also changed the temporal response properties of some units. The present results do not support the conclusions of the previous studies, but suggest that the centrifugal inputs enhance visual responses of the RGC through the neural circuits that are independent of the center-surround organization of the receptive field.

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549.17

UV Light Modulates the Retinal Sensitivity of *Limulus*.

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Ultraviolet light enhances the circadian rhythms in the sensitivity of the *Limulus* lateral eye (Westerman and Barlow, 1983). Ambient UV light is sensed by a pair of frontal eyes called median ocelli. At night, this UV information is transmitted by optic nerve activity to a circadian clock located in the brain. The ocellar input enhances the clock's neural output. Efferent optic nerve fibers feed the clock's output back to the ocelli and lateral eyes enhancing their sensitivity. As a result, retinal sensitivity is 0.5 log units higher at night under UV illumination than in complete darkness.

Nighttime UV illumination of the ocelli enhances retinal sensitivity; daytime illumination does not. Exposing the ocelli to environmental light at any time during the night increases sensitivity of the lateral eyes within minutes. Blocking UV illumination (<430nm) of the ocelli at night decreases lateral eye sensitivity while blocking visible wavelengths (>360nm) has no effect.

The ocellar influence on retinal sensitivity may have a role in the animals' nocturnal mating behavior which is known to involve vision. Both starlight and moonlight contain significant levels of UV light. At night, sufficient amounts of UV light can penetrate the water column to a depth of 10 meters to stimulate the median ocelli . Knowing all this, we still do not understand what role UV light may have in the animals' behavior.

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SUBCORTICAL VISUAL PATHWAYS: CORTICAL INPUTS AND SUBCORTICAL RESPONSES

550.1

PROPERTIES OF CELLS IN AREA 17 PROJECTING TO THE CAT'S LATERAL POSTERIOR-PULVINAR COMPLEX.

C. Casanova. Dept. of Ophthalmology, Faculty of Medicine, University of Sherbrooke, Sherbrooke, Canada, J1H 5N4.

The main visual input of the lateral part of the lateralis posterior nucleus (LPl) comes from the striate cortex. When compared with cortical units, cells in LPl have properties similar to those of complex rather than simple cells. Anatomical studies have indicated that cortico-LPl cells are mainly located in layer V of areas 17 and 18 were most complex cells lie. We have investigated the properties of cortical units antidromically activated from the LPl and from the superior colliculus (SC).

Experiments were performed with anesthetized and paralyzed normal adult cats. Bipolar stimulating electrodes were placed in LPl and SC. A first set of electrodes was placed in the superficial layers of SC. Multi-unit responses were recorded from an electrode placed in successive latero-medial positions in the LGN. The location of LPl was derived from these recordings. Tungsten-in-glass microelectrode was used to record from cells in area 17. Receptive fields were stimulated with drifting gratings.

Preliminary results indicated that cortical cells antidromically activated from LPl responded with a mean latency of 1.6 ms. All cortico-LP units were selective to orientation and were tuned to relatively low spatial frequencies (0.4 c/deg). Most units responded to moving visual noise and had properties similar to the special complex cells described by Palmer and Rosenquist (1974). So far, no cells were antidromically activated from the LPl and the colliculus. These results suggest that complex cells in area 17 projecting to LPl do not project to SC. (Supported by MRC of Canada)

550.2

ULTRASTRUCTURAL DEVELOPMENT OF VISUAL CORTICOTECTAL PROJECTIONS IN THE CAT.

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The corticotectal projection in adult cats has precise topographic and laminar targets. Yet in neonates the projection extends beyond these targets. To determine how the growing cortical axons interact with neurons in the superior colliculus to acquire their adult specificity, biocytin was injected into the visual cortex in animals aged 0 to 35 days. The superior colliculus was processed for electron microscopy.

Examination of labeled cortical axons in the superior colliculus showed that the axons do not follow dendrites, axons, or glia for any significant distance. Labeled contacts were present even at 1 day of age and increased in frequency over several weeks. At the earliest ages most contact zones contained few synaptic vesicles as the only indication of developing synapses. For several weeks following birth, the number of presynaptic vesicles increased and synaptic clefts and postsynaptic densities became more defined. The labeled axons often contacted profiles that contained vesicles. The number of vesicles in these profiles decreased with age. In adults corticotectal axons are believed to synapse with dendrites and thus the neonatal cortical axons may be contacting developing dendritic profiles.

Supported by Health Futures Foundation and Basic Research Support grants.

550.3

CORTICOTECTAL PROJECTIONS IN THE CAT. J.K. Harting, B.V. Updyke, and D.P. Van Leishout*. Department of Anatomy, University of Wisconsin-Madison, Madison, WI 53706 and Department of Anatomy, Louisiana State University, New Orleans, LA 70112

The possible significance of the patchy, mosaic-like organization of afferents within the stratum griseum intermediale (SGI) of the cat superior colliculus is slowly emerging. One particularly fruitful approach has involved comparing the spatial relationships of various tectopetal fiber systems to specific transmitter and enzyme markers and, most important, to each other. The general theme of these light microscopic studies has been that functionally related afferents end in patches, lattices or domains, where they are coordinated in order to influence collicular neurons involved in controlling eye and axial body movements.

As a prerequisite for determining specific compartments and their components, we have been studying the laminar, sublaminar and, in many instances, the patchy distribution of twenty-four corticotectal projections. Our findings reveal that fourteen cortical areas project exclusively to the SGI. All of these corticotectal systems innervate limited regions of the SGI, and the great majority end in patches. One particularly interesting finding is that several corticotectal systems target what we have termed the middle tier of the SGI (Harting & Van Leishout, 1991). Such cortical input arise from SIV, SV, lateral frontal eyefields, cingulate cortex, and area 4. We have previously shown that nigral and trigeminal inputs also end in this tier, but in a complementary, nonoverlapping fashion. At this time we are investigating the specific spatial relationships of several of these "middle tier" corticotectal systems to each other, and to various subcortical collicular afferents.

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550.4

THE MORPHOLOGY AND PROJECTIONS OF CORTICOTECTAL CELLS IN THE OWL'S VISUAL CORTEX. I.C. Gynther and J.D. Pettigrew. Vision, Touch and Hearing Research Centre, The University of Queensland, Qld. 4072, Australia.

In the owl, corticotectal neurons are involved in the control of visually-guided behavior via their influence on the tectum. This, together with the large size, abundance and wide distribution of corticotectals makes them one of the most important classes of output neuron in the owl's visual cortex. In this study, we have combined dye-labelling in the whole brain and in fixed brain slices to determine details of the morphology and projections of corticotectal cells in the visual cortex of tytonid owls. Corticotectal neurons were the largest cell type in cortex and possessed numerous dendritic spines, as is also true in mammalian visual cortex. However, in contrast to mammals, corticotectals in the owl were located above the granular layers and had axons which passed superficially, just below the pia.

An unusual feature of owl corticotectals was that, as a class, they projected bilaterally. This is probably due to the fact that the rostral portion of the tectum deals with the region of binocular overlap and so each tectum would share part of the visual field with each visual cortex. However, although the corticotectal population as a whole projected bilaterally, individual neurons did not, since we rarely found double labelled cells in cortex after placing different retrograde dyes in corresponding regions of each tectum. There was no indication that corticotectal cells were segregated within cortex (e.g. into columns or stripes) based on the laterality of their projections. On the contrary, cells projecting to both tecta were often found side by side. The existence of patches of corticotectal efferent fibres within the tectum (Bagnoli et al., Brain Behav. Evol., 1991, in press) suggests that segregation may occur instead at the output level. Such segregation appears similar in principle to that producing ocular dominance columns although, here, the segregation of efferents in a tectal patch cannot be due to the fibres being derived from a common eye but rather appears to be related to their derivation from the same hemisphere.

550.5

CORTICOSTRIATAL AND CORTICOTECTAL PROJECTIONS FROM THE CAT LATERAL SUPRASYLVIAN CORTEX. J.G. McHaffie, B.E. Stein and M. Norita. Department of Physiology, Medical College of Virginia, Richmond, VA, 23298 and Department of Anatomy, Niigata University Sch. of Medicine, Niigata 951, Japan.

The relationships between the visual cortex and the striatum (ST) of the cat is poorly understood. The present experiments were an attempt to determine if regions along the lateral suprasylvian cortex (LS), known to sent projections to the superior colliculus (SC) also project to the ST, and if so, to determine whether corticostriatial and corticotectal axons arise from the same neurons. Injections of the anterograde tracer biocytin into the posterior portion of LS resulted in dense label in both ST and SC. In ST, labeled fibers and terminals were found predominately in the caudal part of the head of the ipsilateral caudate nucleus and the caudal portion of the ipsilateral putamen. These cortical injections also resulted in label in the superficial and deep laminae of the ipsilateral SC. After paired injections of retrogradely transported fluorescent dyes into ST and SC, numerous labeled LS neurons were observed in lamina V and modest numbers in lamina III; however, whereas corticostriatial neurons were found in laminae III and V, corticotectal neurons were seen only in lamina V. Although labeled neurons from each injections were intermingled in lamina V, very few were double-labeled. These data suggest that while ST and SC receive substantial visual inputs from the same cortical area, the nature of that information may be quite different. Supported by NEI grant EY05554.

550.6

POSTNATAL ONTOGENESIS OF CORTICOSTRIATAL AND CORTICOTECTAL PROJECTIONS FROM THE CAT LATERAL SUPRASYLVIAN CORTEX. M. Norita, B.E. Stein and J.G. McHaffie. Dept. of Anatomy, Niigata Univ. Sch. of Med., Asahimachi Niigata 951, Japan and Dept. of Physiology, Medical College of Virginia, Richmond, VA, 23298.

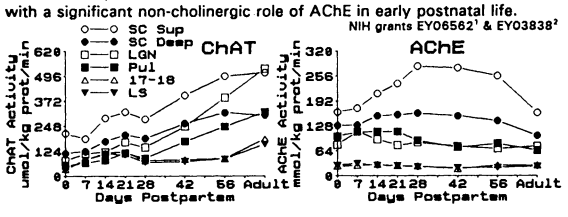
To evaluate the postnatal ontogenesis of corticostriatal and corticotectal projections from the lateral suprasylvian cortex (LS), we injected the anterograde tracer biocytin into LS in neonatal cats aged 1-60 days postnatal (dpn). At the light microscopic level, both corticofugal pathways were already present at birth. Although the terminations of these projections appear to have topographies approximating those of adults, neonatal terminal regions were much more densely labeled. To determine whether these neonatal projections represent actual synaptic endings or merely the presence of labeled fibers, we examined the ontogeny of synapses with the electron microscope. Although labeled axon terminals could be found in both ST and SC at 1 dpn, their number was minimal. During the first postnatal month, the number of labeled synapses gradually increased. We suggest that the protracted synaptic development of these neural pathway underlies the protracted development of visually-guided orientation behavior. Supported by NEI grant EY06562.

550.7

POSTNATAL DEVELOPMENT OF CHAT AND ACHE ACTIVITY IN VISUAL STRUCTURES OF THE CAT. D.D. Dunning[1], J.G. McHaffie[1], B.E. Stein[1] and C.D. Ross[2]. Dept. of Physiol., Med. Coll. of Virginia, Richmond, VA, 23298[1] & Dept. of Otorhinolaryngol., Univ. of Okla., Oklahoma City, OK, 73190[2].

The postnatal maturation of cholinergic enzymatic activity was determined by radiometric assay in select cortical and subcortical visual structures of the developing cat (0-56 days). ChAT activity increased progressively from birth except for a transient decrease about day 28. Adult ChAT activity was similar between the following pairs of structures: superficial superior colliculus (SC) and lateral geniculate (LGN), deep SC and pulvinar (Pul), area 17-18 and lateral suprasylvian cortex (LS). AChE activity increased after birth then decreased gradually to adult levels in all structures except visual cortex, which had a flat profile, and Pul, which had a slightly higher level at birth than adulthood. AChE in LGN and Pul peaked early (day 7) but fell rapidly to adult levels, whereas SC peaked late (day 28), declining gradually to adult levels. The caudal half of SC had higher activities in both superficial and deep layers, beginning at about day 14 for ChAT and day 42 for AChE. Decreasing AChE/ChAT ratios are consistent with a significant non-cholinergic role of AChE in early postnatal life.

NIH grants EY06562[1] & EY03838[2]



550.8

PHYSIOLOGICAL MATURATION OF THE VISUAL TOPOGRAPHY IN THE CAT SUPERIOR COLLICULUS. C.-Q. Kao, J.G. McHaffie, M.A. Meredith and B.E. Stein. Departments of Physiology and Anatomy, Medical College of Virginia, Richmond, VA, 23298.

Anatomical and behavioral studies indicate that central visual fields are likely to develop before peripheral ones. The present experiments were initiated to determine the physiological maturation of the visuotopy in the superior colliculus (SC). Single and multiunit responses were recorded in urethane-anesthetized kittens (7-50 dpn). A systematic exploration of the SC at about the time of eye opening (9 dpn) revealed topographically organized, visually-responsive neurons in superficial layers. Although fewer responsive neurons were found in younger animals, the visuotopy appears to exist prior to eyelid opening. There was no evidence that neurons in a particular region of the SC became responsive before any other; thus neurons with central fields were not active earlier than those with more peripheral fields. The superficial layer topography was evident before most deep layer neurons were responsive to visual stimuli. These data contrast with observations that early visual behaviors depend first on the central retina. Supported by NEI grant EY06562.

550.9

CORTICAL CONVERGENCE ON MULTISENSORY OUTPUT NEURONS OF CAT SUPERIOR COLLICULUS. M.T.Wallace, M.A.Meredith and B.E.Stein, Depts. Physiol. and Anat., Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298

In cat superior colliculus (SC), the convergence of inputs from different sensory modalities (Stein and Arigbede, 1972), and the integration of these inputs by single neurons (Meredith and Stein, 1986) has been well established. Multisensory responses in SC neurons are dynamic, and are likely subject to modulation by experience and behavioral state: functions believed to be derived from higher cortical centers. We examined the accessibility of multisensory SC neurons to cortical inputs by means of orthodromic stimulation. Chronically prepared cats had arrays of stimulating electrodes implanted in visual (PLLS, ALLS, AEV), auditory (Field AES) and somatosensory (SIV) cortices. A total of 249 (from a sample of 413) neurons received cortical input from at least one of these areas. Many multisensory cells (112/189) received input from two or more cortical areas. In general, multisensory neurons receiving convergent cortical input demonstrated a strong interactive effect when stimuli from more than one modality were presented. Antidromic stimulation revealed that most (77%) multisensory cells which received convergent cortical input were also efferent neurons that projected into the tectoreticulospinal tract. These results demonstrate that cortex can modulate the integrated multisensory signals which form a major component of the crossed descending output from the SC, through which attentive and orientation behavior can be mediated.

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550.10

IDENTIFICATION OF CELLS IN THE SUPERIOR COLLICULUS SUBJECT TO WIDESPREAD INHIBITION. R. M. Douglas and K. E. Anderchek,* Depts. of Ophthalmology, Psychology and Physiology, Univ. British Columbia, Vancouver, B.C. V5Z 3N9

A winner-take-all circuit must exist somewhere between the visual and oculomotor systems. We have proposed (Douglas & Vetter, 1986) that an inhibitiory circuit capable of selecting one focus of activity lies in the SC. To identify which cells may be involved, iontophoretic application of glutamate or acetylcholine was used to increase the discharge levels of SC neurons in urethane-anesthestized rats. Only cells lying in the intermediate layers showed strong inhibition to electrical stimulation of the contralateral SC. This inhibition could be blocked by bicuculline. Many of these cells could be activated antidromically from the brainstem. In addition, brainstem stimulation produced inhibition but also revealed that many projection neurons were insensitive to glutamate. The results are consistent with target selection being the last processing step in the SC.

550.11

THE EFFECTS OF SEROTONIN AND ITS ANALOGUES UPON VISUAL NEURONS IN THE HAMSTER'S SUPERIOR COLLICULUS. X. Huang and R. D. Mooney. Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43699-0008

The superficial layers of the rodent superior colliculus (SC) are innervated by serotoninergic fibers from the nucleus raphe dorsalis and contain multiple serotonin (5HT) receptor subtypes, including $5HT_{1A}$, $5HT_{1B}$, $5HT_{1D}$, and possibly $5HT_2$. This raises the possibility that 5HT may exert differential effects in the SC through interactions with different receptors. We recorded single cells with multibarrelled pipettes and characterized their receptive fields. Using micropressure application, 5HT and/or an analogue was ejected from the pipette as the cell responded to electrical stimulation of the optic chiasm or visual patterns. Visual activity was strongly suppressed by 5HT in 81% (35/43) of the neurons tested; only 5% (2/43) were excited and 14% (6/43) showed no effect. No differential effects on cells with particular receptive field types were noted. Two $5HT_{1A}$ agonists 8-hydroxy-2[di-n-propylamino]tetralin (8-OH-DPAT) and 5-carboxamidotryptamine (5CT) had weaker suppressive effects than 5HT: 8-OH-DPAT suppressed 43% (3/7) of cells tested and 5CT suppressed activity in all 3 cells tested, but was less potent than 5HT. The $5HT_{1B}$ agonist m-trifluoro-methyl-phenyl-piperazine (TFMPP) had no effect on visual activity of 3 cells tested. Antagonists for $5HT_{1\,\&\,2}$ receptors spiperone and mianserin blocked the suppressive effects of 5HT in all cells tested (n=8: 4 each agent); while ketanserin, a $5HT_2$ blocker, was ineffective (n=6) and methiothepin, a $5HT_{1A\,\&\,2}$ antagonist, showed no consistent effect (n=4). We conclude that the effects of 5HT on visual activity in the hamster's SC are primarily mediated through $5HT_{1A}$ receptors and a second, as yet unidentified subtype.

EY08015 and EY04170

550.12

ADENOSINE MODULATES RETINOTECTAL TRANSMISSION IN GOLDFISH. W. M. King and J. T. Schmidt. Dept. of Biol. Sci., SUNY-Albany, Albany NY 12222.

Presynaptic adenosine receptors inhibit the release of many neurotransmitters in brain and decrease quantal content at the neuromuscular junction. Since retinal ganglion cells have high levels of adenosine and adenosine receptors on their terminals, Braas *et al.* (P.N.A.S. 84:3906-3910, 1987) have suggested that endogenous adenosine may serve as a coneurotransmitter in the retinal ganglion cells. We evaluated this possibility by testing the effects of adenosine and adenosine agonists and antagonists on retinotectal transmission in an in vitro preparation of goldfish brain.

Concentrations of 100-300 μM adenosine, comparable to those that reduce quantal content at the neuromuscular junction, and 100 μM 2-chloroadenosine, an agonist minimally transported across cell membranes, reduced retinotectal field potential amplitude by 30-50%. They also increased paired pulse facilitation which suggests a presynaptic site of action. These effects were attenuated by the adenosine antagonists caffeine or IBMX (100-200 μM) by 30-60%. Either caffeine or IBMX alone increased the amplitude of the retinotectal field potential, suggesting that endogenous adenosine may limit transmitter release. Since the cAMP analogues dibutyryl-cAMP and 8-bromo-cAMP (1 mM) had negligible effects, the adenosine effect is not likely to be mediated by cAMP. Since we have previously shown that presynaptic nicotinic receptors enhance retinotectal transmission, the present results suggest that transmitter release at optic nerve terminals may be under dual, bidirectional regulation by two neuromodulators acting at distinct presynaptic receptors. (Supported by NIH grant EY03736)

550.13

VISUAL RESPONSES OF NEURONS IN THE NUCLEUS OF THE BASAL OPTIC ROOT OF THE NORTHERN SAW-WHET OWL. Douglas R. Wylie, Steven W. Shaver and Barrie J. Frost, Department of Psychology, Queen's University, Kingston, Ontario, Canada K7L 3N6.

The nucleus of the basal optic root (nBOR) is a component of the avian Accessory Optic System (AOS) which is involved in the analysis of visual "flowfields" resulting from self-motion. The structure of flowfields is qualitatively different for lateral-eyed and frontal-eyed species, thus one might expect differences in the AOS. The AOS has been investigated in the lateral-eyed pigeon, but frontal-eyed birds have not been studied. Using standard extracellular techniques we recorded from neurons in the nBOR of the northern saw-whet owl (*Aegolius acadicus*). Under ketamine anaesthesia this bird has a binocular field measuring 50 X 75 degrees (horizontal X vertical). Neurons in nBOR responded best to large patterns of random dots moving either upward, downward or nasal to temporal (N-T) in the contralateral visual field. Few units preferred temporal to nasal motion. Up units were found in dorsal region of nBOR whereas down units were found ventral to these. N-T units were found in both the lateral margin, and ventral to the down units in the lateral half of nBOR. About half of the units tested responded to stimulation of the ipsilateral as well as the contralateral eye. For all but one cell, the direction preference of both eyes was the same in visual space. In most cases, binocular stimulation resulted in greater modulation of the cells' firing rates than monocular stimulation. These responses resemble those of pigeon nBOR neurons with respect to direction preference; however, few neurons in the pigeon nBOR are binocular. In mammals, there is a greater incidence of binocular neurons in the AOS of frontal-eyed species, compared to those with laterally placed eyes. Together, these data suggest that the avian and mammalian AOS may have developed in a similar fashion with the occurrence of frontal-eye placement.

550.14

MULTISENSORY NEURONS IN THE MIDBRAIN OF THE PIGEON. S.W. Shaver and B.J. Frost. *Department of Psychology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.*

The neural integration of information from different sensory systems is required for a variety of functions, and cells responsive to stimulation of more than one sense (multimodal units) have been identified in several species. Multimodal units were identified using extracellular recordings in the pigeon nucleus mesencephalicus lateralis, pars ventralis (MLv). Most units encountered were multimodal and were either visual-auditory, visual-somatosensory, or visual-auditory-somatosensory. Visually-responsive units preferred small moving stimuli and showed no clear directional specificity. Somatosensory-responsive units were maximally activated by gentle brushing of the feathers and beak. Auditory-responsive units tended to be broadly-tuned, or non-responsive, to tone stimuli, but had low thresholds to noise (20-30 dB SPL). Many units displayed large receptive fields with no clear best area. These units responded to visual stimuli presented in a large area of contralateral visual space (up to 80° X 80°). Similarly, the somatosensory receptive fields often extended over the whole contralateral body surface. The auditory spatial tuning for these units tended to be flat in azimuth and elevation, but for some cells was restricted to the contralateral field. For units with clear best areas, the receptive field maps for each mode of stimulation tended to be in register. For instance, units with visual best areas located 90° lateral to midline tended to have similarly located auditory best areas, and showed peak somatosensory responding to stimulation of the lateral body surface. These multimodal cells in the pigeon MLv appear to be well suited to the mediation of attentional and orientational responses to stimulation.

Supported by the Natural Sciences and Engineering Research Council of Canada

550.15

SOME NEURONS IN THE NUCLEUS ROTUNDUS OF PIGEON COMPUTE TIME TO COLLISION. Y-C. Wang and B.J. Frost. Departments of Physiology and Psychology, Queen's University, Kingston, Ontario K7L 3N6, Canada.

Behavioral studies have shown that the avian tectofugal pathway, which is similar to the mammalian colliculo-pulvinar-MT pathway, is involved in processing several important visual attributes, such as color, intensity, pattern, and motion. Our previous results indicated that cells in anatomical discrete zones within the nucleus rotundus of pigeon exhibited specific visual response characteristics associated with these attributes, and about 17% of cells in the dorsal-posterior subarea of the nucleus strongly responded to a looming stimulus. In this study, standard extracellular responses from dorsal-posterior subarea, EMGs from pectoral muscles, and EKGs were simultaneously recorded from awake birds. We have found that looming sensitive neurons are not only very specific to the 3D direction of motion of a "soccer ball" like stimulus, but give their maximum response at a constant time before "collision", even when the size of the stimulus or its velocity were varied over a large range of values. The EMGs always occurred 100-200 ms after the peak looming cell response, and the heart rate increase followed even later. When the looming stimulus was not on a collision course with the bird, the looming cells' response was dramatically reduced and no EMGs, or increased heart rate occurred. We propose that these looming sensitive neurons signal "time to collision" and provide information necessary for an emergency avoidance response by the bird. Supported by NSERC grant A0353 to BJF.

550.16

THE EFFECT OF POSITIONAL DISPARITY ON MOTION RESPONSES OF NEURONS IN THE ACCESSORY OPTIC SYSTEM OF CAT. K.L. Grasse. Dept. Psychology, York University., North York, Ontario, Canada M3J 1P3.

It is widely accepted that the accessory optic system (AOS) provides visual signals for the control of optokinetic nystagmus (OKN). In frontal-eyed animals, the substantial overlap in the visual fields of each eye and a projection from the visual cortex gives rise to an increased incidence of binocularly responsive neurons in the AOS. Visual cortical input has been shown to mediate ipsilateral eye responses and high speed tuning, and can function independently of the contralateral eye. However, binocular tuning characteristics of AOS cells have not been extensively examined. The present study set out to determine if binocular AOS cells are sensitive to retinal disparity. Single units were recorded from the dorsal terminal nucleus (DTN) of anesthetized, paralysed cats. The positions of areæ centrales were determined by fundus reflection. Convergent and divergent disparities were generated by deviating the visual axis of one eye using wedge prisms ranging from ±1-20 diopters (i.e., base-in or base-out, 1 diopter=0.57 deg). A random-dot pattern composed of multiple gray-levels was moved by computer at a constant velocity in the preferred direction. The responses of DTN units to positional disparity fell into 4 categories: 1) cells with tuning profiles similar to "tuned excitatory" responses consisting of a marked facilitation for a single or a small range of disparities (convergent and divergent); 2) cells relatively insensitive to disparity, showing a relatively flat response profile across the entire range of conditions; 3) cells displaying rather complex tuning profiles which usually contained multiple peaks or troughs at different disparity values; and 4) a small number of cells broadly tuned for inhibition. In summary, this study demonstrates that many AOS cells are sensitive to positional disparity and supports behavioral investigations showing that OKN gain is dramatically affected by changes in depth planes defined by disparity. Supported by a grant from NSERC.

550.17

SENSORY MODALITY SPECIFICITY OF NEURONAL RESPONSES WITHIN THE CAT'S SUPRAGENICULATE NUCLEUS.

G. Benedek*, J. Perényi*, G. Kovács*, Y. Katoh*, B. Csákány*, and M. Norita. Dept. Physiol., Albert Szent-Györgyi Med. Univ., Szeged, Hungary and Dept. Anat. Niigata Univ. Sch. Med., Niigata 951, Japan.

The feline insular cortex contains cells sensitive to visual, auditory and somatosensory stimuli. The suprageniculate nucleus (SGn), receiving the superior collicular afferents, seems to be the main subcortical source of afferents towards the insular cortex. We studied the modality specificity of extracellularly recorded SGn cells in barbiturate anesthetized, immobilized, artificially ventilated cats. Of the 136 cells tested 109 showed unimodal sensory characteristics, 23 was bimodal and only 4 was sensitive to all the three modalities. ***Visual*** sensitivity was found in 100 cells. These showed large uniform receptive fields including the area centralis. No retinotopy could be detected. ***Somatosensory*** sensitivity to tactile stimulation was found in 32 cells, and that to nociceptive stimulation in 5 cells. ***Acoustic*** sensitivity was found in 24 cells. No systematic relationship was found in the anatomical position of cells sensitive to various modalities. Our results show sensory properties in SGn similar to that described in the insular cortex. This is in line with the concept of parallel processing of sensory information along a tecto-thalamo-insular cortex route.

CEREBELLUM II

551.1

DOES THE CEREBELLUM PREFERENTIALLY CONTROL MULTIJOINT MOVEMENTS? H.P. Goodkin and W.T. Thach. Departments of Anatomy & Neurobiology and Neurology & Neurosurgery, Washington University School of Medecine, St. Louis, MO 63110.

Inactivation of the cerebellar dentate nucleus in the monkey impairs reaching and pinching with sparing of trained wrist movements (Kane et. al., 26.6, 1989). This result suggests that dentate control of movement is preferentially concerned with multiple joints rather than single joints. The following study tests this hypothesis.

Two juvenile Rhesus monkeys (*Macaca mulatta*) have been trained to insert their right hand into a "pinch" manipulandum. The apical pads of the thumb and index finger rest on snap-action switches which are closed by flexion (3-5mm) of the distal phalanx of these digits. Digits 3, 4, and 5 are held firmly in place in the manipulandum. Cued by an LED display, the monkey performs 3 tasks: (1) closure of the thumb switch by flexion of the THUMB, (2) closure of the index finger switch by flexion of the INDEX finger, and (3) closure of both switches by the combined simultaneous flexion of the thumb and index finger (PINCH). Force analysis confirms that the THUMB and INDEX tasks are performed, on average, independently. Analysis of EMG (recorded from the shoulder girdle, arm, forearm, and intrinsic hand muscles) shows that the PINCH task is performed using only muscles that are active during the THUMB, INDEX, or both tasks; no muscle is recruited uniquely for PINCH. Thus, the two independent movements are combined into a synergic sum. This combination of two independent movements into a synergic sum is the crucial factor that is missing from previous ablation and single unit recording studies which address the question of whether cerebellar control of coordination is at the single joint or the multijoint level. (NIH grant NS12777)

551.2

INFERIOR OLIVE DISEASE IN MAN IMPAIRS LEARNING NOVEL SYNERGIES W.T.Thach, J.G.Keating, and H.P.Goodkin, Depts of Anatomy & Neurobiology, Neurology and Neurosurgery, and The IWJ Rehab. Institute, Washington University Medical School, St. Louis, Mo. 63110.

In throwing, the eyes (and head) fixate the target, and serve as reference aim for the arm. Coordination between gaze direction and arm throw is a skill: it is developed and maintained by practice. When wedge prism spectacles are placed over the eyes with the base to the right, the optic path is bent to the right, and the eyes (and head) move to the left to fixate the target. The arm, calibrated to the line of sight, throws to the left of the target. With practice, the calibration changes, and the arm throws closer to and finally on-target. Proof that eye (and head) position is the reference aim for the arm throw trajectory occurs when the prisms are removed and the arm throws. The eyes are now on-target, but the eye-head-arm calibration for the previously leftbent gaze persists: the arm throws to the right of target an amount almost equal to the original leftward error. With practice, eye-head position and arm synergy is recalibrated: each throw moves closer to and finally on-target.

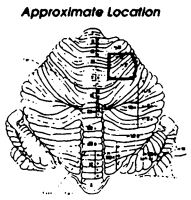
Baizer showed in macaques that the adjustment is abolished by cerebellar lesion (Baizer and Glickstein, 1974). Weiner et al (1983) obtained similar results in patients with cerebellar disease, and showed that adaptation was not impaired in disease of corticospinal or basal ganglia systems. We here confirm these results in patients with pure cerebellar cortical disease (1 infarct, 2 cortical atrophies). We have also studied two patients with MRI-documented inferior olive hypertrophy. Both had palatal myoclonus and progressive ataxia of trunk and gait. Neither showed adaptation on this task, despite near-normal throwing performance (cf also Gauthier et al 1979). Finally, we studied patients with lesions presumed to involve mossy fibers of the middle cerebellar peduncle, who also show impaired adaptation (cases of ataxic hemiparesis, with contralateral lesions of the basis pontis involving leg corticospinal and arm pontocerebellar fibers, according to Fisher, 1978).

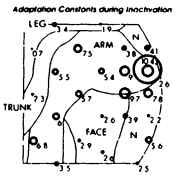
These results indicate that cerebellar cortex, inferior olive, and mossy fibers are all necessary for the adaptation, but they do not distinguish the roles played by each (NIH grant NS12777).

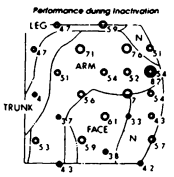
551.3

THE CEREBELLAR CORTICAL AREA REQUIRED FOR ADAPTATION OF MONKEY'S "JUMP" TASK IS LATERAL, LOCALIZED, AND SMALL. J.G. Keating and W.T. Thach. Dept. of Anatomy and Neurobiology, Washington University Sch. Med., St. Louis, MO 63110.

We obtained numerically independent measures of adaptation and performance during adaptation of a wrist ballistic movement to a visual target ("Jump") as described previously (Soc. Neurosci. Abs. 314.15, 1990). An "inactivation map" of cerebellar cortex was made by systematic injection at 24 sites of 2µl of 1µg/µl muscimol and attempting to induce adaptation. Inactivation of a small area (2x2mm) of cortex in the extreme lateral hemisphere resulted in a marked slowing of adaptation (larger circles) with no significant change in performance of the task. Circles are overlaid on a somatosensory receptive field map of units in Purkinje cell layer. (NIH grant NS12777)







551.4

THE KINEMATICS OF THE DECOMPOSITION OF TWO-JOINT ARM MOVEMENT INITIATION IN NORMALS AND PATIENTS WITH CEREBELLAR ATAXIA. S.G. Massaquoi* and M. Hallett. Human Motor Control Section, Medical Neurology Branch, NINDS, NIH, Bethesda, MD 20892.

Seven patients with cerebellar degeneration and seven age and sex-matched controls, all right handed, were asked to make 80 (2 blocks of 40 trials) left to right cross-body arm movements in the horizontal plane such that the hand traced a precisely linear path on a datatablet. As the thorax and wrist were immobilized, the linearity of the movements directly reflected the precision of shoulder-elbow coordination. Linearity was assessed quantitatively during the first 150 milliseconds of movement.

At lower peak velocities (<20 inches/second) there was a very gentle curvature, convex relative to the body, in both normals and patients. At higher peak velocities, both patients' and normals' trajectories demonstrated a component of increased convexity. Patterns ranged from sigmoids (first concave then convex) to convex arcs. Normals typically produced mild, more balanced sigmoids while patients produced heavily convex sigmoids or bowing arcs. Generally, for any level of peak velocity (> 20 i/s), the magnitude of nonlinearity was significantly greater in patients than in normals independent of pattern.

We conclude that during the initiation of attempted linear cross-body arm movements, normals and patients produce systematically nonlinear trajectories at increased hand speeds which differ primarily in the magnitudes of their convex components. This implies a general, velocity-related failure of interjoint coordination in human arm movement which is significantly more severe in patients with cerebellar ataxia.

551.5

LEARNING OF ARM TRAJECTORY FORMATION IN PATIENTS WITH CEREBELLAR DEFICITS.
H. Topka, S.G. Massaquoi*, T. Zeffiro, and M. Hallett. Human Motor Control Section, Medical Neurology Branch, NINDS, NIH, Bethesda, MD 20892

Recent studies suggest that the cerebellum plays a role in adaptation learning of reflexes such as the vestibulo-ocular reflex. To investigate its role in skill learning, we studied 18 patients with cerebellar cortical atrophy and olivo-ponto-cerebellar atrophy and 15 healthy controls.

Subjects were instructed to perform a multi-joint arm movement on a data tablet generating a trajectory connecting 5 viapoints in a given sequence. Movements were to be performed at a constant total movement time and spatial error was determined. After completion of the first 100 trials at a target movement time of 3500 ms, subjects were instructed to continue for another 100 trials at their maximum speed.

At slow speed both patients and controls reduced the error over trials. Rate of improvement was similar. When starting the fast movements, both groups initially showed increased error. Normal subjects then improved rapidly, while patients showed only gradual improvement. When fast movements were requested first, patients improved less rapidly than normals. Errors in patients correlated with mean square jerk of the trajectory.

Patients with cerebellar deficits can exhibit normal rate of skill learning at slow speeds, but are slower than normals for high movement speeds. The problem appears to be a difficulty in improving the details of the execution of their performance.

551.6

FORELIMB REACHING IN CATS WITH LESIONS OF THE DEEP CEREBELLAR NUCLEI: ACCURACY AND INTERJOINT COORDINATION S. E. Cooper & C. Ghez Ctr for Neurobiol. & Behav., Columbia Univ. & NYS Psychiatric Inst., New York, NY, 10032.

Gordon Holmes noted that, while cerebellar hemisphere lesions impaired performance of single-joint movements, multi-joint movements were more inaccurate than the sum of single-joint errors would suggest. Recently, some authors have proposed that the lateral/intermediate cerebellum is concerned with multi-joint movements *per se*.

We have studied a multi-joint forelimb reaching movement in cats (modified from Gorska & Sibirska 1980). We find that reaching to targets of different heights is accomplished by scaling of a common elbow flexion trajectory, but that movements at other joints of the limb (eg. wrist & shoulder), closely coordinated with the elbow flexion, are essential for successful task performance. Interruption of the output of lateral and intermediate cerebellum by lesions of the lateral and interpositus nuclei produced severe inaccuracy in reaching, including both increased variability and systematic directional biases. Much of the inaccuracy was due to failure to produce wrist and shoulder movements that were coordinated with the basic elbow flexion.

Normal cats can alter the pattern of coordination of wrist and shoulder with elbow in order to generate a reaching trajectory which avoids an obstacle. Following deep nuclear lesions, cats could not alter the pattern of coordination to avoid an obstacle: on every trial the paw collided with the obstacle, and only then made a corrective response.

These results suggest that the cerebellum contributes to the coordination between movements at different joints which is essential to accurate multi-joint movement. (NS19205 & GM32099)

551.7

NETWORK PROPERTIES OF THE OLIVO-CEREBELLAR CIRCUITRY DURING SPONTANEOUS ALTERATIONS IN LIMB COORDINATION. Sheryl S. Smith. Dept. of Anat., Inst. for Neurosci., Hahnemann Univ., Phila., PA

The goal of this study was to examine properties of a well- defined neural network across changes in its functional state, as defined by specified behavioral correlates of network activity. The rubro-olivo-cerebellar circuit at the level of the paravermal cerebellum plays a role in coordination of the distal limbs. The present study was conducted to determine the behavior of individual neurons in the circuit, recorded chronically as ensembles of 10- 20, during tests of limb coordination across spontaneous changes in functional state, here defined as changes in the speed and accuracy of limb trajectory. Hormonal (estrous) fluctuations are associated with marked alterations in limb coordination. Limb response to changes in treadmill speed is most accurate on the night of behavioral estrus following endogenous increases in estradiol and progesterone, relative to diestrus (lower hormone levels). For this study, female rats were chronically implanted with arrays of microwires (50 µ dia, Calif. Finewire) to record from Purkinje (Pnj) cells of the paravermal cerebellum and inferior olivary neurons, simultaneously. Neuronal activity was monitored during treadmill paradigms employing i.) constant speed (4 or 11 cm/s) or ii.) variable acceleration. Typically, Pnj cell discharge is correlated with limb movement, while olivary discharge is correlated with errors in placement or transitions in terrain. Increases (42-74%) in cerebellar discharge and decreases (15-24%) in olivary discharge correlated with treadmill locomotion were observed on estrus relative to values obtained on diestrus during both paradigms. In addition, olivary activity tested on estrus during variable acceleration was three- fold higher than corresponding discharge correlated with constant speed locomotion. Cross-correlation analysis at this time revealed increases in the amplitude of Pnj cell responses to olivary triggers. Sensorimotor gating at the level of the DAO (Gellman et al, 1985) may underlie error signal capabilities of this structure. Increased amplitude (by 50%) of olivary responses to forepaw stimulation during non-movement was observed on estrus . In contrast, estrus was associated with a greater decrease (by 30%) in olivary response during movement relative to diestrus values. These results suggest that alterations in the emergent properties of the olivo- cerebellar circuit accompany changes in its functional state associated with the estrous cycle. *(Supported by NS 25809.)*

551.8

COMPLEX SPIKE DISCHARGES IN RELATION TO INITIATION OF VOLUNTARY MOVEMENT IN MONKEY. J.-P. Pellerin, M.-T. Parent* and Y. Lamarre. Centre de Recherche en Sciences Neurologiques, Département de Physiologie, Université de Montréal, Québec, Canada, H3C 3J7.

Climbing fiber responses (CFR) of cerebellar Purkinje cells (Pc) were recorded in the anterior and posterior lobes of the cerebellum in a monkey trained to perform elbow flexion or extension in response to randomly presented visual, auditory and somesthetic cues. In the anterior lobe, Pc showed CFR in relation to the somesthetic cue only. In the lateral part of the posterior lobe, Pc showed CFR only following the visual cue at a latency of 80-200 ms. The probability of firing increased for shorter reaction time (RT) and decreases for longer RT. In the vermal part of the posterior lobe, Pc were activated in three ways: 1) PC showed CFR following the auditory cue at a latency of 50-120 ms. As in the lateral hemisphere, the firing probability is dependent on the RT, 2) some Pc showed CFR 50-120 ms before the onset of the movement in response to visual and auditory cues only, and 3) others were activated in the same way for all three modalities. Moreover, CFR were not associated with oculomotor events, and they were reduced or abolished when the monkey did not respond to the cues or perform a self-paced movement. These results suggest that the olivocerebellar system may be involved in the rapid initiation of movement triggered by an externally presented cue, and this may be accomplished by modulating the Pc responsiveness. (Supported by MRC Group Grant).

551.9

CEREBELLAR CONTROL OF PROPRIOCEPTIVE GAIN: A NEW PARADIGM M. Gorassini and A. Prochazka, Division of Neuroscience, Univ. of Alberta, Edmonton, Alberta, T6G 2S2, CANADA.

It is thought that one of the roles of the cerebellum is to control the gain of sensorimotor transmission in extracerebellar reflex pathways, in particular, the proprioceptive system. Sherrington has called the cerebellum the "head ganglion" of the proprioceptive system. We wish to test the hypothesis that the cerebellum controls the stretch sensitivity of muscle spindle receptors by influencing fusimotor output. Perhaps abnormalities in the control of proprioceptive gain contribute to the ataxia and tremor seen in cerebellar disease. To test this, we have developed a preparation that will allow us to record muscle afferent discharge during reversible inactivation of the cerebellar cortex in the freely moving cat. An injection port chronically placed over the paravermal cortex acts as a guide for the injection of 100 µl of 2% lidocaine onto the cortical surface. Recording microelectrodes are inserted into the L7 dorsal root ganglion and single unit activity is transmitted by telemetry. To date, we have implanted two cats and have observed in both, marked ataxia and large postural deficits in limbs ipsilateral to the injection site. These deficits are reversible and temporary, lasting for only 3 to 5 minutes on average. If fusimotor abnormalities contribute to this ataxia, we will be able to monitor this in the afferent recordings. In support of this, we have observed a marked increase in spindle discharge upon injection of lidocaine and cooling of the cerebellar cortex in two acute experiments. (Supported by MRC of CANADA.)

CEREBELLUM III

552.1

SITES OF MOTOR LEARNING IN THE VESIBULO-OCULAR REFLEX (VOR) PREDICTED BY A DYNAMICAL NETWORK MODEL. T.J. Sejnowski and S.G. Lisberger, The Salk Institute, La Jolla, CA 92037, and Department of Physiology, University of California, San Francisco, 94143.

A dynamical neural network model of the VOR and smooth pursuit eye movements was used to explore possible sites of plasticity of the VOR. The model had processing units that were nonlinear leaky integrators interconnected in a pattern based on the known anatomy of the VOR pathways. There were two vestibulo-motor pathways: a direct one through the brainstem and an indirect one through the cerebellar flocculus. The flocculus also received negative visual feedback related to image velocity and positive feedback related to output eye velocity. We used a gradient descent optimization procedure i) to adjust the parameters of the model so it achieved good tracking during VOR and smooth pursuit trials and ii) to determine how the network would reduce the amplitude of the VOR while maintaining excellent pursuit.

Reductions in the amplitude of the VOR in the model were achieved by decreasing the connection weights in the vestibular input to the brainstem. The connection weights in the vestibular input to the flocculus increased during the early stages of learning, as suggested by M. Ito, but decreased during the later stages, as demonstrated by F. Miles et al. Because gradient descent optimization allowed learning at all sites in the model, we conclude that the pattern of weight changes resulted from other factors, such as the anatomical structure of the network and the requirement for accurate dynamic tracking during visual and vestibular stimulation. Similar factors may determine the sites of local learning mechanisms in the VOR pathways in the brain. (Supported by DARPA through the Office of Naval Research and the Howard Hughes Medical Institute).

552.2

A NETWORK MODEL OF THE CEREBELLUM THAT USES A TRAINED SET OF PATTERN GENERATORS TO CONTROL A SINGLE DEGREE-OF-FREEDOM JOINT. N.E. Berthier, A.G. Barto and J.C. Houk, Dept. of Computer and Information Science, Univ. of Mass., Amherst, MA 01003.

Recently we proposed a model of motor control that considers the cerebellum an array of modules that function as adjustable pattern generators (APGs, Houk et al., *Neural Networks for Control*, Miller, Sutton & Werbos, 1990). The model requires that the correct subset of APGs be used for a given movement. The selection and use of APGs is determined by the postsynaptic effects of proprioceptive and teleceptive parallel fibers that convey information about the internal and external state of the organism. The response of an APG to its inputs is adaptively altered by the action of climbing fibers which are seen as providing private training signals evaluating the APG's contribution to movements.

The current research is an extension of previous work where APGs were trained to control movements of a planer, kinematic arm. In that work a physiologically inspired learning rule was used to adapt parallel fibers conveying target information to a Purkinje cell. Because only one target line was active at a time this was equivalent to adapting a single parallel fiber per trial. In the present experiments, we sought to extend the learning rule to more realistic situations where all the parallel fiber inputs to an APG are adapted and where APGs must act cooperatively to control a single degree-of-freedom joint with nonlinear muscle dynamics. Simulations were performed in which parallel fiber inputs provided redundant, irrelevant, or distracting information. In these simulations, a subset of the APGs could be trained to move the joint to one target, and a different subset trained to move the joint to another target of greater joint angle. (Supported by ONR N00014-88-K-0339 and the McDonnell-Pew Foundation for Cognitive Neuroscience.)

552.3

COMPUTATIONAL MODELS OF THREE REGIONS OF THE CEREBELLUM. M. Kawato and H. Gomi*. ATR Auditory and Visual Perception Research Laboratories, Sanpeidani, Inuidani, Seika-cho, Soraku-gun, Kyoto 619-02 Japan.

In supervised motor learning, one of the most essential and difficult problems is how to convert the error signal measured in the sensory space (trajectory error in the task-oriented coordinates) into the error signal for the motor command in the coordinates of muscles. We proposed the feedback-error-learning approach where the feedback motor command is used as an error signal to train a neural network which then generates a feedforward motor command.

The cerebellum is divided into three functionally distinct parts: the vestibulocerebellum, the spinocerebellum, and the cerebrocerebellum with distinctive anatomical connections although the cellular organization of the cerebellar cortex is simple, regular and uniform. The feedback-error-learning neural network was originally proposed as a model for the cerebrocerebellum. The spinocerebellum receives sensory information from the periphery. We proposed a closed loop control system based on the feedback-error-learning as a model of the spinocerebellum. Flocculus is known to play an essential role in adaptive modification of the vestibulo-ocular reflex (VOR) and the optokinetic response (OKR). Although the neural circuit itself does not contain any feedback controller, the visual system measures the combined eye and head velocity (retinal slip) and plays a role of differential-type feedback controller converting trajectory error into the motor command error. Our combined model of the flocculus and the ventral part of the flocculus (thought to be a part of the flocculus until recently) explains modification of the VOR, OKR and smooth pursuit. This model resolves the long lasting controversy about adaptive roles of the monkey flocculus in the eye movements. Thus, learning functions of all the three parts of the cerebellum can be coherently understood by the feedback-error-learning scheme.

552.4

PRESYNAPTIC CIRCUITRY OF THE DORSAL SPINOCEREBELLAR TRACT IS A PARALLEL DISTRIBUTED NETWORK. C.E.Osborn and R.E.Poppele. Depts of Physiology, Michigan State Univ, East Lansing, MI 48824 and Univ of Minnesota, Minneapolis MN 55455.

We present a new description of the functional organization of the dorsal spinocerebellar tract (DSCT) which accounts for the extensive polysynaptic component of its behavior. A reevaluation of DSCT function was required by several recent findings including: 1) a profound influence of interneurons on DSCT activity evidenced by the polysynaptic activation of 80% - 90% of the DSCT by afferents from a single muscle, and 2) a widespread convergence of affferents from muscle, joint and skin onto interneurons projecting to the DSCT.

We show in a representative population of DSCT neurons in cat that the activity of individual cells is affected by diverse sensory receptors activated by hindlimb movements. Population responses are characterized by a distribution of diverse response types among the units of DSCT. We found no evidence for distinct presynaptic pathways or functional cell types. These data are consistent with a model for the DSCT having a parallel distributed presynaptic circuitry. According to the model, DSCT activity results from a weighted distribution of inputs onto an extensive interneuronal network projecting to DSCT neurons.

Supported by NIH Grant NS 21143.

552.5

A METHOD OF EXTRACELLULAR MICROSTIMULATION WITH COINCIDENT RECORDING. I. D. Hentall. Univ. of Ill. Coll. of Med., Rockford, Illinois, U.S.A. 61107-1897.

To record and stimulate in the extracellular field with one electrolyte-filled micropipette (3M NaCl) requires that sub-millivolt signals be visible <0.5 ms after passing several μA Major problems are capacitative stimulus artifact and transient tip potentials (TTPs). I removed the former by electronic switching between current source and amplifier input, which leaves a tolerable if inconvenient switching artifact. TTPs, which reach 1-20 mV and last >2 ms, were shown to result from depletion of ions entering the microelectrode, and to vary as the square of measured impedance. Pressure ejection of 3M NaCl, and differential recording and stimulation with matched microelectrodes (one in brain, one elsewhere) minimized them. Several discretely recruited spikes were usually detected 0.5 ms after rectangular stimulus pulses (1-3 μA) in the cerebellar cortex of pentobarbital-anesthetized rats. All-or-nothing properties and refractoriness within 2-5ms were evident for the spike of lowest threshold. Longer, complex waveforms were also sometimes found. The technique offers a global search stimulus and improved method of functional brain mapping. (Supported by NINDS grant 26116.)

552.6

TEMPORAL RELATIONSHIPS BETWEEN CEREBRAL CORTICAL AND CEREBELLAR RESPONSES TO TACTILE STIMULATION IN THE RAT. J. Morissette, M. Lee, and J.M. Bower. Computation and Neural Systems Program, California Institute of Technology, Pasadena, CA. 91125

Cerebellar granule cell responses to brief (10 msec) tactile stimuli consist of an initial component with a short 8-10 msec latency, and a second component with a more variable and longer 16-32 msec latency. We have recently shown that the longer latency component of this response is much more resilient to neonatal peripheral lesions than is the short latency component (Morissette et al., Soc. Neurosci. Abst. 16,370.1, 1990). Accordingly, we were interested in determining the pathway responsible for this longer latency cerebellar response.

Several different experimental techniques were used to demonstrate that the long latency granule cell layer response component is dependent on a pathway involving the somatosensory (SI) cortex. Lidocaine injections into SI, local SI ablations, and complete midcollicular sections all substantially interfere with the second cerebellar response without having any effect on the short latency response. Further, when tactile evoked responses were simultaneously recorded in both the cerebellar granule cell layer and layer IV of SI cortex, the latencies of both responses were strongly correlated. Intraperitoneal injections of sodium pentobarbital during these recordings produced a highly correlated increase in latency in both the SI and the second component of the cerebellar responses. These results suggest that the topologically organized SI projection to the cerebellum (Bower et al., Brain Behav. Evol. 18,1-18, 1981) influences cerebellar cortex at late and variable latencies. Dual recordings are currently being used to investigate the influence of SI cortex on the late and variable latency cerebellar climbing fiber responses to tactile stimulation.

Supported by NIH (NS22205) and BRSG grant (RR07003).

552.7

TEMPORAL AND SPATIAL ORGANIZATION OF CLIMBING FIBER TACTILE RESPONSES IN RAT CEREBELLAR CORTEX. M. Lee and J. M. Bower, Division of Biology 216-76, Caltech, Pasadena CA 91125.

The climbing fiber (CF) projection to the cerebellum mediates Purkinje cell complex spike responses to tactile stimulation. The temporal and spatial organization of these responses was studied with reference to the well-characterized map of tactile responses in the granule cell (GC) layer of folium crus 2a in rat cerebellar cortex (Bower & Kassel, *J. Comp. Neurol.* 302:768, 1990).

Temporal organization. CF responses to tactile stimulation are sparse, occuring only about 1/5 of the time for regular (1 Hz) punctate stimulation, as seen in dot-raster displays of spike data. This characteristic appears to be related to the 10 Hz periodicity of CF activity (Sasaki *et al., Eur. J. Neurosci.* 1:572, 1989), since stimulation during "off" phases of the excitability cycle have a much lower probability of producing a spike response. CF responses also occur at latencies later than those of GC multiunit responses to the same stimuli, as seen in PST histograms of spike data: CF response latencies are around 50 ms, as compared to 2-5 ms for GC responses. The CF response often involves a rebound spike at 150 ms. These CF response latencies are, however, on the same order as those of GC-mediated increases in Purkinje cell simple spike activity in response to tactile stimulation (Thompson & Bower, this volume).

Spatial organization. CFs with similar tactile receptive fields are arranged in a "patchy mosaic" that is roughly in register with the underlying map of GC receptive fields. Thus CFs with non-contiguous receptive fields can be located close to each other in the cerebellar cortex. However, CF receptive fields are large, typically several times larger than GC receptive fields, and bilateral, even when receptive fields in the underlying GC layer are unilateral. For example, in the center of crus 2a, GCs have receptive fields on the ipsilateral upper lip, but CFs have receptive fields covering both sides of the upper lip.

Supported by NIH grant NS 22205.

552.8

PROLONGED SIMPLE SPIKE RESPONSES TO ACTIVATION OF THE GRANULE CELL LAYER IN THE RAT CEREBELLUM J.H. Thompson and J.M. Bower. Div. of Biology 216-76, Caltech, Pasadena, CA 91125

For the last several years we have been using our knowledge of the detailed pattern of tactile mossy fiber projections to the granule cell layers of crus IIa to explore the physiological relationships between granule cells and Purkinje cells. Specifically, using *in vivo* extracellular recording techniques, we have monitored Purkinje cell activity in crus IIa of the rat while applying very brief (5 ms) tactile stimuli to perioral regions known to project to underlying or nearby granule cell layer locations. Our results have shown that Purkinje cell responses to these brief peripheral stimuli do consist of a short latency component (5-8 msec) but that there can also be produced a prolonged (100-400 msec) increase in simple spike activity. By discriminating between simple and complex Purkinje cell responses we have been able to show that climbing fibers are also activated by these stimuli at latencies of around 50 msecs (see Lee and Bower, this volume). However, when those trials in which climbing fiber responses do occur (about 1 in 5) are separated from those that have only simple spike responses, the prolonged simple spike response is still present. This result suggests that the mossy fiber / granule cell system is capable of generating prolonged changes in Purkinje cell activity in response to natural tactile stimulation.

We have pursued this result using both *in vitro* intracellular techniques (Jaeger and Bower, this volume) and detailed single Purkinje cell modeling (De Schutter and Bower, this volume).

Supported by NIH grant NS22205.

552.9

A COMPUTER SIMULATION OF PLATEAU POTENTIALS AND SYNAPTIC INTERACTIONS IN PURKINJE CELL SPINY DENDRITES. E. De Schutter and J.M. Bower. Div. of Biology 216-76, Caltech, Pasadena, CA 91125.

EM-reconstruction of granule cell to Purkinje cell synapses has shown a spatial segregation between parallel fiber synapses and synapses from the ascending part of the granule cell axon (G Gundappa-Sulur & JMB, Abstr. Soc. Neurosci. 16: 896, 1990). The position of parallel fiber synapses between the ascending branch synapses and the main Purkinje cell dendrite is compatible with a modulatory role of the parallel fiber segment, with the main excitatory effects coming from ascending fiber inputs from underlying granule cells. Extracellular *in vivo* recordings (J Thompson & JMB., this volume) and intracellular recordings in slices (D Jaeger & JMB., *ibid.*) show that granule cell stimulation generates postsynaptic plateau potentials of 100 to 400 ms in Purkinje cells.

To examine the significance of these plateaus and of the interactions between ascending and parallel fiber synapses we have constructed a detailed compartmental model of a Purkinje cell, based upon anatomical measurements. The model includes sodium, calcium and potassium currents, quisqualate and GABA synaptic conductances and diffusion, buffering and extrusion of calcium. Temporal summation of plateau potentials and the effects of increasing amounts of either random or time-locked parallel fiber and inhibitory stellate cell synaptic activity upon somatic spike firing after an ascending fiber plateau have been examined. The fast increase in internal calcium concentration after application of glutamate on dendrites, and the possible effects of cytoplasmic calcium uptake and release mechanisms have been simulated.

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552.10

ANALYSIS OF PLATEAU POTENTIALS SEEN IN INTRACELLULAR PURKINJE CELL RECORDINGS WITH SYNAPTIC STIMULATION. D. Jaeger and J. M. Bower. Computation and Neural Systems Program, Caltech, Pasadena, CA 91125

For the last several years we have been exploring the effects of granule cells on Purkinje cells within the rat cerebellar cortex using *in vivo* extracellular recording techniques (J. Thompson and J.M. Bower, this volume). Our data has suggested that purely granule cell input, even in the absence of climbing fiber activation, can generate prolonged (100-400 msec) increases in simple spike firing rates. In the present study we have used *in vitro* intracellular recording procedures to further explore these prolonged effects. Specifically, slices were cut in two orientations; parasaggitally to preserve Purkinje cell inputs from the ascending granule cell axon while significantly reducing input from parallel fibers, and horizontally, to preserve both components of the granule cell input.

Our results reveal that synaptic activation by granule cells produces a prolonged membrane depolarization lasting 100-400 msecs in both the soma and dendrites of Purkinje cells. These depolarizations can generate trains of simple spikes and can sum either temporally or spatially. As in the *in vivo* preparation, prolonged responses occur with or without concomitant climbing fiber activation. A careful examination of the interaction between the climbing fiber response and the prolonged depolarizations has provided critical information for modeling the locations and the properties of the conductances underlying these responses in a realistic Purkinje cell model (E. De Schutter and J. M. Bower, this volume). Supported by NIH grant 22205.

552.11

ANALYSIS OF FACE RECEPTIVE FIELDS IN THE CAT CEREBELLUM A.M. Castelfranco*, G. McCollum, L.T. Robertson R.S. Dow Neurol. Sci. Inst. and Dept. Anat., SD, Oregon Health Sci. Univ., Portland, OR 97201.

The encoding of the location and magnitude of climbing fiber responses to tactile stimulation of the face varied for different regions of the cerebellar cortex. An analysis of the face receptive fields was made of 75 climbing fiber responses encountered in the anterior lobe, of 33 responses from the paramedian lobule (PML), and 52 responses from the crus II of the anesthetized cat. The receptive fields in each region had distinctive configurations and were concentrated on different areas of the face.

The encoding of the skin location depends on the activation of an ensemble of climbing fiber responses in various cortical regions. By overlapping the face receptive fields on a planar diagram, the smallest skin areas (i.e. compartments) that the climbing fiber system could resolve were identified. The receptive fields are unions of compartments, although different combinations of compartments occurred in the three regions. In the anterior lobe, contiguous compartments extended from the ear, along the nose, to the chin; in the PML and the crus II, the contiguous compartments involved the lower jaw and chin; and in the crus II, lines of contiguous compartments included compartments from the ear to the vibrissae, from below the eye to the vibrissae, and from above the eye to the nose tip.

The compartments that were most frequently included in the face receptive fields were the chin, nose tip, and cornea, although there were regional differences (face receptive fields in the anterior lobe mainly included the nose and chin, in the PML, mainly the chin, and in the crus II, mainly the cornea). Thus, the magnitude of the climbing fiber response in to stimulation of the cornea, will be larger in the crus II than in the other regions.

552.12

WITHDRAWN

552.13

EXPANSION OF TURTLE RUBRAL SOMATOSENSORY RECEPTIVE FIELDS BY LIDOCAINE APPLICATION TO THE CEREBELLAR CORTEX. *R. Sarrafizadeh, J. Keifer, and J. C. Houk.* Department of Physiology, Northwestern University Medical Center, Chicago, IL 60611-3008.

It has recently been appreciated that rubrospinal neurons receive detailed somatosensory information viâ direct and indirect pathways. A recent theory of motor control suggests that cerebellar Purkinje cell inhibition of interpositus neurons sculpts the spatial and temporal features of activity in the cerebellorubral circuits (J. C. Houk *In*: Models of Brain Function, p. 309, 1989). To investigate the role of the cerebellum in the processing of sensory input to the red nucleus we recorded extracellularly from single rubral neurons in the sodium pentobarbitol anesthetized turtle *(Chrysemys picta)*.

The somatosensory receptive fields of single rubral neurons typically covered one or more limbs and parts of the shell, and were composed of excitatory and inhibitory regions. Units were excited by electrical stimulation of the peripheral fields with response latencies that ranged between 20-36 ms. Placement of a small cotton pellet containing 2% lidocaine over the surface of the contralateral cerebellar cortex while recording from a single red nucleus neuron resulted in increased rubral spontaneous activity after several minutes. Both excitatory and inhibitory receptive fields of individual neurons typically expanded in size. Light touch directed to excitatory and inhibitory regions located within the original receptive field resulted in increased responsiveness of each unit's activity. Rubral spontaneous activity and receptive field size and sensitivity returned towards control measurements several hours after lidocaine was washed from the surface of the cerebellar cortex.

The expansion of rubral somatosensory receptive fields may result a.) from inhibition of cerebellar origin that contributes to the shaping of rubral sensory receptive fields, or b.) from hyperexcitability in brainstem circuits released from cerebellar inhibition. These findings are in contrast to previous studies showing a reduction in rubral receptive field size following cerebellectomy perhaps due to reduced excitability resulting from a lesion of the interpositus nucleus.

552.14

PURKINJE CELL RESPONSE TO CLIMBING FIBER ACTIVATION AND INACTIVATION IN NORMAL AND GENETICALLY DYSTONIC (*dt*) RATS. M.H. Thorstad, S.E. Stratton and J.F. Lorden. Dept. Psychology, Univ. of Alabama at Birmingham, Birmingham, AL 35294.

Activation of the inferior olive by harmaline produces a generalized tremor in normal rats. In the *dt* rat, harmaline does not elicit a tremor, but does induce rhythmic activity in olivary neurons. In normal rats, the increased frequency and rhythmicity of olivary activity is conveyed to the cerebellar cortex where high frequency, rhythmic complex spikes and complete suppression of simple spikes are recorded in vermal Purkinje cells. This response is rarely seen in *dt* rats. Recording of vermal Purkinje cells and microstimulation (20-50 µA, 2-8 Hz) of the contralateral caudal medial accessory olive (cMAO) were combined to determine if Purkinje cells in *dt* rats can follow climbing fiber stimulation and if it modulates simple spike activity. In both normal and *dt* rats, complex spikes followed stimulation reliably at all frequencies and significant decreases in simple spike rate were observed. Complete simple spike suppression, typical of the harmaline response, was seen in 17 of 18 cells in normals but only in 4 of 7 cells in *dt* rats. In normal rats, inactivation of the inferior olive produces large increases in simple spike rate. Preliminary results of lidocaine injections (10%, 0.1-0.2 µL) in the cMAO of *dt* rats indicate that although cMAO inactivation silences complex spikes in *dt* rats, the increase in simple spike rate is small in comparison with that seen in controls. These results indicate mechanisms underlying modulation of simple spikes may be abnormal in *dt* rats but that the failure of harmaline to produce sustained increases in complex spike rate cannot be attributed to a failure of Purkinje cells to respond to olivary input. (Supported by a Dystonia Med. Res. Fdn. grant and grant BNS 90-10187 from NSF.)

CONTROL OF POSTURE AND MOVEMENT IV

553.1

RELATIVE PHASE AND THE COUPLING OF THE REACH AND GRASP COMPONENTS IN THE PROSTHETIC HAND. S. A. Wallace, L.E. Carlson* and T. McCullough. Dept. of Kinesiology and Dept. of Mechanical Engineering*, Univ. of Colorado, Boulder, CO 80309.

Amputees wearing body-powered prostheses have many ways to open and close the prehensor (end effector). Since the cable attached to the prehensor crosses several joints and anchors to the contralateral shoulder, a number of degrees of freedom must be constrained to coordinate the movement of prehensor with the joints responsible for reaching toward an object (for example). In the present case study, we used a relative phase analysis (eg. Kelso, Buchanan & Wallace, in press, Exp. Brain Res.) in an attempt to 'capture' this coordination as the speed of the reach was systemmatically increased. The subject, a below-elbow amputee and an experienced user of a voluntary closing prosthesis, was required to continuously reach forward, momentarily grasp a small cylindric object mounted on a table top and return to the start position. The subject was instructed to synchronize the grasp of the object with a tone produced by a computerized metronome program. Cycling frequency began at .6 Hz and was increased by .06 Hz after every 10 cycles, with five such trials yielding a total of 250 complete reaching cycles. The results indicated that relative phasing of final closing remained consistent at approximately 140 deg across all five transport speeds in spite of clear scaling changes in several kinematic parameters of the reach and grasp. In addition, the standard deviation of the mean relative phase was small (approx. 13 deg) and also consistent, providing evidence for only one stable pattern of coordination. When the subject duplicated these cyclical motions without an object, the relative phase increased nearly 20 deg suggesting a different pattern of grasping later in transport. These results imply that the grasping pattern of coordination in the prosthetic hand is more sensitive to the type of functional goal than on the speed of transport.

553.2

SHORT-TERM SYNCHRONIZATION OF MOTOR UNITS IN HUMAN WRIST EXTENSOR MUSCLES : EFFECTS OF SUPERFICIAL CUTANEOUS STIMULATION. A. Schmied*, J.P. Vedel, and S. Pagni*, Lab. Neurosciences Fonctionnelles-CNRS, 13402 Marseille Cédex 9, France.

Pairs of motor units (MU) were recorded in human subjects during isometric voluntary contraction of the Extensor carpi radialis muscles. Relying on audio and visuo-feedback of the action potentials, the subjects kept both MUs discharging steadily within a range of 4 to 12 imp./s. The MUs were characterized by their contraction times and their recruitment thresholds. The temporal relationships between the MUs discharges were analyzed by cross-correlation. Short-term synchronizations likely to involve common inputs were revealed by the presence of narrow central peaks in the cross-correlation histograms.

Among the population of MU pairs (47%) which presented significant short-term synchronization, 70% included two MUs with rather slow contraction times and low recruitment thresholds and 30% included one or two MUs with faster contraction times and higher recruitment thresholds.

The influence of cutaneous inputs on the short-term synchronization of the Extensor carpi radialis MUs was determined by comparing the cross-correlation histograms obtained during voluntary contraction with or without superfical cutaneous stimulation above the recorded muscle.

Reversible significant changes were observed for more than 65% of the tested MU pairs. Reductions of short-term synchronisation were predominant for the pairs with slow contraction times and low recruitment thresholds, while reductions or increases occurred equally for the pairs with fast contraction times and high recruitment thresholds.

No relation was observed between the opposite effects induced by cutaneous stimulations and the frequency of discharge of the MUs.

553.3

HANDEDNESS, PRACTICE ORDER, AND ASSIMILATION EFFECTS IN RAPID BIMANUAL MOVEMENT. D.E. Sherwood, Motor Behavior Lab., Department of Kinesiology, University of Colorado, Boulder, CO 80309.

When subjects make rapid bimanual aiming movements over different distances, assimilation effects are typically shown as overshoots in the shorter-distance limb. The effect of practice order and handededness on assimilation effects were assessed by giving 60 right-handed and 32 left-handed male and female subjects practice on 20° (Short) and 60° (Long) reversal movements first separately, then together. Subjects were randomly assigned to a practice order of either Short-long or Long-short and peformed the Short and Long movements in either the left or right hand. For all subjects, overshooting was shown on the dual trials in the shorter-distance limb, showing assimilation effects. Assimilation effects were greatest when the Long movement was practiced just prior to the dual trials, showing practice order effects. Also right-handed subjects showed greater assimilation effects in the non-dominant limb, relative to the left-handed subjects. The findings suggest that assimilations effects can be influenced by practice variables.

553.4

COORDINATION OF INDEX FINGER MOVEMENTS. W.G. Darling, G.F. Miller, K.J. Cole. Dept. of Exercise Science, University of Iowa, Iowa City, IA 52242.

The EMG and kinematic patterns of index finger motions involving all 3 joints (metacarpophalangeal - MCP, proximal and distal interphalangeal - PIP, DIP) have received little quantitative study. Previously, kinematics and EMGs (of 4 muscles) of two joint (MCP, PIP) index finger motions were investigated (Darling and Cole 1990 - J Neurophys, 63, 1098-1108). In this report we have studied EMG patterns in all index finger muscles during various motions to examine coordination of index finger motion and posture.

EMGs were recorded using hooked wires inserted into 6 or 7 index finger muscles (extensor indicis EMG was not always recorded). Movements were recorded opto-electronically with infrared emitting diodes placed over the base of the 2nd metacarpal and the centers of the 3 finger joints. Subjects performed 6 types of discrete motions involving: flexion or extension of all joints, flexion or extension at the MCP joint while the interphalangeal IP joints were voluntarily held in flexion, and flexion or extension at the PIP and DIP joints while the MCP joint was voluntary held in flexion. Analysis of velocity profiles showed that there is coupling of PIP and DIP motions during flexion or extension of these joints. The EMG data show: (1) maintenance of flexed MP or interphalangeal joint angles while moving the other joints requires coactivation of several muscles, suggesting that joint stiffness is increased to compensate for dynamic interaction torques and (2) synergist muscle activities are coordinated differently according to the task, and exhibit higher correlations when acting as agonists than as antagonists.

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553.5

ALTERATIONS IN THE BEHAVIOR OF HIGH-THRESHOLD MOTOR UNITS IN A HUMAN HAND MUSCLE OF OLDER SUBJECTS.
R. M. Enoka, P. M. Barreto* and A. J. Fuglevand. Depts. Exercise & Sport Sciences and Physiology, Univ. Arizona, Tuscon, AZ 85721.

Senescence can be characterized by a decline in the number of motor units in a muscle and, through reinnervation, an increase in the innervation ratio for some motor units. The purpose was to determine if these changes influence the behavior of high-threshold motor units. Seventeen healthy subjects (64-79 years) volunteered for the study. We measure the abduction force exerted by the left index finger. A fine wire bipolar electrode was inserted subcutaneously over the first dorsal interosseous (FDI) muscle and used to measure the potentials of 1-3 motor units. Subjects were instructed to grade the isometic force to various levels (5-100% MVC) and to sustain the force briefly (3-20 s) or to the endurance limit. The presence of action potentials with large amplitude (4 mV) and polyphasic shapes indicated that there had been some motor unit reorganization with age. These changes were accompanied, in some subjects, by altered features of behavior among high-threshold motor units: (1) a reduced ability to smoothly grade force at moderate-to-high force levels; (2) altered recruitment patterns; (3) an increase in the upper limit of recruitment. Despite these changes in high-threshold motor units, neither the MVC (32±8 vs 29±9 N) nor the endurance time of FDI at a sustained target force of 35% MVC (196±86 vs 227±103 s) was statistically different for old (n=17) and young (n=53) subjects, respectively. Although we found age-related changes in the behavior of high-threshold motor units in FDI, the functional significance of these adaptations is unknown.

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553.6

FIRST REACHES IN HUMAN INFANTS: A KINEMATIC, KINETIC, AND EMG ANALYSIS. E. Thelen, D. Corbetta*, J. Konczak*, K. Kamm*, K. Schneider*, & R.L. Zernicke, Dept. of Psychology, Indiana University, Bloomington, IN 47405.

All normal infants learn to reach, but the processes by which they acquire control over early, undirected arm movements are not known. Here we report on a longitudinal study of 4 normal infants, followed weekly from week 3 as they reached for a toy. We collected 3-D coordinate data from the shoulder, wrist, elbow, and hand of each arm using WATSMART, and used these data to generate kinematic and kinetic descriptions (Schneider, et al 1990). We also collected EMG signals from arm extensors and flexors and two postural muscles.

Infants reached successfully at 12, 15, 22, and 22 weeks. The two earlier reachers produced rapid swipes at the toy embedded in ongoing movements. They damped motion-dependent forces by stiffening through co-contraction. The two later reachers had fewer undirected movements, which generated low motion-dependent torques. They used muscles to counteract gravity.

Reaches were assembled within an ongoing movement context, which differed among the infants. Since all infants were proficient reachers at 6 months, different developmental pathways may lead to similar functional outcomes.

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553.7

THE RELATIONSHIP BETWEEN SPATIAL PATTERNS OF MUSCLE ACTIVATION AND THE INTRINSIC MECHANICS OF THE ELBOW JOINT.
R.F. Beer, J.P.A. Dewald, T.S. Buchanan and W.Z. Rymer, Depts. of Biomedical Eng. and Physiology, Northwestern University, Chicago, IL 60611.

A functional relationship exists between the intrinsic joint biomechanics provided by passive tissues and the muscle activation patterns dictated by the CNS. This relationship can theoretically be quite simple—the CNS may activate muscles to provide the required torque(s) about the joint axis(es) of rotation while allowing passive tissues to equilibrate torque components in other directions. Alternatively, muscle activation patterns may reflect a strategy designed to reduce passive tissue loading. In this study we have investigated the relationship between activation of the primary elbow musculature and the intrinsic mechanics of the elbow joint.

EMGs were recorded from the muscles of the elbow using surface and intramuscular electrodes while subjects isometrically generated force against a 3-DOF load cell (flexion/extension, varus/valgus, and pronation/supination) located distally on the forearm. In the first set of experiments EMGs were recorded for eight levels of flexion/extension torque ranging from 5-40% of MVC. In a second set of experiments, torque magnitude was maintained constant while the direction of loading was varied over a full range of 360° in the flexion/extension-varus/valgus plane in increments of 15°. Pronation/supination torque was zero in all experiments. The arm was fixed at 90° elbow flexion, neutral forearm position and 90° shoulder abduction.

Although a number of elbow muscles have moment arms about the varus-valgus axis, polar plots of muscle EMG activity as a function of load direction were well predicted using the flexion/extension component of each torque and the torque-EMG relationship established in the first set of experiments. For muscles with linear torque-EMG relationships the polar plots were well fitted by a cosine function with a peak orientation in the flexion-extension direction rather than the muscle's direction of maximum mechanical advantage. These results suggest that for static loads of low to intermediate magnitude the activation of motor nuclei serving the elbow was not modulated to assist the passive tissues in equilibrating varus-valgus torques and argue against significant input to these nuclei from passive tissue (e.g. ligament) based sensory receptors under the stated conditions.

This work was supported by NIH grants NS19331 and AR40408.

553.8

TRUNK MUSCLE ACTIVITY DURING VERTICAL PLANE RAPID ARM MOVEMENTS WHILE SITTING. A. E. Tyler and Z. Hasan. Dept. of Physiology, Univ. of Arizona, Tucson, AZ 85724.

Arm movements are known to be associated with postural adjustments in standing subjects. We have examined in sitting subjects the EMG activity of trunk muscles (paraspinals and abdominals on the right side) to determine the relationship of initial trunk muscle activity to arm movement parameters. Subjects performed a variety of rapid movements of the right arm in a parasagittal plane.

Preliminary data showed distinct and consistent EMG bursts in trunk muscles for all arm movements, with trunk and shoulder muscle onsets approximately synchronous. When the arm was moved downward (from hand above the shoulder to hand in front of the shoulder), the initial trunk muscle activity was consistently abdominal. There was increased paraspinal activity toward the end of the movement to accommodate the forward displacement of the body's center of gravity (CG). For upward arm movements (from hand at side to hand in front of the shoulder), the trunk muscle initially activated depended on whether the target position was within arm's reach or required that the trunk move forward, even though in both cases the CG moved forward. We conclude that the muscle activity pattern chosen by the nervous system to initiate a given arm movement is not determined by the requirements for equilibrium against gravity at the final position.

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553.9

RAT SKILLED REACHING: MOVEMENT COMPONENTS AND NEOCORTICAL ORGANIZATION Ian Q. Whishaw, Boguslaw P. Gorny* and Sergio M. Pellis, Department of Psychology, University of Lethbridge, Lethbridge, Alberta, Canada, T1K 3M4

Rats are skilled at reaching for and grasping food objects using one limb. The movement can be divided into a subcomponents. Food is localized using olfaction. Using movements of the upper arm, a limb is the lifted and aimed at the food, transported to the food, and the paw is pronated over the food. The digits are opened during the transport phase and are closed after palm contact initiates the retraction phase of the reach. During retraction, the paw is supinated at the wrist in two 90^{o} movements, one occurs during retraction and one after retraction, to present the food to the mouth. Some of these movements can be disrupted by neocortical motor cortex area damage but whole body movements can be used to substitute for the missing reaching movements. On the basis of the behavioral results, cortical anatomy, and cortical electrophysiology, it is suggested that the different components of reaching have partially independent cortical representations. It is also proposed that the similarity of some of the components or rat reaching to human reaching argue for parallel development or homology.

553.10

THE EFFECTS OF PREDICTABILITY ON THE INITIATION OF REACHING TO A MOVING TARGET. P. van Donkelaar *, R.S. Gellman, and R.G. Lee. Dept. of Clinical Neurosciences, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

Normal human subjects were required to either track a moving target with their extended index finger or reach out and intercept a moving target. In each case, target velocity was either predictable or unpredictable from trial to trial. The position of the index finger was monitored using a WATSMART system. In the tracking task, when target velocity was unpredictable, the movement velocity traces superimposed during the first 50-60 msec. This superimposition was not present when target velocity was predictable. In the interception task, the initial trajectories overlapped for a similar duration when target velocity was unpredictable, but diverged much sooner in the predictable condition.

In both tasks, the initial latency increased as target velocity decreased, suggesting a threshold mechanism for the detection of target motion. Regression analysis (of latency *vs* 1/velocity) revealed that the minimal processing time, as determined by the y-intercept, was greater in the interception task than in the tracking task. Thus, in the interception task planning the appropriate movement took longer than in the tracking task. These results coincide with what has been found for smooth pursuit eye movements, suggesting that the nervous system uses similar strategies to control the initiation of both eye and the limb movements.

553.11

MOTOR UNIT ACTIVITY DURING FATIGUING HUMAN ARM MOVEMENTS. S.J. Garland, T. Ohtsuki*, J.D. Cooke. Departments of Physical Therapy and Physiology, University of Western Ontario, London, Canada, N6G 1H1

During sustained, submaximal, isometric contractions, motor units (MUs) in human biceps brachii muscle exhibited a diversity of discharge patterns (Garland et al, 1989). The present experiments were performed to determine if MUs have similar patterns during fatiguing elbow movements.

Subjects performed 100 flexion-extension elbow movements (40°, 0.6 s), utilizing a symmetrical phase-plane tracking paradigm. A constant torque of 2 Nm. opposing extension was applied to the handle moved by the subject. MU activity was recorded from the lateral head of triceps using a branched bipolar fine wire electrode inserted subcutaneously overlying the muscle fascia.

During extension, MUs were phasically active at movement onset and tonically active at the end of the movements. The activity of MUs, which were strongly active in early trials, remained unchanged or decreased with fatigue. Other MUs, which were rarely recruited in early trials, became more active with fatigue. A given MU could be phasically active at movement onset even if its tonic activity was reduced during fatigue. Since the movement performance remained constant despite fatigue, our data suggest that the performance was preserved by the recruitment of additional MUs. These data concur with MU data from fatiguing, sustained, isometric contractions.

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553.12

DEPENDENCE OF MOTOR UNIT FIRING ON ACCELERATION - DECELERATION CHARACTERISTICS OF HUMAN ARM MOVEMENTS. J.D.Cooke, T.Ohtsuki*, J.Garland. Depts. of Physiology and Physical Therapy, University of Western Ontario, London, Ontario, Canada N6A 5C1

Recent studies have shown that surface electromyographic (EMG) activity is related to the acceleration - deceleration characteristics (ADCs) of the desired movement. We have recorded from single motor units (SMUs) in humans performing movements with specified ADCs.

SMU activity was recorded from the lateral head of the triceps muscle using branched, bipolar fine wire electrodes inserted subcutaneously over the muscle fascia. Utilizing phase plane tracking, subjects made flexion-extension movements about the elbow in which the durations and magnitudes of acceleration and deceleration were varied systematically.

Depending on movement ADCs, a given triceps MU could be phasically active during either or both acceleration and deceleration in extension movements and during deceleration in flexion movements. In extension movements a given MU's activity could be switched from movement initiation to movement termination by altering acceleration and deceleration magnitudes. If, however, the mean acceleration magnitude was constant, any active MU continued to be active for all acceleration durations tested. There was no evidence that changes in the duration of surface EMG bursts were produced by recruitment of new MUs late in the burst period. Rather the duration of MU firing varied with the acceleration and deceleration durations.

A given motor unit can thus be active both when its muscle is acting as an agonist and as an antagonist. When and whether the unit is active depends on the desired acceleration and deceleration characteristics of the movement.

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553.13

LOSS OF PROPRIOCEPTION PRODUCES DEFICITS IN INTERJOINT COORDINATION. R.L. Sainburg*, H. Poizner, Center for Molecular and Behavioral Neuroscience - Rutgers University, Newark, NJ, 07120. C. Ghez, Center for Neurobiology and Behavior, New York Psychiatric Institute, New York, NY 10032.

Specific large fiber sensory neuropathies (LFSN) produce severe disorders of posture and movement. Recent studies (Neurosci. Abst. 14: 953) have revealed large direction-dependent *spatial errors* in the feedforward control of multijoint movement. Proprioceptive information about the limb was shown to be necessary to compensate for directional variances in inertia that arise because of its multiple degrees of freedom (Neurosci. Abst. 16:1089). In the present study we asked whether proprioceptive input is also necessary for the *temporal coordination* of joint motions since motion at individual limb segments may produce torques at other segments. We studied the trajectories of repetitive "slicing" gestures made by a 43 y/o woman with LFSN and by 9 normal controls. Hand, wrist, elbow and shoulder points were digitized using a WATSMART system. The patient was unable to maintain the normal linearity and planarity of individual or successive movement cycles and the velocities at the different joints were temporally decoupled. A large anomalous velocity disruption at the wrist occurred at the end of shoulder flexion before shoulder extension when the inertial resistance to ongoing motion is maximal. This error resulted from a lack of synchronization of shoulder and elbow joint motions. It reflected an interaction torque at the elbow which was not adequately compensated by active muscle contraction. The anomalous forearm deflection was velocity dependent, increased with added mass and decreased when the subject could visualize her arm during movement. We conclude that in multijoint movements, proprioceptive input is critical to assure the temporal coordination of muscle commands needed to counteract or to efficiently utilize movement-dependent torques.

553.14

ORGANISATION OF CUTANEOUS FACILITATION OF HAND MUSCLE RESPONSES TO TRANSCRANIAL MAGNETIC STIMULATION. P.H. Ellaway, N.J. Davey*, D.W. Maskill*, and P. Romaiguere*. Dept. of Physiology, Charing Cross and Westminster Med. Sch., London W6 8RF, U.K.

Changes in motoneurone excitability and reflex responses to cutaneous stimulation depend critically on the location of skin stimulation. We have examined the effect of cutaneous stimulation on the response of adductor pollicis (AP), first dorsal interosseus (FDI) and abductor digiti minimi (ADM) muscles to transcranial magnetic stimulation (TMS) of the brain. EMG activity was recorded using surface electrodes. The motor cortex was stimulated using a Novametrix Magstim 200 with a 9 cm coil, centred at the vertex, at a strength just suprathreshold for a response in the relaxed muscle, in the absence of cutaneous stimulation. The EMG responses were rectified and the average voltage calculated. Mechanical stimulation of the skin, using a stiff brush, elicited facilitation of TMS responses in 9 of 11 normal subjects (aged 22-64 yrs). Brushing alone elicited no EMG activity. The degree of facilitation was dependent on the site of cutaneous stimulation. Facilitation of muscles was particularly strong (up to 450% in AP) from skin overlying the metacarpo-phalangeal and carpo-metacarpal joints. Brushing the palmar and dorsal surfaces of the little finger strongly facilitated the ADM response to TMS.

Electrical stimulation of digital nerves up to 5 times perceptual threshold, as reported by Day et al., (J. Physiol. 399:68P, 1988), could inhibit responses to TMS. In the same subjects brushing the skin produced facilitation of TMS responses but not inhibition.

We conclude that stimulation of the skin overlying joints associated with the action of a certain muscle is particularly efficacious in facilitating the response of that muscle to TMS.

553.15

CHARACTERISTICS OF HAND PATH CURVATURE FOR PLANAR, MULTIJOINT ARM MOVEMENTS. C.M. Karst, Department of Therapeutic Science, University of Wisconsin-Madison, Madison, WI 53706.

There are conflicting reports in the literature regarding the kinematic characteristics of the hand paths associated with multijoint reaching movements. The aims of this study were to: (1) better characterize the kinematic details of the hand path for a wide variety of planar reaching movements; and (2) attempt to relate those characteristics to the muscle activation patterns responsible for the movements.

Normal, adult subjects performed self-paced reaching movements in 12 different directions and from different initial limb configurations. Kinematic data were obtained using a WATSMART motion analysis system, and EMG activity was recorded from shoulder and elbow muscles involved in the movements. Hand paths were characterized by the direction and extent of the major curvature and by the number and location of inflections. These kinematic characteristics were found to vary in a consistent manner relative to the initial limb configuration and the spatial direction of movement. These direction-dependent variations in hand path characteristics were then studied in relation to initial muscle activation patterns. Changes in the direction of curvature appeared to be associated with qualitative changes in the EMG pattern (e.g. the switch from initial flexor activity to initial extensor activity at one joint), while changes in the extent of curvature and the location of inflection points were associated with variations in the relative timing and magnitude of the initial EMG activity at the shoulder and elbow.

553.16

ARM AND TRUNK SYNERGIES IN POINTING MOVEMENTS IN NORMAL SUBJECTS AND PATIENTS WITH CEREBELLAR DEFICITS. A.M. Iannone, M. Hallett, S. Stanhope* R. Vail*. Human Motor Control Section, NINDS, Bethesda and MCO, Toledo.

The coordination of unconstrained multijoint movement in three dimensional space is a difficult task and one that gives patients with cerebellar deficits particular difficulty. We studied the kinematics of one such movement and compared the performance of patients with that of normals. Subjects sat on a chair and rested their right arms on a table in front of them. Pointing movements were made to targets at shoulder height and distances that were 80%, 100% and 120% of arm length. Movements were made at freely chosen "fast" and "slow" speeds. Anterior-posterior movements of the trunk and angles of the shoulder and elbow were measured using the Vicon System.

The movements of the different body parts was consistent in each individual for each task, but clearly changed with movements of different distance. For example, the trunk movement was backwards for the short distance, minimal for the middle distance and forward for the long distance. Movements of the trunk, shoulder and elbow were simultaneous and were coordinated to produce a nearly linear trajectory of the finger to the target. While the patients usually showed normal simultaneous movements of the different body segments, the coordination was impaired resulting in misdirected, multisegmented trajectories. These trajectories showed variable errors in the ratio of forward and vertical movement needed for a smooth trajectory. In particular the movement of the trunk in the long distance movement was abnormal because it moved twice the distance of normal subjects. The reason for this is not clear.

The coordination of these multijoint movements is characterized by similar time course of the individual components. Patients with cerebellar deficits are abnormal not because of timing errors, but by virtue of inaccurate component movements.

553.17

EFFECTS OF STATIC TORQUE LOAD ON POSTURAL ACTIVITY ASSOCIATED WITH RAPID WRIST MOVEMENT AND ARM HOLDING IN MAN. F. Aoki Department of Rehabilitation Research, Tokyo Metropolitan Institute of Gerontology, Itabashi-ku, Tokyo 173.

Effects of static torque loading on phasic postural activity associated with rapid wrist movement were examined and compared with effects on tonic postural activity during arm holding. Subjects, holding their arm in a horizontal plane with the forearm supinate, made a rapid wrist flexion in response to a tone signal. Motor tasks were executed in 6 conditions, involving combinations of 3 loads to the wrist (flexor load, extensor load or no load) and two modes of support to the forearm (with or without support). Phasic activities of the forearm and upper arm muscles related to the rapid wrist flexion and tonic activities related to the arm holding were compared with respect to the load and support effects. The tonic activities of both the forearm and upper arm muscles during the arm holding were influenced by the load condition, and activities of the upper arm muscles were affected by the support condition. In contrast to the tonic activities, the phasic activities of the forearm and upper arm muscles related to the rapid wrist flexion were not affected by the load or support condition. These results suggest that tonic postural activities are influenced by static load condition, including the state of support, but phasic postural activities associated with rapid movement are controlled independently of static torque condition.

553.18

OPTIMAL ARM MOVEMENTS. M.Dornay, M.Kawato, Y.Uno* and R.Suzuki*. Cognitive Processes Dept. ATR, Kyoto 619-02 Japan and Dept. of Mathematical Engineering, Tokyo University, Tokyo, 113 Japan.

In order to control a voluntary hand movement, the CNS must determine: (**A**) which trajectory (hand path and velocity) should be used while moving the hand. (**B**) What motor commands should the muscles receive to specify each intermediate state of the hand while it is moving. Two main antagonistic approaches have been proposed to explain the control strategies used by the CNS: The *minimum-jerk model (MJM)* proposes a solution only to the trajectory determination problem (problem **A**). An antagonistic approach, the *minimum-torque-change model*, takes into account the structure and dynamics of the arm and predicts simultaneously both the hand trajectory and the joint torques. Both models were criticized for their inability to reproduce some experimental arm movement (Uno et al. 1989; Flash 1990). However, Uno et al. (1989) found that the *minimum-muscle-tension-change model* reproduced human trajectory data quite well based on a simple 2-link 6-muscle model.

The following study is based on our belief that the structure and dynamics of the arm must play a role in the determination of hand trajectory. A detailed dynamic model of the rhesus monkey's arm is used to investigate the possibility that minimizing the rate of change of: A) motor commands to the muscles, B) torque created by each muscle, C) tension created by each muscle, D) potential energy stored by each muscle, during a planar movement in front of the body, can produce by itself realistic trajectories. As a preliminary stage, the *MJM* is utilized for trajectory determinations (problem **A**), and a dynamic local minimization approach is used for motor command determination (problem **B**). Initial trajectories with 100 intermediate cartesian points simulated entirely by the *MJM* are compared to trajectories in which 50 intermediate points are simulated by the *MJM* and 50 intermediate points are predicted by a dynamic optimization approach. Since the *MJM* has good prediction abilities for the tested movements, the results give a hint which of the above dynamic strategies is a good candidate for being utilized by the CNS.

553.19

THE EFFECTS OF TARGET VELOCITY ON HUMAN PREHENSION. H. Carnahan. Dept. of Physical Therapy, Elborn College, University of Western Ontario, London, Ontario, Canada, N6G 1H1.

The purpose of this study was to examine how the kinematic characteristics of the reach and grasp are influenced when individuals reach towards moving targets of various velocities. Subjects were required to reach towards, grasp, and pick up a small object which rolled down a ramp that was aligned directly along the subject's midline. Movements of the wrist, index finger and thumb were monitored with the WATSMART system. Results showed that movement time and time to peak velocity decreased as target velocity increased. As well, peak velocity of the transporting limb increased as target velocity increased, however, at the slowest target speed, peak velocity was actually lower than the peak velocity in the stationary control condition. These results demonstrate that the movement kinematics of the target strongly influence the movement kinematics of the subject's limb. Target movement however, had no influence on the size of peak aperture suggesting that control of aperture may be a "higher order" control variable. (Supported by NSERC).

553.20

OVERSHOOT IN THREE-DIMENSIONAL POINTING MOVEMENTS. O. Fookson, M. Berkinblit*, S. Adomovich*, and H. Poizner, Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ 07102, Academy of Sciences, Moscow.

Pointing movements were executed by right-handed normal subjects with their eyes closed using their right index finger or a pointer 30 cm in length. Most of the movements were executed from an initial position in which the upper arm was vertical and the elbow strongly flexed, so that the index finger was close to the shoulder joint. A programmable robot arm was used to position 10 targets in 3D space. The movements of the subjects and the robot arm were digitized in 3D with a Watsmart system, and analyzed numerically and graphically. The subject had to begin the movement immediately after closing his eyes and was instructed to touch the target on the tip of the robot's arm during a restricted period of time. Since there was no instruction for the subject to come to the target with a zero velocity, overshooting of targets often occurred. The radial "error" was larger when the distance from the shoulder to the target was longer. However, the distance in 3D space between the target and the final position of the tip of the finger or pointer (3D "error") was found to depend upon the target's location. The 3D "error" could be larger when the target was closer, and shorter when the target was more distant. Finally, for one of the subjects, a 5 sec. delay was introduced between the time the subject closed his eyes and the sound signal to move was given. This delay caused no essential decrease in the movement's efficiency. The possible mechanisms of the observed overshoot in pointing movements will be discused with respect to dynamic regulation of limb posture.

553.21

ARE THERE DIFFERENCES IN POSTURAL SUPPORT STRATEGIES FOR SIMPLE BALANCE TASKS VS TASKS REQUIRING PRECISE HAND STABILIZATION? H. Sveistrup*, J.Massion*, S. Moore, M.H. Hu* and M. Woollacott. University of Oregon, Eugene, OR 97403.

During normal behavior, we are faced with situations in which we must regulate the position of an object in space, eg, when standing and holding a cup of water. When stance disturbances occurs in this situation, the hand position in space is kept within narrow limits to avoid spilling the water. This constraint is required in spite of postural displacements of the body occurring at the same time. Thus a conflicting situation occurs between the two goals of balancing and holding the cup steady. Experiments were designed to determine at what level control strategies change when subjects balance under these conditions. Balance was perturbed by moving the platform on which subjects stood. Instructions were to 1) hold the fingertip steady in space using visual (EOP) or tactile (ECT) cues, 2) hold a cup of water on a tray with no spills (EOTr), or 3) hold the above forearm/arm position but keep the elbow angle stable (EOH, control condition). Both leg, trunk and arm EMG's and body segment positions were recorded. For EOP and ECT conditions, horizontal hand displacement was reduced to 20% of EOH values (4mm vs 20mm), while for EOTr it was reduced less (65% of EOH). However, the movement velocity was slower in EOTr than in EOH conditions. Control of hand position in space was related to a reduced shoulder displacement and to a shortening of the hand-shoulder distance. No clear-cut stiffness change at the ankle joint was noticed prior to disturbance onset but some uncoupling between arm and trunk was suggested by the fact that displacement of hand and elbow markers starts earlier under EOP, ECT and EOTr (see Droulez & Berthoz, 1986). There were no significant changes in the latency or amplitude of EMG responses in leg/trunk muscles across conditions. However minor changes were observed in those of the shoulder and arm.

Results indicate that accurate control of hand position in space during stance disturbance is the result of multilink adjustments involving arm and shoulder which occur without detectable changes in muscle responses of the leg/trunk.

553.22

A MODEL OF HUMAN TARGET-DIRECTED HORIZONTAL ARM MOVEMENT J.R. Flanagan, A.G. Feldman and D.J. Ostry. McGill University, Montréal, Canada, Institut de génie biomédical, Université de Montréal, Montréal, Canada.

Last year we presented experimental and simulated trajectories of rapid horizontal arm movements directed towards stationary and displaced visual targets. Simulations were generated with a biomechanical model of two-joint planar arm motion. The model, which is based on the equilibrium point hypothesis, assumes that (a) arm movements are planned in coordinates which specify the equilibrium position (EP) of the endpoint and (b) that the EP of the endpoint shifts at a constant rate towards the presently illuminated target. This year the model, which has been extended to include accurate muscle moment arms, is used to predict both kinematic and electromyographic (EMG) patterns. EMG activity was recorded from biceps, triceps, posterior deltoid, and pectoralis major and arm positions were recorded in 3-D with WATSMART. Movements of varying rate to both single- and double-step visual targets were examined. The results further support the hypothesis that horizontal reaching movements are planned in coordinates of the EP of the endpoint. Moreover, the findings suggest that movements of different speeds are achieved by shifting the EP of the endpoint at different rates. The model is used to examine the interaction between the rate of shift of the EP and the level of co-contraction, both of which are regulated by central commands. The issue of whether kinematic and EMG patterns of double-step movement trials can be predicted by the superposition of patterns of single-step movement trials is investigated.

553.23

SYSTEMATIC DIRECTIONAL ERRORS IN PLANAR ARM MOVEMENTS ARE REDUCED BY VISION OF THE ARM. M.F. Ghilardi*, J.Gordon, and C. Ghez. Ctr. for Neurobiol. & Behav., Prog. in Phys. Ther., Columbia Univ. and NYS Psych. Inst., New York, NY 10032.

We have previously shown that in targeted arm movements normal human subjects make systematic direction-dependent extent errors which result from incomplete compensation for variations in the inertial field of the arm. We now report 2 types of systematic errors in movement direction. Subjects moved a cursor from a central starting position to 24 equidistant radial targets on a horizontal digitizing tablet at shoulder level. Target and cursor positions were displayed on a computer screen; the screen cursor was blanked during movement. Starting hand position was varied in different blocks of trials. Vision of the arm was allowed in some blocks and prevented in others. All subjects showed *direction-dependent directional errors* manifest as systematic clockwise (CW)errors in certain directions and counter-clockwise (CCW) errors in others. These resulted in clustering of responses in 4-5 directions, most consistently along the axis of least inertia (orthogonal to the distal segment). These errors were superimposed on *direction-independent directional biases* that varied with the initial hand position. Mean constant errors were CW with initial hand position to the right of the body midline, near zero at the midline, and CCW to the left. These biases were large when subjects could not see their arms but significantly reduced when they viewed their arms during task performance. Direction-dependent errors represent inaccuracies in the specification of movement dynamics, since they vary with limb inertia. Direction-independent errors represent inaccuracies in the specification of joint kinematics as though the nervous system programs joint rotations assuming a midline starting position of the hand and neglecting proprioceptive information about limb orientation. Vision of the arm is needed to bring the intrinsic coordinate system into register with extrinsic coordinates of target location. (NS 22715)

553.24

MUSCLE ACTIVATION WAVEFORMS AND ARM MOVEMENTS IN THREE-DIMENSIONAL SPACE. M. Flanders. Dept. of Physiology, Univ. Minnesota, Minneapolis, MN 55455.

This study related the temporal waveforms of muscle activation to the direction or velocity of the movement of a human arm to targets in three-dimensional space. For the study of direction, targets in different directions were placed in the sagittal plane and the frontal plane, the initial arm position and movement amplitude were constant, and the velocity was moderate (movement time was about 500 ms). For the study of velocity, movement times varied between about 200-1000 ms. Statistical techniques were used to quantify temporal patterns of electromyographic (EMG) activity in 7-9 elbow and/or shoulder muscles. These techniques included a best covariance procedure to calculate temporal delays and a principal component analysis to view the shape of basic EMG waveforms.

The analysis revealed simple neural strategies for generating mechanically complex movements, e.g., basic EMG waveforms are scaled in amplitude and delayed in time to generate movements in different directions.

553.25

A MASS-SPRING MODEL OF TWO-JOINT ARM MOVEMENTS. Nicholas G. Hatsopoulos*, W. H. Warren, Jr.*, and J. A. Anderson. Dept. of Cog. and Ling. Sciences. Brown Univ., Prov. RI 02912.

The mass-spring model (Asatryan & Fel'dman, Biophizika 10) of limb control is extended to two-joint movements and tested against kinematic data from Morasso (Exp. Brain Res. 42). It incorporates a synergy discovered by Mussa Ivaldi (In P. Morasso & V. Tagliasco (Eds.), Hum. Mov. Understand.) which is manifested in a static hand stiffness field that is always oriented toward the shoulder regardless of the position of the hand in the workspace. Two versions of the model are compared. In the first version, the equilibrium position changes abruptly while the torque generated at the joints is gated with a rise-time function. In the second version, the equilibrium position is updated gradually as suggested by various researchers (Bizzi et al., J. Neurosc. 4). The model involves the numerical integration of the equations of motion of a two-joint arm with dissipative "springy" muscles. It is shown that the gradual shift in equilibrium version performs better in matching Morasso's kinematic data. Moreover, the model is able to qualitatively match variable-speed data collected by Atkeson and Hollerbach (J. Neurosc. 5). Finally, two target-switching hypotheses are tested and compared with actual data. The first hypothesis assumes that the first target command is terminated and replaced with the new target command. The second hypothesis states that the first and second target commands are added together vectorally (Flash et al., unpub. man.). The second hypothesis is shown to not only agree with human data as reported by Flash and her colleagues but also to form smoother paths and velocity profiles.

553.26

DISCRETE MOVEMENTS IN RESPONSE TO UNPREDICTABLE CONTINUOUS TARGETS IN HUMAN HAND TRACKING TASKS. F.E. Tendick* and L. Stark. Graduate Group in Bioengineering, University of California, San Francisco and Berkeley campuses, 481 Minor Hall, Berkeley, CA 94720.

Human subjects used a joystick to track an unpredictable two-dimensional target, presented on a CRT display, which had constant speed but varying direction. Subjects responded to the continuous target with brief discrete movements. These movements occurred as rapidly as one per 100 milliseconds, significantly shorter than the response delay. The fast movements consisted of velocity bursts which did not come to rest. These results indicate the possibility of significant parallelism in visual-motor processing. Also, motor programs can be initiated according to a feedforward estimate of position error, without benefit of visual feedback of error. Experiments with brief unpredictable pulse targets support these results.

553.27

COMBINED NEURAL NETWORK AND MATHEMATICAL LARYNGEAL MODEL FOR VOICE PRODUCTION CONTROL. G.R. Farley. Boys Town National Research Hospital, Omaha, NE 68131.

Control of the vocal tract during vocalization involves a complex set of CNS structures. Insight into the function of these structures might be enhanced by the interplay between theoretical models of their function and neural data obtained during vocal behavior. To advance this effort, a two stage model has been developed to control vocal fold tension, a parameter important to fundamental frequency control during vocalization. The first stage of the model is an artificial neural network whose input is the desired vocal fold tension and whose output is analogous to the neural activation of three intrinsic laryngeal muscles: the thyroarytenoid (TA) and the cricothyroid pars oblique (CTo) and rectus (CTr). The second stage of the model calculates from these activation levels the position of the thyroid cartilage relative to the cricoid cartilage, along with accompanying vocal fold tension and length. Network training minimizes the difference between desired and produced vocal fold tension using a genetic training algorithm. When trained, the model can generalize to produce graded vocal fold tensions across the entire available vocal fold tension range. However, different strategies of muscle control can be adopted during training to produce these tensions. Additional constraints now being studied may force the neural network to adopt control strategies limited to those actually used by vocalizing organisms. Nonetheless, the current study demonstrates the feasibility of developing such vocal control models for furthering knowledge of real CNS control systems. [Supported by NIH research grant NS-19624-06.]

INVERTEBRATE MOTOR FUNCTION II

554.1

DUAL INHIBITION OF THE CRAYFISH'S CLOSER MUSCLE BY THE STRETCHER AND COMMON INHIBITORS. T.J. Wiens. Dept. of Zoology, Univ. of Manitoba, Winnipeg, Canada R3T 2N2.

A study of the roles of inhibitory motoneurons in the control of the crayfish claw revealed that the stretcher inhibitor (SI) motoneuron, previously thought to inhibit only the stretcher muscle, also innervates many fibers of the closer muscle strongly and consistently; it does not inhibit any further claw muscles. The same applies to the SI's of the crayfish's walking legs. The claw's common inhibitor (CI) likewise routinely inhibits parts of the closer and stretcher muscles and all other cheliped muscles, with one exception: CI input to the claw's opener muscle could only be demonstrated in some 25% of the claws examined. The CI's of walking legs were confirmed to innervate all muscles including the opener. SI's and CI's unusual distributions may represent adaptations to the closer muscle's size advantage over the opener. The opener inhibitor (OI) was confirmed to innervate only the opener muscle.

SI's and CI's somata were identified intracellularly and stained in claw-ganglion preparations. Both somata lie near the midline, CI's being slightly contralateral and nearer the posterior end of the ganglion. Their form and function suggest homology between the three crayfish limb inhibitors and those of the locust (Watson et al. 1985).

I thank W.J. Heitler, St Andrews, for facilities.

554.2

Spiking activity of the common inhibitory motoneurone in crustacean leg. Synaptic connections with excitatory motoneurones and leg sensory afferences.
M. Bévengut* and F. Clarac. Laboratoire de Neurosciences Fonctionnelles, CNRS-LNF2, B.P. 71, 13402 Marseille Cedex 9, France.

The motoneuronal command of the leg muscles in crustacean is composed by excitatory motoneurones specific for each muscle and by a common inhibitory motoneurone (CI) which innervates every leg muscle. During rhythmic locomotory behaviors, such as swimming and walking, excitatory motoneurones display rhythmic bursts of spikes patterned to ensure coordinated muscle contractions while the CI motoneurone fires tonically with an increase of firing frequency at the end of the return stroke. Pre- and post-synaptic inhibition of CI at the neuromuscular junction are performed to suppress contraction of the slow muscle fibers and to speed up relaxation of the fast muscle fibers during leg movements. In isolated nervous preparations, central synaptic relations between excitatory and CI motoneurones are few and correspond to weak excitatory monosynaptic and/or weak inhibitory polysynaptic connections. However leg sense organ afferences have strong mono- and poly-synaptic connections with the CI motoneurone. These results suggest a separate central control of the spiking activity of the excitatory and the CI motoneurones and a specific peripheral control by the sense organs during locomotory behaviors.

554.3

INNERVATION OF THE SWIMMERETS OF CRAYFISH: A PAIR OF MODULAR PATTERN-GENERATING CIRCUITS IN EACH ABDOMINAL GANGLION. B. Mulloney, W.M. Hall*, C. M. Sherff, G. Braun-Weninger* and A. Chrachri*. Zoology and Neurobiology, Univ. California, Davis CA 95616 USA.

Each swimmeret on the abdomen of the crayfish, Pacifastacus leniusculus, is innervated exclusively through one of the paired first segmental nerves, N1, in its segment. Backfills revealed that each N1 contains axons of sixty eight swimmeret motor neurons, two non-spiking stretch receptors and many other sensory afferents. Cell bodies of these motor neurons were located ipsilateral to their axons; none were located in other ganglia. Every motor neuron had dendritic branches in the lateral neuropil. Unilateral nonspiking local interneurons that are components of the swimmeret pattern-generating circuit also have dendritic arbors restricted to the lateral neuropils.

These are anatomical features of a pattern-generating circuit within each half ganglion, a module of the swimmeret system. To test the ability of a module to function independently, we recorded from motor neurons on contralateral sides of one ganglion and activated the swimmeret system by perfusion with proctolin or proctolin plus TTX. With proctolin alone, membrane potentials of contralateral motor neurons oscillated precisely in phase. With proctolin plus TTX, the phases of their oscillations drifted, and bouts of oscillations occurred in one motor neuron when the other's membrane potential was constant. This uncoupling was reversible.

These results would be predicted if each abdominal ganglion contained a pair of modular pattern-generating circuits. Each module consists of all the motor neurons to one limb and all the pattern-generating unilateral local interneurons that synapse with them. A module generates the characteristic alternating bursts of impulses in antagonistic motor neurons. Each is capable of independent operation, but normally is constrained to operate in a coordinated manner.

554.4

NEURAL PATHWAYS UNDERLYING BEHAVIORAL PLASTICITY IN RESPONSE TO NERVE CORD TRANSECTION IN THE CRAYFISH. R. Glidden* and M.D. Kirk. Div. Biological Sciences, Univ. Missouri-Columbia, Columbia, MO 65211

Escape swimming in the crayfish consists of alternating extensions and flexions of the abdomen (tail). These movements are driven by nongiant fast extensor and fast flexor motoneurons of the abdomen and require an intact rostral nervous system (as shown by loss of swimming motor output following complete nerve cord transection below the subesophageal ganglion). However, as previously demonstrated (Lee and Wine, 1984), three to four weeks after nerve cord transection between the thorax and abdomen (a T-A cut), the abdominal fast flexor motoneurons and other nongiant motor elements again respond to sensory stimulation in a manner resembling nongiant escape swimming. This behavioral plasticity which is referred to here as hyperreflexia, occurs without regeneration across the transection. We investigated where within the chain of six abdominal ganglia these changes take place. For instance, are there important interganglionic interactions or can a single ganglion express hyperreflexia?

EMG recordings from posterior telson flexor muscles (PTFs) and behavioral observations were made following nerve cord transections at different levels within the abdomen. We found that hyperreflexia occurs in segments posterior to transections between the third and fourth ganglia, as well as between the fifth and sixth ganglia. The latter result indicates that a single abdominal ganglion (i.e., the sixth) is capable of expressing hyperreflexia. However, in T-A cut animals exhibiting hyperreflexia, a second cut between the fifth and sixth ganglia eliminated the response in the PTFs of the tailfan. Thus, intersegmental interactions are important under these conditions. Supported by the HHMI.

554.5

MRO POSTURAL REFLEXES IN LOBSTERS DEPEND UPON CENTRAL PROGRAM. S.C. Sukhdeo and C.H. Page. Dept. Biol. Sci. Rutgers Univ., Piscataway, NJ 08855.

Stretch stimulation of the abdominal muscle receptor organ (MRO) of the lobster, *Homarus americanus*, initiated spike discharge of its tonic sensory neuron (SR1). This sensory response evoked a series of reflex responses in the motor efferents of the superficial extensor and flexor muscles of the abdominal postural system. The type of motor response varied according to whether a flexion or extension pattern of spontaneous activity was generated by the postural efferents.

During spontaneous centrally-initiated flexion motor programs, SR1 discharge elicited an assistance response which included excitation of a medium size flexor excitor (f3) and the peripheral extensor inhibitor (e5), and inhibition of at least one extensor excitor. While during spontaneous centrally-initiated extension motor programs, SR1 spiking triggered a resistance response that included excitation of the extensor excitors and the flexor peripheral inhibitor (f5) only, f3 and e5 spontaneous activities were unchanged. Thus reflex reversal occurs between assistance (flexion) and resistance (extension) reflexes.

554.6

MORPHOLOGY OF GIANT AXONS AND THEIR TARGET NEURONS IN LOBSTERS. *P.M.Ma, Dept. Neurobiology, Harvard Medical School, Boston, MA 02115.* In lobsters and crayfish, serotonin and octopamine are neurohormones that modulate many behaviors, including escape tail-flips mediated by the medial (MG) and lateral (LG) giant axons in the ventral nerve cord. For example, serotonin decreases the excitability of giant axons by modulating their primary afferent inputs. To explore the possibility that amines also modulate escape behavior on the output side, studies were performed to examine the structure of giant axons and to locate and identify their target neurons. The giant axons of larval and juvenile lobsters were dye-injected and the branching patterns of the axons within each ganglion were analysed. Dye-coupled neurons were tentatively considered to be electrically coupled. MG axons originate in the brain and project, with few branches, to the caudal ganglion. Coupling between the pair of MG axons occurs in both the brain and the caudal ganglion. LG axons are a syncytium with contributions from neurons in each thoracic and abdominal ganglion. These axons extend through the entire nerve cord and are coupled in each thoracic and abdominal ganglion. Stereotyped dendritic processes are seen in each abdominal ganglion; processes are sparse in thoracic ganglia. A few phasic abdominal motoneurons are dye-coupled to the giant axons. The most heavily and consistently coupled neuron appears to be a swimmeret motoneuron since its axon can be traced to the 1st abdominal root. Identification and localization of these neurons should facilitate electrophysiological studies on the modulation of output of giant axons. (Supported by Harvard and NIH)

554.7

BIOTIN-STAINING IN THE LOBSTER GIANT-NEURON SYSTEM. *H. Schneider, P.M. Ma & E.A. Kravitz, Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115.*

The lateral (LG) and medial giant (MG) neurons in the lobster nervous system play well known roles in escape behavior. Anatomically, these cells are characterized by large-diameter axons extending through the entire length of the ventral nerve cord. We found that these cells also are characterized by a high endogenous content of the vitamin biotin.

Due to specific and high affinity binding of biotin to avidin, labelled avidin was used to visualize biotin in whole mounts of the lobster nerve cord. With this method a pair of large ventral midline cell bodies in the brain, and pairs of posterior lateral cell bodies were stained in each thoracic and abdominal ganglion. In double-label experiments Lucifer Yellow was injected into LG or MG axons before Texas Red coupled to streptavidin was applied. The results show clearly that the double-labelled cell bodies are those of the MG and LG neurons. Within the giant neurons biotin also was found on filamenteous structures in the LG and punctate structures in the MG axons.

Biotin is known to serve as a cofactor of 4 carboxylases involved with pathways of carbohydrate and fat metabolism. One or more of these enzymes, therefore, seem to be highly concentrated in the giant neurons. This might be important for the function of these highly specialized cells. (Supported by NINDS, Harvard and DFG Schn 368/1-1)

554.8

SIMULATION OF THE APLYSIA SIPHON WITHDRAWAL REFLEX CIRCUIT: SLOW COMPONENTS OF INTERNEURONAL SYNAPSES CONTRIBUTE TO THE MEDIATION OF REFLEX DURATION. W.N. Frost, L.G. Wu*, and J. Lieb*. Dept of Neurobiology and Anatomy, Univ of Texas Medical School, Houston, TX 77225.

While traditional electrophysiological approaches can yield detailed circuit diagrams for particular behaviors, it often remains difficult to discern the exact functional roles of the different circuit components. Such insights can, however, be achieved through the use of appropriately realistic circuit simulations. The Aplysia siphon withdrawal reflex has been a useful model system for studies of the cellular mechanisms underlying learning and memory. While circuit modifications related to learning have been identified, interpreting their behavioral significance would be helped by a better understanding of the functional roles of the different circuit components.

We have constructed a simulation of the siphon withdrawal circuit consisting of the LE sensory neurons, the L29, L30 and L34 interneurons, and the LFSa, LFSb, LBS and LDS motor neurons. Each neuron was modeled individually, using an integrate-and-fire scheme, from data collected on its intrinsic electrophysiological properties. Each synapse was modeled from intracellular recordings made of identified synaptic connections.

We are currently using this simulation to evaluate the circuit components responsible for the long lasting firing of the LFS siphon motor neurons which occurs in response to siphon stimulation. Our initial finding is that the slow portion of the two-component fast/slow L29-LFS epsp contributes significantly to LFS firing duration in this circuit. Since the L29 interneurons are strongly excited by siphon stimulation, this connection appears to have an important role in translating short bursts of activity in sensory neurons and the L29 interneurons into a long lasting behavioral response.

554.9

MECHANISMS OF BEHAVIORAL CHANGE INDUCED BY BAG CELL NEURONS ON RESPIRATORY MOTOR SYSTEMS IN *APLYSIA*. JLM Morgan, SH Ligman, PH Brownell. Dept of Zoology, Oregon St Univ, Corvallis, OR 97331

Impulse discharge in the bag cell neuroendocrine system of *Aplysia* is known to trigger complex changes in behavior related to onset of egg laying. While much is known about the synthesis, release and neuronal actions of bag cell transmitters, less is known about the behavioral expression of these effects. Several of the ganglion neurons affected by bag cells and their peptide transmitters are motor neurons innervating respiratory organs, the gill and siphon. We have systematically quantified the actions of bag cell activation (BCA) and bag cell peptides infused individually into the ganglionic vasculature. Our results show that BCA and bag cell peptides affected the tonic firing rate of gill and siphon motor neurons, but that no overt changes in tonus of the gill and siphon were associated with the modulated firing frequencies. For example, BCA generally increased the tonic firing rates of gill and siphon motor neurons (LB and LD cells) by 20 to 140 spikes/minute. Similarly, ganglionic infusion of 1-10 μM egg-laying hormone (ELH) tonically excited LD_{G1}, LB_G, and LB_a but did not increase the tonus of the respiratory organs. Likewise, infusion of α-bag cell peptide inhibited all gill and siphon motor neurons at different thresholds, but was not correlated with relaxation of the gill and siphon. However, both bag cell peptides and BCA affected the amplitude and frequency of respiratory pumping, a phasic contraction of the mantle organs mediated by a network of interneurons. These results suggest bag cell peptides act globally to modulate excitability of motor circuits, facilitating their responsiveness to coordinated synaptic inputs without directly influencing behavior of their target organs. (NIH NS18681, OR Heart Assoc. 80-962, Sigma XI).

554.10

SEROTONIN - SENSITIVE NEURONS IN THE PEDAL GANGLION OF *Pleurobranchaea californica* ARE SEROTONIN IMMUNOREACTIVE. Lee Sudlow and Rhanor Gillette. Dept. of Physiology and Biophysics, Univ. of Ill, Urbana, Ill. 61801.

Locomotion associated with the appetitive and aversive behaviors of the sea slug, *P. californica*, is governed by the pedal ganglion (PedG). We sought to identify the effector cells of the PedG involved in locomotion. Cobalt backfills of the pedal nerve labelled 11 somata at the medial lobe and numerous others at the anterior pole of the PedG. Many of these neurons are known to have a serotonin-activated cAMP-dependent Na^+ current ($I_{(cAMP\text{-}Na^+)}$; Huang & Gillette, Soc. Neurosci. Abs. 13:459, 1987). Single channel analysis of $I_{(cAMP\text{-}Na^+)}$ yielded an opening time constant of 2.27 ± 0.12 msec with a conductance of 40 pS. It was surprising to find that many of the neurons also exhibited serotonin immunoreactivity. Self-re-excitation of these serotonergic neurons in the PedG may contribute to the prolonged changes in the locomotory activity of the slug.

554.11

CHEMOSENSORY RESPONSE TO METAMORPHIC INDUCER ACTIVATES A CENTRAL PATTERN GENERATOR IN A LARVAL MOLLUSC. E.M. Leise and M.G. Hadfield*. Pacific Biomedical Research Center, Kewalo Marine Laboratory, University of Hawaii, Honolulu, HI 96813.

Planktic larvae of the sea slug Phestilla sibogae settle and metamorphose in response to a small (<500 dalton), water-soluble, organic molecule produced by their adult food source, the coral Porites compressa. Sensory receptor neurons in the larval epidermis are probably responsible for recognizing the inducer compound. We hypothesize that excitation of the putative chemoreceptor neurons triggers metamorphosis by activating controlling circuits in the larval CNS. The events of metamorphosis include relatively rapid phenomena, such as loss of the ciliated swimming organ and detachment of the larva from its shell, as well as sequential tissue rearrangements and cell movements. To test our assumptions we are studying the responses of the larval NS to the metamorphic inducer.

Experiments are conducted on 10 day-old metamorphically competent larvae. Extracellular and intracellular recordings are made in vivo from unanesthetized, deshelled and restrained larvae or from isolated larval heads in vitro. The tissue is bathed in filtered seawater (SW), SW containing the inducer compound (I-SW), or SW conditioned by exposure to non-inducing corals. I-SW elicits several alterations in the activity of the CNS and velum, the larval swimming organ. One to 2 mins after addition of I-SW, the frequency and duration of compound spikes indicative of velar ciliary arrests change. Spontaneous bursts of action potentials from smaller units become rhythmic (~0.5 Hz) after the preparation is exposed to the inducer for 1 to 2 hrs. Experiments on larvae with only the visceral hump removed suggest that the rhythmic bursts are associated with the stereotypical posture taken by larvae initiating metamorphosis.

554.12

Correlations of specific neural patterns and buccal muscle contractions with dynamic stages of the feeding cycle of Helisoma trivolvis. B.C. Arnett and A.D. Murphy Dept. of Biol. Sci., Univ. of Illinois at Chicago,Chicago, Il. 60680

Rhythmic feeding behavior in Helisoma is characterized by dynamic stages of protraction, retraction, and hyper-retraction of the feeding apparatus. This behavior is generated by a buccal pattern generator composed of three neuronal subunits (S1, S2 and S3). Each subunit is an independent oscillator but can be temporally linked to either one or two of the other subunits to produce a variety of motor patterns. The neuromodulators serotonin, dopamine, and GABA can reliably phase-lock the subunits to evoke particular neural patterns. In order to correlate these resulting neural patterns with consequent behaviors specific for each neuromodulator, I have simultaneously videotaped movements of the buccal mass and recorded intracellularly from identified buccal motoneurons that provide a monitor of the functional state of the pattern generator. The results are consistent with our hypotheses that: S1 activity mediates the protraction of the radula and odontophore cartilage and flattening of the buccal mass; S2 activity mediates retraction of these oral structures; and S3 activity results in the hyper-retraction of these structures. Supported by NIH R01 NS26145.

554.13

CHARACTERIZATION OF A FEEDING NEURON CONTAINING THE NEUROPEPTIDE APGWa IN THE POND SNAIL, LYMNAEA STAGNALIS. RP Croll[1], CR McCrohan[2]* and MW Baker[1]. [1]Dept. Physiol. Biophys. Dalhousie Univ.,Halifax, Canada B3H 4H7 and [2]Dept. Physiol.Sci.,Univ. Manchester, Manchester M13 9PT, UK.

The tetrapeptide, APGWa, has been localized in the CNS of several molluscs (Van Minnen et al, this volume). One neuron which exhibits APGWa-like immunoreactivity in Lymnaea is located on the ventral surface of each cerebral ganglion between the bases of the median and superior lip nerves. The cell sends a single axon into the ipsilateral cerebral-buccal connective and from there into the buccal ganglia where it produces numerous immunoreactive varicosities in the neuropile. We have recently identified a cell with similar location and anatomy which receives excitatory synaptic input and is active during the radula protraction phase of the feeding cycle. Sustained depolarization of this cell appears to inhibit feeding related activity in buccal neurons. The observation that APGWa is found primarily in neurons controlling male reproduction in Lymnaea suggests that the cell might help suppress feeding during mating.

SENSORIMOTOR INTEGRATION: MUSCLE II

555.1

INABILITY OF HUMANS TO FULLY ACTIVATE MUSCLE DURING SUSTAINED CONTRACTIONS AT DIFFERENT FORCE LEVELS. A.J. Fuglevand, K.M. Zackowski*, K.A. Huey*, and R.M. Enoka. Depts. Exercise & Sport Sciences and Physiology, Univ. Arizona, Tucson, AZ 85721

During a constant-force submaximal contraction, there is a gradual decline in the force capacity of active motor units. The nervous system, therefore, can increase motor unit recruitment and discharge rate in order to maintain the desired force. The purpose of this study was to examine changes in neural drive to muscle, as reflected in the surface EMG, during sustained contractions. Subjects (n = 32) were instructed to sustain an isometric abduction of the index finger to exhaustion at one of three force levels: 20%, 35%, or 65% of maximum voluntary contraction (MVC) force. Surface EMG and abduction force of first dorsal interosseous were monitored throughout the fatiguing task. Endurance times for the three force levels were significantly different from one another (534±195 s, 246±110 s, and 66±23 s for the 20, 35, and 65% MVC groups). The mean power frequency of EMG declined in parallel for all three groups, reaching a similar value at exhaustion (49.7, 42.6, and 44.7 percent of initial for 20, 35, and 65% groups). EMG amplitudes at exhaustion, expressed as a percent of MVC EMG, were 45.2, 54.4, and 81.0% for the 20, 35, and 65% MVC groups. These results suggest a force-level-dependent inability of the nervous system to fully activate muscle at exhaustion during sustained contractions. This incomplete activation parallels the decline in neuromuscular propagation observed during submaximal contractions (Fuglevand et al., *3rd IBRO World Congr. Abstr.*, in press).

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555.2

INFLUENCE OF ELECTROMYOSTIMULATION (EMS) AND EXERCISE HISTORY ON VOLUNTARY FORCE IN MEN.

T. Hortobágyi, N.J. Lambert† and R.G. Israel. *Human Performance Laboratory and †Department of Physical Therapy, East Carolina University, Greenville, NC 27858.*

Prior reports have suggested that maximal voluntary isometric and lengthening forces may be neurally inhibited in sedentary men. We have examined the influence of activation and strength-training history on the force-velocity relationship in 6 strength-trained and 6 sedentary men, hypothesizing that EMS added to voluntary effort would not further enhance voluntary isometric, shortening, or lengthening forces in the trained men. Over four days, maximal isometric (MVC), tetanic, and shortening and lengthening constant angle isokinetic forces at 6 velocities were measured in the arm flexors under voluntary condition and with EMS (2.5 kHz sine wave, 50 burst·s^{-1}, 50% duty cycle) superimposed on the voluntary muscle actions. Trained men were 31% stronger than untrained controls ($P < .05$). Voluntary shortening forces decreased with increasing shortening velocities and were less than MVC in both groups ($P < .05$). EMS + voluntary forces never exceeded voluntary forces. The EMS + voluntary force-velocity curve was similar to the voluntary curve. Lengthening forces, as expected, exceeded MVC only in the trained men ($P < .05$). EMS + lengthening forces were also greater than EMS + MVC in untrained men ($P < .05$). These data suggest that a lack of neural inhibition may allow trained men to volitionally exert lengthening forces to a full capacity. However, it is inconclusive whether inhibition or other factors hamper untrained men to attain lengthening forces greater than MVC.

555.3

EFFECTS OF AGING ON THE ESTIMATED NUMBER OF FUNCTIONING HUMAN MOTOR UNITS. T.J. Doherty*, A.A. Vandervoort*, A.W. Taylor* and W.F. Brown. Faculty of Kinesiology and Dept of Clinical Neurological Sciences, University of Western Ontario, London, Ontario, Canada, N6A 3K7.

This study investigated the age associated loss of motor units in the biceps brachii-brachialis muscles and the resultant effect on force production.

The number of functioning motor units was estimated with the spike-triggered averaging technique (Brown et al, Muscle & Nerve, 11, 423-432, 1989) in 18 active older adults (age 68 +/- 5 yrs) and 15 active younger controls (29 +/- 5 yrs). The peak to peak amplitude of the compound muscle action potential (M-wave)was significantly lower in older subjects as compared to controls ($p < .001$) and the surface recorded single motor unit potential was significantly greater ($p < .05$). Therefore, as expected, older subjects had significantly fewer motor units (195 +/- 85) than controls (352 +/- 81) ($p < .001$) The electrically evoked isometric twitch contraction did not differ significantly between old and young subjects whereas the maximal voluntary contraction was significantly lower in the older subjects.

555.4

SYNAPTIC MECHANISMS UNDERLYING SHORT-TERM SYNCHRONIZATION IN HUMAN MOTONEURONS. K.S. Türker, T.S. Miles and M.A. Nordstrom, Department of Physiology, University of Adelaide, SA 5000, Australia.

During a steady, voluntary contraction, there is a small but significant tendency for active motor units to discharge synchronously, rather than completely randomly with respect to one another's activity. These synchronous occurrences are manifest as a central peak in the cross-correlation histogram of the spike trains of most pairs of active units. It has been suggested that this "short-term synchronization" is the result of common post-synaptic potentials from stem neurons that branch to innervate many motoneurons. We have used a new approach to investigate the synaptic events that are responsible for this synchronization. The spike trains of two concurrently-active motoneurons were recorded in human masseter or tibialis during a steady voluntary contraction. Synchronous occurrences of spikes were identified, and the durations of the interspike interval (ISI) preceding the synchronous spikes in both units were compared with random interspike intervals from the same spike train. In some units, the pre-synchronous ISIs were significantly shorter than randomly-selected ISIs from the same spike train. This strongly suggests that the nett synaptic effect which synchronized both units was excitatory. We conclude that the short-term synchronization manifest as a peak in the cross-correlogram is due to common, excitatory synaptic potentials whose amplitudes are significantly greater than those of the normal synaptic potentials that are driving the cell during tonic excitation.

This work was supported by the National Health & Medical Research Council of Australia.

555.5

ELECTROMYOGRAPHIC (EMG) AND RANGE OF MOTION (ROM) COMPARISONS BETWEEN STATIC STRETCHING AND PROPRIOCEPTIVE NEUROMUSCULAR FACILITATION (PNF) STRETCHING METHODS IN HUMANS. B.R. Etnyre and S. Overholt*. Human Performance Laboratory, Rice University, Houston, TX 77251

Static stretching and two common PNF techniques were compared for hip flexion ROM and the phasic and quantitative muscle activity of stretched femoral muscles. The stretching techniques for each of 15 subjects were: static stretch (SS), contract-relax (CR), and contract-relax-reversal (CRR). Hip flexion ROM was measured before and after stretching. EMG was recorded from the subject's rectus femoris and biceps femoris muscles. Unlike most previous studies, we precluded electrode "cross-talk" between antagonist muscles by establishing independence of activity of each muscle before testing. The EMG signals were integrated (IEMG) and peak spectral density was determined from fast Fourier transform (FFT) of each trial. All three stretching techniques yielded significant gain in hip flexion ROM compared with the initial condition, $F(3,27) = 9.05$, $p < .001$, (initial = 75°, SS = 82°, CR = 85°, CRR = 85°), with no significant differences was between stretching techniques. Over all stretching conditions only one subject demonstrated biceps femoris activity during stretching (CRR condition: IEMG = 90.6% of control, peak FFT = 67 Hz). Rectus femoris activity during the reversal stretching resulted in average IEMG over subjects of 25.8% and FFT = 50.4 Hz. Although the PNF methods resulted in the greatest gain in ROM they were not significantly different from the SS method. While controlling for cross-talk between electrodes of antagonist muscles, co-contraction between antagonist muscles during the reversal method was observed, but it was uncommon, contrary to results of previous studies.

555.6

EVIDENCE FOR FAILURE OF NEUROMUSCULAR TRANSMISSION IN THE NEWBORN DIAPHRAGM. A. R. Bazzy-Asaad, D. F. Donnelly, and G. G. Haddad, Dept. of Pediatrics, Section of Respiratory Medicine, Yale Univ. Sch. of Med., New Haven, CT 06510.

Although neuromuscular transmission (NMT) failure can contribute to diaphragmatic fatigue, it is not clear what its relative contribution is in the newborn as compared to the adult. To examine this question we studied NMT in-vitro in the rat phrenic nerve-hemidiaphragm preparation from animals ages 4-7d (n = 17) and adults (n = 10). Force was generated by the muscle by either stimulating it directly or indirectly via the phrenic nerve at frequencies ranging from 10-100 Hz for a brief period of 1 second. We found that, at a given frequency, the adult muscle generated the same level of force via either route of stimulation. In contrast, muscles from newborn rats generated less force when stimulated via the phrenic nerve than when stimulated directly at all frequencies used. In addition, at frequencies ≥ 40 Hz force generated indirectly could not be maintained during the one second stimulus and fell to about 50% of the peak value at a given frequency. To determine whether this loss of force was due to failure of some muscle fibers to respond to nerve stimulation, we recorded action potentials (AP) intracellularly from single muscle fibers in both age groups using flexible microelectrodes of resistance of 80-100MΩ filled with 3M KCl. We found that in 30% of the newborn fibers (n = 6), in response to a given stimulation frequency, fewer than the expected number of action potentials was recorded. In other cases (even within the same train), end-plate potentials or no change in membrane potential was found. In adult muscles, (n = 4) the expected number of APs was always found. We conclude that in the newborn diaphragm 1) NMT failure occurs in response to brief periods of stimulation over a wide range of frequencies and 2) this failure may be secondary to a decrease in release of neurotransmitter from the nerve terminals.

555.7

THE LENGTH-TENSION RELATIONS OF NINE DIFFERENT SKELETAL MUSCLES. M. Solomonow, R. Baratta, H. Gareis, R. Best, & R. D'Ambrosia. Bioengineering Laboratory, LSU Medical Center, New Orleans, LA 70112.

The length-tension relations of nine different muscles in the hind limb of the cat was determined with special emphasis on possible differences that may be function and architecture related. Electrical stimulation at tetanic rates was applied to the sciatic nerve while monitoring isometric force with an FT-10 transducer mounted on a crank operated platform.

Crank operation allowed the measurements of isometric forces from the muscle when completely collapsed (zero force) and up to maximal length when it ruptured. Data from each muscle was normalized with respect to its maximal active force and the length at which it occurred (optimal length). Data from all preparations of the same muscle were pooled and smoothed with a five point moving average.

The results show that the length-tension curves varied widely among the muscles. The FDL, MG, LG, and TP had a symmetric active force, whereas, the Soleus, EDL, PL, and PB had asymmetric active force. The TA had double peaked active force. The passive force started always just before the optimal length except for the Soleus, EDL, and LG in which there was passive force over the full length. The elongation range varied from ±15% to ±30% Lo in most muscle while the TA, PB, and PL had asymmetric range. It is concluded that different muscles have different length-tension relations reflecting their function and structure.

555.8

IN-SERIES COUPLING OF SPINDLE RECEPTORS. R. Delgado-Lezama*, E. J. Muñoz-Martínez, J. G. Raya*, R. Cueva-Rolón* and J. Aguilar-Linares*. Dept. Physiol., Biophys. & Neurosci. CINVESTAV-IPN. Apdo. Postal 14-740. 07000-México,D.F. MEXICO

Spindle discharges during muscle contraction (loading response; LR) and/or relaxation were investigated in the soleus of anaesthetized cats. Single Ia spikes were recorded in dorsal roots (L7-S1) filaments. Spindles location was found by pressing the muscle with von Frey hairs (12.5 mg). Perpendicular muscle vibration (250 Hz, variable intensity) was applied to spots 1 mm apart. Maximal sensitivity was restricted to a single spot. Ia fibers showing LR to nerve stimulation were located near either tendon but those showing typical unloading responses (UR) were located at the medial third of the muscle. Contraction of discrete muscle bands were produced by direct stimulation. UR and relaxation firing (RF) were found stimulating on the receptor but stimulating 2-4 cm away in the longitudinal direction, LR appeared and RF decreased or disappeared. Lateral stimulation only gave UR. Thus, spindles are coupled both in-series and in-parallel with extrafusal fibers.

555.9

BIOMECHANICAL ACTIONS OF MULTI-ARTICULAR MUSCLES IN THE CAT NECK. W.S. Selbie*, D.B. Thomson, and F.J.R. Richmond. MRC Group in Sensory-Motor Physiology, Queen's University, Kingston, Ont. K7L 3N6 CANADA.

Our goal is to describe biomechanical features of head movement in the cat. In an earlier study we determined that the centres of rotation in the sagittal plane were stationary for all cervical joints; thus, we can consider the cat neck to be a set of rigid segments linked by pin joints. In the present study we have added seven major neck extensors and flexors to our model and we have examined the effect of different postures on those muscle paths. The sites of attachments of these muscles were estimated by dissecting feline cadavers. Many muscles had broad origins that spanned several vertebrae and thus required several independent representations. Muscle paths were computed initially as a straight line from origin to insertion as suggested by the literature. The resultant muscle paths for some long muscles actually passed through the cervical column in some postures and thus required the addition of path constraints such as tethers and pulleys. Even a simple, short extensor such as rectus capitis posterior had a moment arm (at skull-C1) whose magnitude changed by more than a factor of three throughout the physiological range of motion. We are now attempting to determine the minimal path complexity necessary for adequate representation of sagittal plane motion so that eventual extension to three dimensions will be tractable.

(Supported by the Canadian MRC)

555.10

FORCE-LENGTH RELATIONSHIP OF FELINE ANTERIOR SARTORIUS RESULTS FROM A COMPLEX INTERACTION OF PASSIVE AND ACTIVE CONTRACTILE ELEMENTS. S.H. Scott, D.B. Thomson, F.J.R. Richmond and G.E. Loeb. MRC Group in Sensory-Motor Physiology, Queen's University, Kingston, Ont. K7L 3N6 CANADA

In glycogen-depletion experiments we have shown that the proximally- and distally-directed nerve branches entering anterior sartorius supply asymmetrically distributed motor-unit territories. These territories are organized partly in parallel, partly in series. Force-length relations of anterior sartorius have now been examined by stimulating either the parent nerve bundle or the individual nerve branches supplying the muscle while the whole muscle was clamped at different lengths. When the proximally- or distally-directed nerve branch was stimulated, the end of the muscle closest to that branch actively shortened while the opposite end lengthened. The resulting force-length curves were not simply a scaled-down version of the force-length curve produced by stimulating the parent nerve bundle; at long whole-muscle lengths, force of the partially activated muscle was enhanced whereas at short whole-muscle lengths, it was diminished. The force-length relations suggested that a complex interaction of both passive and active elements contributed to total force. It remains to be confirmed whether selective recruitment occurs amongst the different subpopulations of motor units, as suggested by reflex studies of Sherrington (1910). The complex mechanics arising from recruitment of asymmetrically distributed motor units raises interesting problems for the neural control of this and possibly other long muscles.

(Supported by the MRC and Muscular Dystrophy Association of Canada)

555.11

MOTOR-UNIT DISTRIBUTION IN FELINE ANTERIOR SARTORIUS MUSCLE. E. Smits, M.J. Heron*, P.K. Rose, T.T. Gordon and F.J.R. Richmond. MRC Group in Sensory-Motor Physiology, Queen's University, Kingston, Ont. K7L 3N6 and Dept. of Pharmacology, University of Alberta, Edmonton, Alt. T6G 2E1 CANADA.

The success of functional electrical stimulation to reanimate paralyzed muscles will depend on the ability to position stimulating electrodes appropriately within target muscles. Long muscles such as gracilis and sartorius pose a particular challenge because of their complex architecture. Forces produced by their short, tapering, in-series fibers are exerted not onto tendon, but onto other fibers that may or may not be concurrently active. If individual muscle units are not distributed evenly along the length of the muscle, mechanical instabilities may arise. In this study, single motor-units were glycogen-depleted by stimulus trains delivered either intracellularly to motoneurons or extracellularly to single ventral-root axons. In all motor units studied so far (n=8), fibers were distributed asymmetrically, with a relatively high density at one end or in the middle of the muscle, decreasing or disappearing over the remaining length of the muscle. The muscle-unit territories were also confined laterally within long, relatively narrow strips comprising only a fraction of the muscle width. The implication from these studies is that multiple sites of stimulation may be required to ensure a synchronous and mechanically stable distribution of contractile forces in long muscles.

(Supported by the Canadian MRC and the Network of Centers of Excellence for Neural Regeneration and Functional Recovery)

555.12

MECHANICAL CHARACTERISTICS OF THE FELINE ANKLE MUSCULATURE. R.P. Young, S.H. Scott and G.E. Loeb. MRC Group in Sensory-Motor Physiology, Queen's University, Kingston, Ont. K7L 3N6 CANADA.

The potential contribution of each muscle to the production of a given movement depends on the instantaneous magnitude and direction of its moment arm and the distribution of motion between its muscle fibers and series-elastic connective tissues. As is typical of many joints, the ankle has multiple muscles acting upon multiple degrees of freedom. Many of the ankle muscles have very short moment arms combined with highly pinnate architectures, which makes direct measurement of these critical parameters difficult and unreliable. We have employed sonomicrometry to measure small increments of motion between bone, tendon and muscle fascicles when the ankle is clamped in various positions of extension/flexion and inversion/eversion during both passive and active conditions of the various muscles. Independent estimates of moment arms were obtained from this relative motion and from the ratio of muscle tension to plantar force produced at the foot clamp. For example, peroneus brevis was found to have a small but constant moment arm contributing to eversion and extension over most of the normal range of motion (as a result of a pulley around the lateral maleolus) whereas the peroneus longus had a larger but changing moment arm contributing to eversion and dorsiflexion (as a result of its tethered tendon path). These morphometric and biomechanical data can now be combined with the available EMG data to assess the relative sensory and motor contributions of these muscles to natural behaviors.

(Supported by the MRC and the Muscular Dystrophy Association of Canada)

555.13

A COMPUTER-MODELING ENVIRONMENT FOR THE ANALYSIS OF KINESIOLOGY, BIOMECHANICS AND SENSORIMOTOR CONTROL IN MULTIARTICULATED LIMBS. G.E. Loeb, Z. Hu*, P. Chiang* and W.S. Levine*. Bio-Medical Engineering Unit, Queen's University, Kingston, Ont. K7L 3N6 CANADA and Dept. of Electrical Engineering, University of Maryland, College Park, MD 20734 USA.

Body movements are the result of a complex interplay between the intrinsic mechanical properties of musculoskeletal systems and their dynamic modulation by the nervous system. For all but the simplest and most constrained systems, an appreciation of these relationships requires quantitative integration and presentation of large amounts of diverse data regarding morphometry, kinematics and dynamics. We have developed a user-interactive environment on the Macintosh II personal workstation that supports the following functions:

Morphometric spreadsheet - generalized system for describing and dimensionless scaling of 3D musculoskeletal architecture;
Analog and video data acquisition - timecode-synchronized spreadsheet files of EMG, transducer and joint position data;
Kinesiological data analysis - editing, temporal normalization and averaging of cyclical data;
Relational database - user-specified data transformations, e.g. estimation of muscle torque from morphometry, EMG, and force-length, force-velocity and active state dynamics;
Iconographic stick-figure display - includes muscle- and joint-specific items selected by the user from the database;
Special functions interface - couples to external code, e.g. equations-of-motion solvers and regulator designers.

(Supported by NIH Grant #NS27193)

555.14

THE INFLUENCE OF MUSCLE LENGTH ON MUSCLE FIBRE CONDUCTION VELOCITY (MFCV) AND MUSCLE FATIGUE. L Arendt-Nielsen[1], T Sinkjær[1] and N Gantchev[2]*, [1]Department of Medical Informatics, Aalborg University, Denmark, [2]Bulgarian Academy of Sciences, Sofia, Bulgaria.

Introduction. Theoretically, it has been found that the muscle fibre conduction velocity increase when the diameter increase. During dynamic muscle load the muscle length change continuously, but the importance of length changes for the development of fatigue is not known. The electromyogram (EMG) can be used to clarify some of these aspects. The present study investigated the influence of muscle (vastus lateralis) length on MFCV and on muscle fatigue.

Methods. The EMG responses were evoked by electrical stimulation of the motor point and recorded by a tripolar surface electrode array aligned along the muscle fibre direction. The MFCV (determined by cross-correlation) was measured for 5, 45, 90 and 120 degrees knee flexion at three different background extension torques (0, 25, 50% maximum voluntary contraction). In the second experiment the EMG activity to a static fatiguing contraction (80% MVC) was measured at 45 and 90 degrees knee flexion and the MFCV calculated.

Results. The MFCV declined for increasing muscle length, and increased for increasing background torque from 0 to 90 degrees knee flexion. The MFCV can change approximately 2 m/s dependent on the background force and muscle length. During fatigue the MFCV decline rate (0.047 m/s/s) was significantly higher for the 90 degrees knee angle compared to 45 degrees (0.024 m/s/s).

Conclusion: The muscle length is an important parameter for the propagation velocity of action potentials and for the development of static muscle fatigue.

555.15

LEFT-RIGHT COMPARISON OF HOMOLOGOUS MUSCLES WITH REGARDS TO TIME AND FREQUENCY ANALYSIS OF EMG SIGNALS. M. Bilodeau, A.B. Arsenault, D. Gravel, D. Bourbonnais, F. Kemp*. Research Centre, Montreal Rehabilitation Institute and School of Rehabilitation, Faculty of Medicine, University of Montreal, Montreal, Quebec, Canada, H3C 3J7.

The aim of this study was to contrast EMG characteristics of homologous muscles of the right and left sides of normals. Surface EMG signals (16-800 Hz) of anconeus, brachioradialis, triceps and biceps brachii (16 subjects) were recorded during 5-s ramp elbow flexions and extensions. The median and mean power frequency of the power spectrum and the root mean square values of the raw EMG computed at different force levels (10, 20, 40, 60 and 80% MAX) were analysed using two-way ANOVAs for repeated measures. Slopes of the EMG/moment relationships were also computed bilaterally and contrasted using paired t-tests. No significant differences were found ($p>0.05$) in any parameters between right and left homologous muscles. It thus appears that these parameters taken from surface EMG signals can be considered potentially useful measures when comparing the unaffected to the affected side of populations such as hemiplegics.

M. Bilodeau is funded by a MRC studentship.

LEARNING AND MEMORY—PHYSIOLOGY VI

556.1

EFFECTS OF DORSAL NEOCORTICAL STIMULATION ON PERFORANT PATH EVOKED FIELD POTENTIALS IN THE DENTATE GYRUS OF THE RAT. D.L. Korol, B.W. Leonard, B.L. McNaughton, and C.A. Barnes. Department of Psychology and ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

Modulation of neural excitability in the hippocampus results from activation of various subcortical inputs. Prestimulation of the medial septal and supramammillary nuclei facilitates the population spike (PS) of dentate granule cell field potentials evoked by perforant path (PP) stimulation, probably by suppressing inhibitory interneurons (Mizumori et al., 1989). To address the question of how cortical afferents other than the primary input from entorhinal cortex affect hippocampal dynamics, we examined the effects of prestimulation (at 25 msec) of various neocortical areas on PP-evoked field EPSPs and population spikes in anesthetized rats. Substantial facilitation of the population spike, with little or no change in the field EPSP, can be evoked by prestimulation of some cortical sites, whereas inhibitory effects are observed at other sites. Preliminary mapping studies suggest a clustering of excitatory fields in frontal (motor) fields and inhibitory sites in the parietal visual (OC2m) and somatosensory (HL) areas; however, the effects are clearly at least disynaptic, and state-dependent. More data will be required to determine whether this clustering is consistent. Cortical activation alone often elicited field potentials with latencies varying from 3-16 msec that were correlated with the incidence of facilitation; however, the cortical potential failed to exhibit polarity reversal in the molecular layer, whereas the simultaneously evoked PP potential did reverse polarity. This suggests that the cortically evoked potential is not generated in dentate gyrus.

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556.2

REDUCED HIPPOCAMPAL LTE IN AGED RATS FOLLOWING CHRONIC ADMINISTRATION OF ACETYL-L-CARNITINE S. Davis, A. L. Markowska[1], C. A. Barnes, B. L. McNaughton, and D. S. Olton[1]. Department of Psychology and ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724; Department of Psychology, Johns Hopkins University[1], Baltimore, MD 21218.

Acetyl-l-carnitine (AC), an ester of the endogenous compound carnitine appears to improve, for example, the morphological changes that normally occur in the hippocampus of aged rats (Ramacci et al, Drugs Exptl. Clin. Res., XIV(9), 593-601, 1988), behaviour on the Barnes circular platform and long-term memory performance in the split-stem T-maze (Barnes et al., Neurobiol. Aging, 11, 499-506, 1990), and is under clinical evaluation for treatment of cognitive and neural dysfunctions in aged humans. We have investigated the effects of chronic administration of AC in aged rats on their performance in a spatial version of the Morris water task and on long-term synaptic enhancement (LTE) in the dentate gyrus, using high-frequency electrical stimulation of the perforant path afferents. Three groups of Fischer 344 rats were tested: young (N=5) and aged (N=7) controls and an aged group (N=5) administered AC (75mg/kg/day) for at least 8 months. The data from the behavioural tests suggest that AC-treated rats show small improvements in spatial learning. In these same animals, however, the magnitude of LTE was greatly reduced in the AC-treated rats compared with old and young control animals, although its decay rate appeared to be the same as controls. These unexpected results suggest that AC may interfere with certain mechanisms of synaptic plasticity; however this will need to be verified in a replicate study (presently underway).

Supported by Sigma Tau Corp..

556.3

AN AGE COMPARISON OF SHORT-TERM EXPLORATORY MODULATION OF RAT PERFORANT PATH SYNAPTIC EFFICACY. C.A.Erickson, C.A.Barnes, B.L.McNaughton, D.F.Gibbons*. Dept. Psychol. & ARL Div. of Neural Systems, Memory and Aging, Univ. Arizona, Tucson, AZ 85724.

Old rats show accelerated forgetting of spatial information. This is correlated with faster decay of electrically induced synaptic enhancement (LTE/LTP) in fascia dentata (Barnes, 1979). Recently, naturally-occuring synaptic changes, termed "short-term exploratory modulation" (STEM), have been identified in rat hippocampus during bouts of exploration (Sharp et al., 1989; Green et al., 1990). Exploration results in an increase in the EPSP component of perforant-path evoked responses and a reduction in the population spike. STEM is cumulative, outlasts the behaviors which produce it, and is blocked by MK-801 (Croll et al., 1990; Erickson et al., 1990). We compared STEM induction in 24 mo and 9 mo old rats chronically prepared for recording perforant path evoked potentials. The rats were plugged into the recording equipment 2 hours prior to the recording session. Perforant path stimuli were delivered every 5 sec to alternate hemispheres and behaviors were scored as either exploratory or non-exploratory. After 1 hr of baseline recording in the home cage, in which the rats typically rested quietly, they were placed for 30 min on platforms containing junk objects. Rats were reinforced for exploration with chocolate milk. Following the exploration phase, the rats were returned to their home cages for the remainder of the 3 hr session. The old rats exhibited significantly less STEM than the young rats; however, the old rats also explored significantly less. Because of the strong dependency of STEM on the cumulative recent history of exploration, we tentatively conclude that the expression of STEM is not substantially affected by the aging process.

Supported by AG03376.

556.4

RAPID INDUCTION OF *ZIF* 268 mRNA IS PRESERVED IN HIPPOCAMPUS OF OLD F-344 RATS FOLLOWING LTE-INDUCING STIMULATION. C. A. Barnes, C. A. Erickson, B. L. McNaughton, R. Bhat, J. M. Baraban, and P. F. Worley. Dept. of Psych. and ARL Div. Neural Systems, Memory and Aging , Univ. Arizona, Tucson, AZ 85724 and Depts. Neurosci., Neurology and Psychiatry, Johns Hopkins Univ., Baltimore, MD 21205.

The transcription factor *zif* 268 can be rapidly activated in granule cells in the hippocampus by patterned electrical stimuli that induce LTE in both anesthetized and chronically-prepared young rats. Like LTE, *zif* 268 induction is dependent on NMDA receptor activation. In old rats, mechanisms of LTE induction appear to be normal, but the maintenance of LTE is deficient. Accordingly, it was of interest to determine whether mechanisms of *zif* 268 activation are defective in old rats. As a first step we examined 4 young (10 mo) and 4 old (26 mo) rats 2 weeks following bilateral implantation of electrodes into the fascia dentata and perforant pathway. A total of 100 stimulus pulses were given to each hemisphere of each rat: one hemisphere received low frequency (LF) stimulation at 0.1 Hz; the other high frequency (HF) at 400 Hz. *Zif* 268, *c-fos*, *jun* B, and *c-jun* mRNA levels were assessed by *in situ* hybridization, and related to the level of LTE 30 minutes following the HF stimulation. As previously reported, there was no difference between age groups in LTE induction (EPSP change: old = 0.41; yg = 0.44), and no change from baseline in the LF stimulation hemisphere (old = 0.08; yg = 0.07). The new finding here is that transcription factor (TF) responses are similar in the LTE hemispheres of both young and old rats. These data suggest that signal transduction leading from NMDA receptor activation in the dendrite to somatic mRNA responses are intact in old rats, and that factors downstream from TF activation may be disrupted.

Supported by AG09219, MH00897 and Kligenstein Foundation.

556.5

AGE-RELATED DECREASE IN CA1 EPSP TO FIBER POTENTIAL RATIO. G. Rao, C. A. Barnes, T. C. Foster and B. L. McNaughton. Department of Psychology and ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

We previously reported an age-related decrease in sensitivity to exogenously-applied AMPA in hippocampal subfield CA1, based both on the ability of this glutamate synaptic receptor agonist to attenuate *in vitro* evoked field EPSPs, as well as on a reduction of extracellular field potentials induced by focal application of AMPA in stratum radiatum (Soc. Neurosci. Abs., 16, 776, 1990). If a decrease in post-synaptic sensitivity underlies this observation, it should also be manifested in a decrease in the amplitude ratios of the CA1 EPSP and fiber potential components.

Hippocampal slices were prepared from F-344 male rats at ages 6 and 27 mos (n = 7 rats per group). A recording pipette and a stimulating electrode were positioned in CA1 stratum radiatum and five extracellular evoked potential responses were collected at four intensities (50, 100, 200 and 400 μA). The amplitude of the initial phase of the EPSP and the area of the fiber potential were subsequently analyzed. At all four intensities, the EPSP to fiber potential amplitude ratios were smaller in the old animals. The decline was significant ($p<.005$) at the 100 and 200 μA intensities. This result is consistent with the AMPA results described above.

Experiments employing minimal stimulation to activate single afferent fibers and quantal analysis to estimate mean quantal parameters are in progress to determine whether the reduced AMPA responses and EPSP to fiber potential ratios are accompanied by a reduced apparent quantal size. Supported by AG03376.

556.6

QUANTAL ANALYSIS OF LTE: FACTORS WHICH MODIFY EXPRESSION. T.C. Foster, B.L. McNaughton. Dept. Psych. and ARL Div. Neural Systems, Memory and Aging, Univ. Arizona, Tucson, AZ 85724.

We reported that a 30% increase in mean EPSP amplitude during long-term synaptic enhancement (LTE) is due to an increase in quantal size (q) in the absence of changes in transmitter release (m) (Foster & McNaughton, *Hippocampus*, 1991). Malinow and Tsien (*Nature*, 1990) reported a substantial EPSP increase (200%) due to a large increase in m and a small increase in q. We have attempted to determine the factors responsible for the disparate results, including: 1) immature (3-5 wk) rats, 2) reduced temperature (22°C), 3) elevated Ca^{++}, 4) method for LTE induction (pairing 40 pulses, 2 Hz, with postsynaptic depolarization), and 5) low stimulation rates. No single factor accounts for the differences in the magnitude or quantal parameters of LTE observed between the two studies. Significant effects of age and temperature were observed on baseline m. Young rats showed low m values and m was larger at 22°C. The larger m at 22°C could be reduced by using a lower stimulation rate. Whereas, in our studies, there was no average change in m, the magnitude and direction of changes in m following LTE stimulation were negatively correlated with baseline m. Changes in m were mainly due to changes in p. Since p cannot be negative, a floor effect may result from initially low values. The initial value of m, calculated from the mean number of transmission failures for 5 cells reported by Malinow and Tsien, was substantially lower than in most other studies of these synapses. Thus the discrepancy between the two studies may be related to differences in the initial resting p; however, we have no explanation for why Malinow & Tsien did not observe increased m after stimulation in the absence of depolarization. Supported by NS08585 to TCF and NS20331 to BLM.

556.7

ADDITIVE RELATIONSHIP BETWEEN PERFORANT PATH LTE/LTP AND STEM SUGGESTS INVOLVEMENT OF DIFFERENT SYNAPTIC POPULATIONS. B.L. McNaughton, C.A. Erickson, C.A. Barnes, G.D. Stevenson*. Department of Psychology and ARL Div. of Neural Systems, Memory and Aging, University of Arizona, Tucson AZ, 85724.

Exploratory behavior leads to a short-term (15-30 min) elevation of synaptic responses evoked in the fascia dentata by electrical stimulation of the perforant path (Sharp et al., Psychobiol., 17:257-269,1989; Green et al., J. Neurosci., 10:1455-1471,1990). Although not permanent, this short-term exploratory modulation (STEM) long outlasts the behaviors that induce it, and has been dissociated from the locomotor component of exploration using a motorized treadmill. Unlike stimulus induced long-term enhancement (LTE), STEM is not due to electrical stimulation *per se*, although both phenomena can be blocked or attenuated by systemic MK801 (Croll et al., Soc. Neurosci. Abst., 16:263, 1990; Erickson et al., Soc. Neurosci. Abst., 16: 442, 1990). To address the question of whether STEM and LTE share a common mechanism of expression we studied the manner in which the two phenomena interact. Possible interactions are occlusion, suggesting a common mechanism, a multiplicative relationship, possibly due to one pre and one postsynaptic effect, and an additive relationship, suggesting independent sites of expression. The absolute, STEM-related EPSP growth (mean 43%) was virtually identical (slope = 1.03) before and after LTE induction (mean 42%), indicating a purely additive relationship. Moreover, whereas LTE was characterized by an increase in EPSP slope, STEM involved an apparent reduction in EPSP onset latency. These data suggest that STEM and LTE may occur at different synapses, and, as suggested by Green et al. 1990 on other grounds, that STEM could represent a rather large growth at a small proportion of synapses. Supported by O.N.R. &AG03376.

556.8

40-HZ "FLUTTERING" OSCILLATIONS IN THE RAT HIPPOCAMPUS. W. E. Skaggs, B. L. McNaughton and C. A. Barnes. Depts. Neurosci. and Psych. and ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724. Email: bill@nsma.Arizona.edu

The hippocampus, because of its large number of cells and laminar architecture, is capable of generating strong extracellular electric fields. In addition to the well studied 6-8 Hz "theta" rhythm, we find that it also gives rise to higher frequency oscillations. These can be heard on an audio monitor as a distinctive "fluttering" sound when a recording electrode passes through the molecular layer and approaches or enters the granule cell layer (the audible component is transient, and appears to be generated by local neuronal injury discharge, which occurs in phase with the underlying EEG oscillation). A series of studies were conducted to characterize these higher frequency EEG oscillations. They are highest in amplitude (about 0.2 mV) in the upper molecular layer of the fascia dentata, but can be detected over a wide region, including the cortex above CA1, and are highly correlated even between the two hemispheres. They appear in anesthetized as well as waking animals, but in waking animals they fluctuate in frequency from 40 to 70 Hz, while under Nembutal anesthesia they are slower (35-45 Hz) and more regular. They increase in amplitude when an animal is actively exploring its environment. These oscillations appear to be generated both in CA1 and in the fascia dentata. It is hypothesized that the oscillations are caused by "ringing" of a feedforward excitation -- feedback inhibition network of glutamatergic granule cells or pyramidal cells and GABAergic basket cells, and that the timing of the oscillation is set by the decay time constant of GABA-a receptor channels. Supported by NIMH grant MH46823.

556.9

A HIGH-DENSITY MINIATURE MICRODRIVE ARRAY FOR CHRONIC STIMULATION AND RECORDING OF PATTERNED MULTIPLE SINGLE-UNIT ACTIVITY IN THE RAT HIPPOCAMPUS. M.A.Wilson, B.L. McNaughton, K. Stengel*. Department of Psychology and ARL Division of Neural Systems, Memory and Aging, University of Arizona, Tucson, AZ 85724.

Understanding spatial and temporal structure of ensemble activity in the brain has long been a primary objective of neurophysiological research. To record ensemble activity of single units, we have developed an electrode array consisting of 16 independently adjustable microdrives. Each drive houses a recording or stimulation electrode, which can be positioned at any one of 60 penetration sites with 250 μm separation over a 3 mm diameter region. The microdrive array is lightweight and compact enough for use on rats. Independent z adjustment allows optimal placement of individual electrodes for recording single units at various depth-distributed sites in the brain. The use of multi-wire stereotrode or tetrode technology, coupled with multi-dimensional cluster analysis, allows isolation of 3 or more cells at each electrode site. Therefore, this approach provides the means to record simultaneously from 50 or more individual units at sites distributed across the hippocampus. The array has been successfully tested in both acute and chronic preparations in the rat. The development of the microdrive array is being coordinated with the concurrent development of a large-scale multi-channel amplifier/digital signal processing system for waveform acquisition and analysis. The successful application of this system should provide insights into ensemble activity in the hippocampus of the behaving rat and enable the evaluation of systems level hypotheses concerning network coding and function. Supported by NIMH, NSF and the McDonnell-Pew Foundation.

556.10

PROGRESSIVE DECREASE OF BEHAVIORAL MODULATION OF HEAD-DIRECTION CELLS IN THE PATHWAY FROM MEDIAL PRESTRIATE TO RETROSPLENIAL CORTEX. L. L. Chen,[1] L.-H. Lin,[1] B. L. McNaughton,[2] C. A. Barnes,[2] and D. F. Gibbons*[2]. Univ. Colorado, Boulder, CO, 80309[1] and ARL Div. of Neural Systems, Memory and Aging, Univ. Arizona, Tucson, AZ, 85724[2].

Cells tuned to allocentric head-direction (HD) are found in both neocortex (posterior parietal cortex; Chen et al.,1988) and periallocortex (post-subiculum; Taube et al., 1990), suggesting a role of these areas in spatial cognition. Of 662 single units from medial prestriate cortex (Oc2M) and retrosplenial cortex (RSA and RSG) in freely-moving rats, 41 HD cells were identified by the criterion of maintained directional bias in the absence of cues or in the dark (RSG-8.5%; RSA-8.4%; Oc2M-2.7%). In some HD cells, directional tuning was modulated by head angular velocity or linear running velocity; there was a progressive decrease of such modulation from neocortex to periallocortex (Oc2M-57%; RSA-15%; RSG-0%), suggesting that neocortical egocentric movement information may be transformed into an allocentric directional representation in the periallocortex. This supports the hypothesis proposed by McNaughton, Chen, & Markus (1991) that an allocentric direction sense might be associatively maintained by combining angular velocity and orientation signals. Also, the preferred direction of most (78%) HD cells persisted in a spatial working memory-radial maze task, irrespective of the presence of salient visual cues or cue rotations; however, during passive rotations, the directional bias of most HD cells was often unrelated to that in the working memory task, and the tuning of many cells rotated when the salient cues were rotated. Thus, task variables such as stimulus significance, motor efference copy, or attention, are also important determinants of HD cell firing. Supported by NS20331.

556.11

SPATIALLY SELECTIVE FIRING IN HIPPOCAMPAL PYRAMIDAL CELLS IS AFFECTED BY THE MAGNITUDE OF THE VISUALLY ACCESSIBLE ENVIRONMENT. B. Gaese, M. Jung, B.L. McNaughton, C.A. Barnes. Dept. Psychology & Div. Neural Systems, Memory and Aging, Univ. Arizona, Tucson AZ, 85724.

Leonard (Ph.D Thesis, 1990) noted that, in experiments in which animals had extensive experience with the environment beyond the test apparatus, substantially fewer hippocampal pyramidal cells had discernable "place fields" on the apparatus. Leonard also noted that cells of animals with extramaze experience sometimes had apparent place fields as the animal was carried passively through the environment. These observations, however, were subject to a number of possible alternative explanations, due to confounding variables such as task demands and behavior. In this experiment, we inquired whether size of the visually accessible space would affect the distribution of place specific firing within a small circular apparatus. Two identical 76 cm diameter platforms were used, one with a high cylindrical wall that restricted the animal's access to distal visual stimuli. The other cylinder was transparent. The opaque wall was covered with complex patterns. In the transparent condition, similar patterns were mounted on the room walls. The same cells were tested in both conditions. Behavior (random search for food pellets) was the same in both conditions. Mean firing rates in both conditions were highly correlated (.98) and not different; however, the presence of clear place fields differed substantially. In the distal cue condition, 4/49 cells had high spatial selectivity. In the proximal-only case, 15/49 cells were highly selective. A paired t-test on the spatial selectivity scores was significant ($p < .0007$). We hypothesize that, even without direct physical experience, place fields spread out to occupy the known space. Supported by NS20331

556.12

AUDITORY AND HIPPOCAMPAL NEURONAL ACTIVITIES DURING PERFORMANCE OF AUDITORY WORKING AND REFERENCE MEMORY TASKS IN THE RAT. Y. Sakurai. Dept. Psychology, Toyama Med. & Pharm. Univ., Sugitani, Toyama 930-01, Japan.

The auditory cortex (AC) and medial geniculate body (MGB) are related to retention processes and the hippocampal CA1 and CA3 are related to a comparison process in an auditory working memory task (Sakurai, Behav. Neurosci., 104:253, 104:856, 1990). The present study examined more precisely the roles of the auditory and hippocampal regions by recording from single units in them while the rats performed both auditory working and reference memory tasks in a single situation. The working memory task was continuous nonmatching-to-sample with two, high and low tones. The rat pressed a panel (Go) if the tone for the current trial was different than the tone for the preceding trial. The reference memory task was continuous discrimination. The rat made a Go response if the tone for the current trial was high. The apparatus, tone stimuli and the sequence for continuous presentation of the tones were identical between the working and reference memory tasks. Some of the units had mnemonic correlates during tone presentations or delay periods in one of the tasks. These and other behavioral correlates of the units provide more information about how the cortical and limbic regions processes memory. (Supported by Grants 02255208 and 02610040 from the Japanese Ministry).

556.13

EFFECTS OF THE NMDA ANTAGONIST, MK-801, ON SHIFTS IN THE EPSP TO SPIKE RELATIONSHIP FOLLOWING LONG-TERM POTENTIATION OR SHORT-TERM EXPLORATORY MODULATION OF DENTATE GYRUS EVOKED POTENTIALS. S.D. Croll and E. Bostock. Dept. of Psychology, Queens College-CUNY, Flushing, NY 11367.

We examined whether shifts in the EPSP to spike (E-S) relationship occur following induction of long-term potentiation (LTP) or short-term exploratory modulation (STEM) of dentate gyrus evoked potentials, and whether MK-801 had any effects on the observed shifts. Rats were chronically implanted with stimulating electrodes in the perforant path and recording electrodes in the dentate hilus. Stimulus intensity series were used to assess changes that occurred in the E-S relationship following induction of LTP or STEM. In addition, the effects of .10 mg/kg sc MK-801 (injected 30 min prior) on these effects were examined. Following LTP induction, a significant leftward shift in the E-S relationship was observed. This shift was blocked and reversed to a rightward shift following MK-801 administration. Animals receiving no LTP following vehicle or MK-801 administration showed no shift in the E-S relationship. During STEM, a significant rightward shift of the E-S relationship was observed. This effect persisted for at least 20 min. This rightward shift was significantly reduced by MK-801 administration. These findings suggest a role of the NMDA receptor in the E-S relationship shifts observed following induction of either LTP or STEM.

556.14

INDUCTION OF HIPPOCAMPAL PKC EXPRESSION BY SPATIAL DISCRIMINATION LEARNING IS ABOLISHED BY AMYGDALOID KINDLING. H.J.A. Beldhuis,* H. Everts,* E.A. van der Zee* B. Bohus and P.G.M. Luiten, Dept. of Animal Physiology, University of Groningen, P.O. Box 14, 9750 AA HAREN, The Netherlands. (SPON:European Brain and Behaviour Society)

Recent evidence suggests the involvement of protein kinase C (PKC) in the molecular mechanisms by which the hippocampus exerts its influence on information processing. Normal functioning of the hippocampus is of importance for these processes. In the following experiment we investigated the contribution of PKC to spatial discrimination performance. We were interested whether abnormal functional activity indeed impairs spatial learning and the correlated changes appearing in the neural substrate. Adult male Tryon Maze Dull S3 rats (n=16; 300 gr) implanted with bipolar electrodes in the medial amygdala were used 1 week following surgery. They were subjected to a spatial discrimination task (SDT) in a hole board every other day. Daily tetanizations up to 30 days were applied to one group of rats (n=8) at least 2 hours after performing SDT. After completion of the SDT brain sections of 20 μm were processed for immunocytochemistry (ICC) with a monoclonal antibody raised against PKC gamma isozyme. Naive rats were used as controls in the ICC procedure. Learning curves for reference memory (RM) were identical for control and tetanized rats until motor seizure stage 3 had appeared in the tetanized rats. RM of the tetanized rats failed to improve further. PKC expression was increased in the non-tetanized control rats which learned the task, and was mainly located in the granular cell layer. In the tetanized rats PKC expression resembled the pattern as found in the naive controls. The present study shows a correlation between performance in a SDT and PKC expression in the granular cells. These alterations probably reflect molecular processes that provide optimal basis for learning and memory. Disturbance of hippocampal functioning induced by propagated amygdaloid tetanizations prevents these alterations in the neural substrate, and impairs spatial learning. These investigations were supported by the TNO Research Committee on Epilepsy (CLEO A65;HJAB) and Troponwerke (Bayer;EAvdZ).

556.15

DIFFERENTIAL CHANGES IN PROTEIN KINASE C EXPRESSION IN RAT CORTEX, HIPPOCAMPUS AND AMYGDALA DETERMINED BY TYPE OF LEARNING TASK. *P.G.M. Luiten, E.A. van der Zee*, H.J.A.Beldhuis*, B. Roozendaal* and S. Cazaubon**. Dept. of Animal Physiology, University of Groningen, The Netherlands.

Protein kinase C (PKC) is a key enzyme for signal transduction and various neuronal plasticity mechanisms. Changes in hippocampal PKC content have been reported after non-invasive conditioning tests. Here, we examined the expression of PKC in rats after different types of learning tasks: Spatial Orientation (SO), Active (AA) and Passive Avoidance(PA). PKC expression was displayed by immunoreactivity (IR) to a monoclonal antibody against the gamma-isoform. Animals were sacrificed 24h after the last performance. PKC was analyzed in neocortex, hippocampal dentate gyrus (DG) and cornu ammonis (CA), and central (Ce) and cortico-medial (CoMe) amygdala of naive (n=20) and learned rats (SO n=20; AA n=20; PA n=10). Region-specific changes of task-induced PKC expression were consistently observed according to the following semi-quant. pattern:

Structure	SO	PA	AA
Neocortex	++	+++	+
Hipp DG	+	o	o
CA	+++	o	+
Amyg Ce	o	o	--
CoMe	o	o	++

In the neocortex increased PKC appeared in a columnar pattern. The hippocampus revealed high PKC levels in clusters of principal cells in DG and CA, while PKC in the central amygdala was strongly decreased after active avoidance learning. The differential changes in PKC expression indicate region and task specificity of PKC.

556.16

HIPPOCAMPAL RESPONSES DURING JAW MOVEMENT CONDITIONING FOLLOWING PREEXPOSURE TO UNPAIRED STIMULI. R.L. Borgnis*, L.J. Dreshfield, M. Cahall*, & S.D. Berry. Dept. of Psychology, Miami Univ., Oxford, OH 45056.

It has been documented that preexposure to conditioning stimuli delays the acquisition of classically conditioned responses (Lubow, 1989; Solomon & Moore, 1975). Since hippocampal neural activity precedes and models the behavioral conditioned response, we hypothesized that preexposure to both CS and UCS would delay the acquisition of behavioral and hippocampal conditioned responses. Bilateral recordings were made during jaw movement two-tone discrimination training (TTD). Group TTD animals received 8 days of TTD training while Group UNP animals received 5 days of unpaired presentations of the same stimuli and then 8 days of TTD training. In the TTD task, one tone (CS+ 8KHz) signalled a saccharin solution; another tone (CS- 1KHz) signalled no saccharin. The UNP group was presented a quasi-random sequence of two tones and saccharin and therefore received 240 preexposures of each stimulus before TTD training started. While we did not find a significant difference in behavioral acquisition rates during TTD training, our preliminary neural results for the UNP group indicate an inhibition of firing just after tone onset in early trials that disappears by the end of the first day of conditioning.

556.17

OPTICAL MEASUREMENT OF NEURONAL ACTIVITIES OF HIPPOCAMPUS *IN VIVO*. T. IIJIMA and G. MATSUMOTO. Electrotechnical Lab., Tsukuba, Ibaraki 305, Japan.

It has been believed that the hippocampus is composed of multiple "lamella units", each of which includes basically similar excitatory pathway. However, we found the heterogeneity of neuro-circuit in each "lamella unit" through *in vitro* experiments using hippocampal slice preparations.

To reveal the real information processing in hippocampus, it is necessary to obtain the neuronal activities from the multisites of an intact hippocampus. For the purpose we tried the optical recording of neuronal activities from the surface of guinea pig hippocampus *in vivo*. The hippocampal surface was exposed by removing a part of cortex. The neuronal activities were recorded from the alvear surface of the hippocampus stained with a voltage-sensitive dye RH 795. When a stimulus was applied to ipsilateral hippocampus, neuronal activities were obtained from a part of hippocampus as the fluorescence change of about 0.3%.

LEARNING AND MEMORY—PHARMACOLOGY: MONOAMINES

557.1

8-OH-DPAT IMPAIRS RATS' PERFORMANCE IN PASSIVE AVOIDANCE AND WATER MAZE TASKS: ROLE OF POST-SYNAPTIC 5-HT_{1A} RECEPTORS. M.Carli*, L. Cervo*, E.Tatarczynska*, M.T. Tacconi, R. Samanin*. Istituto "Mario Negri", Milan, Italy.

Administered subcutaneously 5 min after the training trial, 100 and 300 µg/kg 8-OH-DPAT significantly increased the retention latencies 24 h later in a one-trial passive avoidance task. A similar effect was found by administering 8-OH-DPAT only before the training trial or before the retention test, but anxiolytic effects or changes in motor activity could be involved in these conditions. At 100 and 300 µg/kg, 8-OH-DPAT significantly impaired choice accuracy in a two-platform spatial discrimination task in the water maze. The effects were potentiated in animals injected intracerebroventricularly with 5,7-dihydroxy-tryptamine (150 µg/20 µl) to destroy serotonin-containing neurons. Administration of 8-OH-DPAT (5 µg/1 µl) in the CA1 region of the dorsal hippocampus, 10 min before the training trial in a passive avoidance task, significantly decreased retention latencies 24 h later. No antidepressant- or anxiolytic-like effects were found with intrahippocampal 8-OH-DPAT. The results suggest that activation of 5-HT_{1A} receptors in the hippocampus interferes with mechanisms involved in learning and memory.

557.2

THE EFFECTS OF 8-HYDROXY-2-(DI-N-PROPYLAMINO)-TETRALIN (8-OH-DPAT) ON DELAYED MATCHING-TO-SAMPLE PERFORMANCE IN RATS.
D. Shurtleff and S.T. Ahlers. Naval Medical Research Institute, Bethesda, MD 20889.

The hippocampus, an area important in working memory, contains a high density of serotonin 5-HT_{1A} receptor subtypes. In the present study the effects of the 5-HT_{1A} agonist 8-OH-DPAT on working memory were evaluated using a delayed matching-to-sample (DMTS) task. Rats (n=4) were required to press one of two front wall levers (right or left) cued by an illuminated light. By pressing the correct lever, they extinguished the light and initiated a delay interval. Following a randomly selected delay of 1, 3, 5, 10, or 15 secs, lights above both levers were illuminated, and the rat was required to choose the previously cued lever. During the delay rats were required to respond on the back wall lever to prevent the use of simple mediating behaviors, such as standing in front of the previously cued lever during the delay. Following saline administration, matching accuracy decreased as a function of delay and ranged from an average of 93% correct at the 1 sec delay to 65% correct at the 15 sec delay. All doses of 8-OH-DPAT reduced matching accuracy relative to saline. The dose of 0.03 mg/kg 8-OH-DPAT (ip) decreased accuracy at the 5, 10 and 15 sec delays, while the 0.1 mg/kg dose decreased accuracy at all delays greater than 1 sec, and the 0.25 mg/kg dose decreased accuracy at all delays. These data indicate that the 5-HT_{1A} agonist 8-OH-DPAT impairs DMTS performance, suggesting the involvement of this receptor subtype in working memory.

557.3

THE SEROTONIN 5HT_{1B} AGONIST TRIFLUOROMETHYLPHENYLPIPERAZINE (TFMPP) IMPAIRS PERFORMANCE ON A DELAYED MATCHING-TO-SAMPLE TASK IN RATS.
S.T. Ahlers[1], J.R. Thomas[1], and J.L. Davis[2]. [1]Naval Medical Research Institute, Bethesda, MD 20889 and [2]Office of Naval Research, Arlington, VA 22217.

Recent research has drawn attention to the interaction of 5-hydroxytryptamine (5HT) and acetylcholine (ACh) in the modulation of memory. Of particular interest are studies demonstrating that specific agonists to the 5HT_{1B} receptor inhibit the release of ACh in the hippocampus. The present study examined whether the 5HT_{1B} agonist TFMPP would impair performance on a delayed matching-to-sample (DMTS) task in rats. Five adult rats were trained on a DMTS task in which they were required to respond on one of two levers (sample stimulus) cued by an illuminated light above the lever on the front wall of a standard operant chamber. Following a random delay of 1, 3, 5, 10 or 15 seconds, both lights were illuminated and rats were required to correctly respond on the lever previously cued. During the delay interval rats responded on a fixed-interval schedule on the back wall lever to prevent position bias or the adoption of simple mediating response patterns. When rats were given saline injections, accuracy decreased from 93% at the 1 sec delay to 71% at the 15 sec delay. Administration of 0.1 mg/kg TFMPP (ip) decreased accuracy at the 3, 10, and 15 sec delays while doses of 0.25 mg/kg and 0.5 mg/kg shifted the delay gradient downward across all of the delays (88% at 1 sec to 55% at 15 sec). These results indicate that short-term or working memory is impaired by the low dose of the 5HT_{1B} agonist. Because higher doses of TFMPP lowered accuracy at the short delays, TFMPP may have impaired acquisition as well.

557.4

5-HT DEPLETION DOES NOT AFFECT SPATIAL MEMORY.
H.P.Davis, C.Hendrix*, D.H.Park, A.C.Towle, & B.T.Volpe. Dept. Psychol. Univ. Colorado, Colorado Springs, CO 80907 and Dept. Neurol. & Neurosci., Cornell Univ. Med. Ctr. @ Burke Inst. Med. Res.

The neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) may play an important role in learning and memory. It has also been suggested that 5-HT abnormalities may mediate some aspects of the cognitive disorders associated with Korsakoff syndrome and Alzheimer's Disease. We evaluated the effect of intracisternally applied serotonergic neurotoxin, 5,7-dihydroxytryptamine (5,7-DHT) on learning and memory in rodents. Three day old rats were treated with pargyline (40 mg/kg, ip) followed by 5,7-DHT (50 ug/pup) and returned to the dam for a month. At 53 days of age the tryptophan hydroxylase (TPH) activity was assayed radioenzymatically with ^{14}C-tryptophan in the dorsal raphe nuclei, caudal brainstem and hypothalamus in some animals. 5,7-DHT treated animals demonstrated marked reduction (90%) in TPH activity compared to matched controls. At 75 days of age, rats were tested on a learning set problem in the Morris water maze for 5-days followed by 30 days of testing in a 12-arm radial maze with 8 of the 12 arms baited. In the Morris water maze, the latency to locate the hidden platform and the swim path length did not significantly differ for 5,7-DHT treated and control rats ($F < 1.0$). Similarly, 5,7-DHT treated rats did not re-enter arms more frequently than control rats (working errors), or enter unbaited arms more frequently (reference errors) ($F < 1.0$). These results suggest that 5-HT does not play a critical role in processing of spatial information.

557.5

METHYLENEDIOXYAMPHETAMINE: EFFECTS ON BEHAVIOR AND SEROTONIN. A.G. Romano, J.A. Harvey, S.E. McMaster* and V.J. Aloyo. Dept. Pharmacol. Med. College of Pennsylvania, Philadelphia, PA 19129.

Previous studies have demonstrated that the hallucinogen methylenedioxyamphetamine (MDA) enhances the acquisition of conditioned responses (CRs) during Pavlovian conditioning of the rabbit's nictitating membrane response. This study examined whether the enhanced acquisition of CRs produced by MDA was related to its known neurotoxic actions on serotonergic neurons or to its ability to produce a presynaptic inhibition of 5-HT neurons. A dose of MDA (10 μmol/kg) that enhanced CR acquisition during a one hour acquisition session also produced an increase in frontal cortex content of 5-HT and a decrease in 5-HIAA that persisted for the first two hours after injection. The turnover of 5-HT as indexed by the 5-HIAA/5-HT ratio was also significantly decreased. These effects were not seen in hippocampus. Immunocytochemical methods failed to reveal any evidence of a neurotoxic action of MDA at doses of 10 or 20 μmol/kg. We suggest that the enhanced acquisition produced by MDA may be due to an inhibition of 5-HT neurons resulting from the presynaptic release of 5-HT. (Supported by NIDA grant DA04944-04).

557.6

SCH 23390 ATTENUATES AMPHETAMINE-PRODUCED ENHANCEMENT OF RESPONDING FOR CONDITIONED REWARD IN RATS. R. Ranaldi* and R.J. Beninger. Dept Psych, Queen's Univ, Kingston, Canada, K7L 3N6.

Dopamine (DA) D1 receptors may play a role in reward. The present study evaluated the effects of a D1 antagonist on amphetamine (AMPH)-produced enhancement of responding for conditioned reward (CR). Rats were exposed to two levers, one producing a 3-s lights-off (LO) stimulus and the other a tone (T), for 5 40-min sessions (S). The levers were removed and during 4 Ss rats were exposed to 80 presentations of LO (on a random time 45-sec schedule) paired with food. Testing consisted of two Ss with the levers again present. Results showed that the saline group increased responding for LO more than for T from pre- to post-pairing, confirming that LO became a CR. AMPH (0.1 to 5.0 mg/kg, ip, 5 min before test Ss) produced a dose-dependent enhancement of responding specifically for the CR. SCH 23390, a D1 antagonist (5.0 ug/kg, sc, 2 h before test Ss), shifted the locus of rise in the AMPH dose-response function toward higher AMPH doses. These results implicate the D1 receptor in reward. (Funded by N.S.E.R.C.).

557.7

DELAYED-NON-MATCH-TO-SAMPLE PERFORMANCE IN THE RADIAL ARM MAZE: EFFECTS OF DOPAMINERGIC AND GABAERGIC AGENTS. T.C. Napier and J.J. Chrobak. Department of Pharmacology, Loyola University Chicago, Maywood, IL 60153

Central dopaminergic transmission has been implicated in memory processes. The delayed-non-match-to-sample radial arm maze (DNMTS-RAM) is a useful tool for studying memory in the rat, allowing for an examination of neurobiological treatments on time-dependent memory processes using within-subject designs. The present experiments examined the effects of several direct acting dopaminergic agents on performance of this task. Pretesting administration of the dopamine agonists, apomorphine (D1-D2; 0.25, 0.5 and 1.0mg/kg), quinpirole (D2; 0.1mg/kg), or SKF38393 (D1;3mg/kg) decreased the rate of responding (seconds/choice), but did not affect any indice of accuracy at a 1hr retention interval. Similarly, posttraining administration of quinpirole (0.1, 0.2, 1.0 and 2.0mg/kg), SKF38393 (0.3, 3.0 and 6.0mg/kg), sulpiride (D2 antagonist; 3, 10 and 30mg/kg) or SCH23390 (D1 antagonist; 0.01, 0.1 and 1.0mg/kg) did not affect accuracy, although quinpirole produced a dose-dependent decrease in response rate, assessed 10 hrs post-treatment. For comparison, pre- and post-training administration of the benzodiazepine chlordiazepoxide (CDP) was also tested and produced dose-dependent (1-5mg/kg) impairments in accuracy at both a 1 and 4 hr retention interval. The effects of CDP are consistent with evidence indicating that GABAergic agents can influence memory processes. In contrast, the present findings indicate that alterations of dopaminergic neurotransmission are not sufficient to alter the memory processes required for accurate performance of this DNMTS-RAM protocol. (Supported by DA05255 to TCN and an Ill. Dept. Public Health grant to JJC).

557.8

ALTERED SOCIAL ODOR PRODUCTION AND OLFACTORY ADAPTATION IN DSP-4-TREATED RATS. C. Cornwell-Jones, J. Green*, D. Krasenbaum*, T. Palfai* and E. Byer*. Behavioral Neuroscience Lab., Syracuse Univ., Syracuse, NY 13244.

Two experiments examined possible reasons for impaired adaptation to new home-cage odors previously observed in DSP4-treated rats. In the first experiment, male rats were injected s.c. with either water or the NE neurotoxin DSP-4 on the day of birth and at the time of weaning were placed in a new bedding type in either small or large cages. Olfactory preferences were measured 10 days later. DSP-4-treated rats housed in small cages preferred the old bedding odor to the new one, and therefore failed to adapt. All other groups showed no preference. In a second experiment, normal infant and juvenile rats preferred soiled nest shavings from control rats, to shavings which had housed DSP-4-treated animals. The results suggest that DSP-4-treated rats produce abnormal odors which, when concentrated in a small area, may interfere with olfactory learning. Supported by NSF grant #DIR-8900931 to CC-J.

557.9

SYSTEMATIC ELIMINATION OF DISCRIMINATION CUES MAY PROVIDE A MODEL OF DEPRESSION. A. L. Beggs, S. Barbay*, and S. Hotard*. Department of Psychology, University of Southwestern Louisiana, Lafayette, LA 70504

A test apparatus designed after that of Harlow was used to present discrimination trials to rhesus monkeys. Two stimulus objects covered food wells that were randomly baited, keeping stimulus object and reinforcement congruent. Initially, the stimulus objects differred in color, size, and form. On subsequent days, stimulus objects were changed so as to eliminate all discrimination cues. On the final day of experimentation animals were injected with imipramine or saline and tested with all discrimination cues available. Although no significant difference was found between groups on test performance, linear trend analysis revealed that fewer correct responses were made as discrimination cues were removed. Also, as discrimination cues were removed, responses to the same side of the tray tended to increase. In a second experiment with the same 12 subjects, with treatment conditions reversed, a new set of stimulus objects was employed but color, size, and form relationships were maintained. L-tyrosine given to subjects orally increased the number of correct responses over control subjects. Again, correct responses decreased as discrimination cues were eliminated and responses to the same side of the tray increased.

557.10

EFFECTS OF D-AMPHETAMINE ON EXTRACELLULAR NOREPINEPHRINE LEVELS IN THE AMYGDALA OF FREELY MOVING RATS AS MEASURED BY *IN VIVO* MICRODIALYSIS. **M.H. Meseches and J.L. McGaugh. Center for the Neurobiology of Learning and Memory and Department of Psychobiology, University of California, Irvine, CA 92717.**

It is well established that retention is influenced by posttraining administration of drugs, such as amphetamine, which affect catecholaminergic systems. Extensive pharmacological evidence suggests that such memory-modulating influences may be mediated, in part, by the release of norepinephrine (NE) within the amygdala. To examine this implication the present experiment examined the effects of peripherally administered amphetamine on release of amygdala NE.

One week prior to the experiment, guide cannulae were unilaterally implanted dorsal to the central nucleus of the amygdala in male Sprague-Dawley rats. The microdialysis probe was inserted 1 mm past the tip of the guide cannula and artificial cerebrospinal fluid (CSF) was perfused (0.5 μl/min) through the probe overnight. Thirty minutes prior to the collection of the first sample, the CSF flow rate was adjusted to 2.0 μl/min, and samples were collected every 15 minutes. After establishing a stable baseline, d-amphetamine (0.2 or 2.0 mg/kg; s.c.) was administered. Samples continued to be collected until NE levels returned to baseline. NE was quantified with HPLC-EC. NE levels increased after systemic administration of d-amphetamine. Previous findings demonstrate that peripheral, but not central, administration of amphetamine facilitates retention of an inhibitory avoidance task. Taken together with our previous findings, these results suggest that the effect of amphetamine on learning and memory may be due to increased noradrenergic activity in the amygdala.

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557.11

ADRENERGIC SYSTEMS AND DNA SYNTHESIS IN THE RAT BRAIN. C.Lamberti-D'Mello[2]*, A.G.Sadile[2], A.Cerbone[2], T.Menna*, C.Buono*, F.Rafti*, S.Amoroso[*4], L.Annunziato[4] and A.Giuditta[3]* (SPON: European Brain and Behaviour Society). IIGB, CNR, Naples; [3]Dip. Fisiologia Generale ed Ambientale; [2]Dip. Fisiol. Umana "F.Bottazzi"; [4]Dip.Farmacol., II Fac. Medic. & Chir., Univ. Naples "Federico II", Naples, I.

To study the role of brain adrenergic systems in brain DNA synthesis (BDS), adult male Sprague-Dawley rats were randomly assigned to one of six groups. The control rats received the radioactive precursor only or the drug's vehicle two hours before the precursor. The experimental rats received at time 0 the α-adrenergic antagonist phentolamine (5 μg), the β-antagonist propranolol (10 μg), the α-agonist phenylephrine (1 μg) or the β-agonist isoproterenol (12.5 μg). All rats were given 2 h later 50μCi ^{3}H-thymidine intraventricularly and sacrificed 0.5h later. BDS was measured in several brain regions by the incorporation of ^{3}H-thymidine into DNA. The vehicle decreased BDS in neocortex, and increased it in the septum, neostriatum, hypothalamus, cerebellum and rest of the brain. It had no effect in the hippocampus and midbrain. In comparison with the group receiving the drug vehicle, α- and β-agonists and antagonists induced several significant effects, depending on the brain region. The results suggest a modulatory role of central adrenergic systems in brain DNA synthesis. **(Supported by CNR and MURST 40% grants).**

557.12

ROLE OF THE DORSAL NORADRENERGIC BUNDLE IN BRAIN DNA SYNTHESIS IN RATS. A.Cerbone[3], A.G.Sadile[3], C.Lamberti-D'Mello[3]*, T.Menna*, C.Buono*, F.Rafti*, S.Amoroso[*4], L.Annunziato[4] and A.Giuditta[2]*, IIGB, CNR, Naples; [2]Dip. Fisiol.Gen. & Amb.; [3]Dip. Fisiol. Umana "F. Bottazzi"; [4]Dip. Farmacol., II Fac. Medic. & Chir., Univ. Naples "Federico II", Naples, I.

To study the role of brain adrenergic systems in brain DNA synthesis (BDS), adult male Sprague-Dawley rats bilaterally lesioned in the dorsal noradrenergic bundle (DNB) by injection of 6-OH-dopamine or were sham-lesioned. One week later they were given at time 0 the α-agonist phenylephrine (1 μg), the β-agonist isoproterenol (12.5 μg), or the drug's vehicle. Two hours later all rats received an intraventricular injection of 50μCi ^{3}H-thymidine, and were sacrificed 0.5h later. BDS was measured in several brain regions by the incorporation of ^{3}H-thymidine into DNA. DNB-lesioned rats showed a higher BDS in the hippocampus, neocortex and hypothalamus which was reversed by the α- or β-agonist. No effect occurred in the septum, neostriatum, cerebellum and remaining brain. The results suggest (i) a tonic inhibitory effect of DNB on BDS in the hippocampus and, indirecttly, in the hypothalamus mediated by α- and β-adrenergic systems; and, (ii) a phasic inhibitory effect mediated by both α- and β-systems in the neocortex, and by the β-system in the cerebellum. Thus, a modulatory role of adrenergic systems on brain DNA synthesis is inferred. **(Supported by CNR and MURST 40% grants).**

557.13

SPATIAL ASSOCIATION OF VASOPRESSIN AND β ADRENERGIC RECEPTORS IN THE LIMBIC SYSTEM OF THE RAT BRAIN.

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Associative learning and memory have been shown to be dependent upon spatial and temporal contiguity of paired stimuli (Pavlov, 1927). This work explores whether principles of associative learning observed at the behavioral level of analysis, such as the requirement of spatial and temporal contiguity of paired stimuli, also occur at the molecular level. To investigate this question we have used the association between the neurotransmitters, norepinephrine (NE) and vasopressin (AVP). Together, these neurotransmitters have been found to influence memory of an associative task (Kovacs et al., 1979). Moreover, vasopressin has been found to potentiate noradrenergic-induced accumulation of cAMP in hippocampal slices (Brinton and McEwen, 1989).

Autoradiographic mapping of the distribution of β [1] NE and V[1] AVP receptors was conducted in the rat brain limbic system. Analysis of these receptor maps was undertaken to determine the spatial association between these two receptor systems. Results of these studies showed a dramatic rostral-caudal gradient for V[1]AVP receptors, with the density of V[1] AVP receptors lowest in the rostral portions of the hippocampus and amygdala and greatest in the rostral-caudal transition regions of the hippocampus and amygdala. β [1] NE receptors showed a rostral caudal gradient distinct from that for AVP receptors. The area showing the greatest degree of spatial association between the two receptor systems occurred in the rostral-caudal transition region of the hippocampus particularly in theposterior rostral portions of the dorsal CA subfields and dentate gyrus and in the ventral CA[1] region and in the amygdalo-hippocampal zone. Supported by NIH grant MH46036 to R.E.B.

NEURAL PLASTICITY IV

558.1

A BRIEF BURST OF ACTION POTENTIALS CAUSES A LONG-LASTING TRANSITION FROM QUIESCENCE TO EITHER PACEMAKER OR OSCILLATORY ACTIVITY IN A SPECIFIC *HELISOMA* NEURON. F.H.Bahls and P.G. Haydon. Dept. of Zoology and Genetics, Iowa State University, Ames, IA 50011.

Brief periods of intense synaptic activity can lead to long-term modifications in the strength of synaptic connectivity, a process thought to underlie learning and memory. We have asked whether brief periods of electrical activity also lead to long-term modifications in neuronal membrane properties which affect the integrative nature of a neuron.

To address this question, single identified neurons B19 of the pond snail *Helisoma* were plated in primary cell culture. After 3-7 days in culture, intracellular recordings revealed that these cells were either electrically silent or exhibited low frequency pacemaker activity. Quiescent cells were depolarized for 1-5s to evoke a brief burst of action potentials. After termination of the depolarizing current pulse, the electrical state of neurons changed from quiescence before the burst to pacemaker activity which could last for greater than 30 minutes.

When depolarizing stimuli were provided for shorter durations which did not induce the long-lasting pacemaker activity, a characteristic decaying afterdepolarization was revealed which we suggest underlies the electrical state change.

Networks of electrically coupled neurons B19 were also investigated. A burst of action potentials evoked a change from quiescence to oscillatory bursts of action potentials throughout the network. These observations indicate that brief periods of electrical activity can cause long-lasting changes in the intrinsic membrane properties which regulate action potential firing patterns and are capable of triggering oscillatory activity in neural networks.

558.2

SHORT AND LONG TERM CHANGES IN SYNAPTIC PHYSIOLOGY IN THE DENTATE GYRUS DURING ASSOCIATIVE LEARNING IN THE RAT. S. Laroche, V. Doyère* and C. Rédini Del Negro*. Dépt de Psychophysiologie, PN2, CNRS, 91198 Gif-sur-Yvette, France.

Synaptic transmission, paired-pulse inhibition and facilitation, and the ability of perforant path-to-dentate granule cell synapses to sustain long-term potentiation (LTP), were examined during classical conditioning. Fifteen rats were chronically implanted with a recording electrode in the dentate gyrus and a stimulating electrode in the ipsilateral perforant path. Animals were submitted to 4 sessions of 8 paired or unpaired tone-footshock presentations. Associative learning was measured by conditioned suppression of lever-pressing for food. Potentials evoked in the dentate gyrus were monitored during and for 1 hr after each conditioning session. Associative learning was accompanied by an increase in the slope of the field EPSP which persisted for more than 20 min after conditioning trials. Only a slight increase in EPSP slope was observed at early stages of pseudoconditioning. This increase however never outlasted trial presentation and was followed by a persistent depression of synaptic transmission. A lasting reduction in spike amplitude was observed in both groups although the effect was more pronounced in pseudoconditioned rats. A paired-pulse paradigm with varying delays and pulse intensities was used to examine alterations in facilitation, early and late inhibition. LTP induced 5 days after learning (6x400Hz-20ms trains) was significantly increased in conditioned relative to pseudoconditioned rats. The main results show that associative learning is accompanied by an increase in synaptic efficacy in the dentate gyrus that outlasts the training episode and a prolonged increase in the capacity of the synapses to support LTP. The differential between synaptic potentiation in conditioning and synaptic depression in pseudoconditioning provides evidence that synaptic strengthening or weakening can be determined by contingencies between environmental events.

558.3

VARIANCE OF PERFORANT-PATH/DENTATE-GYRUS (PP/DG) EVOKED POTENTIAL (EP) MEASURES ACROSS THE I/O CURVE DURING BEHAVIORAL IMMOBILITY AND MOVEMENT IN RATS. E.L. Hargreaves and D.P. Cain. Dept. Psychology, Univ. of Western Ontario, London, Ont., CANADA, N6A 5C2.

Nine EP measures were evaluated across the I/O curve during immobility and wheel walking/running. Measures were 1) EPSP slope: a) average b) maximum c) prior to pop-spike roll-off; 2) pop-spike: d) onset-to-peak amplitude e) peak-to-tangent amplitude f) area; 3) onset/timing: g) latency of pop-spike onset h) duration of pop-spike i) ratio of onset-to-peak pop-spike duration / peak-to-offset pop-spike duration. Adult male rats were implanted with stimulating and recording electrodes in the PP/DG system. After recovery full I/O curves were recorded (10 intensities; 50-1000 Uamps; 10 sweeps/intensity/behavior). Individual sweeps were analysed and the measures averaged and plotted for each animal. Results indicate that the variability of the EP measures was reduced at the higher intensities, except for 1), which showed the opposite trend. Most measures revealed differences between recording during behavioral immobility versus movement, with immobility typically having a steeper slope, higher amplitude, or more synchrony. A reversal of this pattern was seen in g, where EPs recorded during movement had a shorter onset. This difference was also more pronounced in measures 1), than in the other measures, with b showing largest difference. Additionally, this difference was more reliable at the higher intensities, and in 1) was also greater at these intensities. Finally, EP measures displayed more variability during immobility than during movement. Other measures are now being examined. Supported by a grant from NSERC to D.P.C.

558.4

ACTIVITY-DEPENDENT EFFECTS ON INTERNEURON EXCITABILITY REGULATE DENTATE GRANULE CELL RESPONSES TO AFFERENT STIMULATION Steffensen, S.C.*, Moneta, M.E.* and Henriksen, S.J., Research Institute of Scripps Clinic, La Jolla, CA 92037

To further our understanding of dentate inhibitory circuitry we have studied the effects of intra-, inter- and extra-hippocampal stimulation on extracellularly recorded field potentials and single-unit activity in the anesthetized rat. Interneuron cell types were differentiated from dentate granule cells (DGCs) and hilar "mossy cells" by the following criteria: 1) location and morphology (biocytin labeling), 2) driven response and 3) spontaneous activity. DGCs were found approximately 50-75 µm below reversal of the population EPSP (pEPSP), had a perforant path evoked threshold spike latency of 3.5 ms ± 0.2 S.E.M., followed high frequency mossy fiber stimulation (5 pulses, 2.5 ms intervals) and exhibited little bursting activity. Hilar "mossy cells" were encountered approximately 200-400 µm below reversal of the pEPSP, were not driven by perforant path stimulation and fired in bursts with spike amplitude and interval decrement. Interneurons were found appoximately 75-150 µm below reversal of the pEPSP, had a perforant path evoked threshold spike latency of 8.8 ms ± 0.5 S.E.M., did not follow high frequency mossy fiber stimulation, discharged multiply outside the envelope of the evoked field potential and exhibited little bursting activity. The number of interneuron discharges was dependent on PS amplitude and PP interval. Single stimulation of either the ipsilateral hilus, contralateral hilus or the medial septum elicited a single interneuron spike in the dentate, markedly increased perforant path evoked PS amplitudes (210% of control at 80ms conditioning interval) and decreased perforant path PP inhibition. Brief high frequency stimulation of these inputs (3 pulses, 2.5 ms intervals) markedly decreased perforant path PS amplitudes (10% of control) and PP inhibition. The number of perforant path evoked interneuron discharges was unaffected by stimulation of these afferents. The ability of these inputs to increase perforant path evoked PSs and decrease PP inhibition may be explained by their activity-dependent differential effects on interneuron somata and terminals. This work was supported by NIDA DA03665 and DA00131to SJH.

558.5

KINDLING-INDUCED HYPER-RESPONSIVENESS OF NEURONS *IN VIVO*. G.C. Teskey, and R.J. Racine. Department of Psychology, McMaster University, Hamilton, Ontario, Canada L8S 4K1.

The alterations in brain function that accompany kindling may in part be based on an increase in the bursting properties of neurons. Previous reports addressing this possibility have utilized intracellular recordings from either a hippocampal or amygdala-piriform coronal slice preparations. The present study examined the effects of kindling on neuronal response patterns *in vivo*. We also investigated changes in baseline, and spontaneous firing patterns as opposed to discrete evoked or spontaneous burst responses. Male hooded rats were implanted with a stimulating electrode in the amygdala and extracellular recording stereotrodes in the perirhinal and piriform areas. After recovery from surgery, EEG and unit activity was recorded using the Discovery software (Brainwave, CO) which allows the discrimination of single unit responses from a multiunit record. Rats were then kindled to a stage five seizure with either a massed kindling procedure which consisted of several daily 60 Hz, 1 sec, 400 µA stimulations or a rapid kindling procedure which consisted of one 3 Hz, 1000 µA stimulation approximately 60 sec in duration. One, three and seven days following kindling, unit activity was reassessed. Our preliminary observations indicate that kindling caused an increase in the baseline firing rate of units as well as the shorter mean latency inter-spike intervals which reflect the increase bursting response. (Supported by NSERC, and Epilepsy Canada)

558.6

KINDLING INDUCES LONG - LASTING ALTERATIONS IN RAT NEOCORTICAL EVOKED POTENTIALS. C.A. Chapman, G.C. Teskey, and R.J. Racine. Department of Psychology, McMaster University, Hamilton, Ontario, Canada L8S 4K1.

Prior attempts to induce a long-lasting potentiation of evoked responses in the rat neocortex with high frequency stimulation have met with limited success. Here we report that kindling reliably induces a long-lasting alteration of the callosal-neocortical evoked response in the rat. Male hooded rats were chronically implanted with a bipolar stimulating electrode in the corpus callosum and a bipolar recording electrode in the ipsilateral neocortical sensorimotor area. Two weeks later, a series of four baseline input/output (I/O) response curves were recorded over an eight day period. Rectangular 100 µsec biphasic pulses were applied at 12 different intensities to the callosum. The potentials evoked in the neocortex were analyzed for onset latency, onset slope, maximum peak amplitude, and latency of maximum peak. Four groups of animals were tested, one group did not receive kindling stimulation while animals in the other three groups received either one, seven, or twenty-five daily stimulations (600 µA, 60 Hz, 1 sec train duration). Post-manipulation I/O's were recorded for up to two months. Our results indicate that 7 AD's induce an increase in the amplitude of the early component and the appearance of a late component, while 25 AD's induce an apparent depression in the amplitude of the early component and a further enhancement of the late component. Thus, kindling, but not high frequency stimulation, can readily induce a long-term alteration in the evoked callosal-neocortical response. (Supported by NSERC)

558.7

LONG LASTING EFFECTS OF MPOA KINDLING INDUCTION OF SEXUAL BEHAVIOR IN NON-COPULATING MALE RATS. R. Paredes, E. Basañez*, A. Abraham* and V. Romero*. Escuela de Psicología, Universidad Anáhuac. México. D.F.

It has been shown that sexually inactive male rats display sexual behavior when electrical kindling is established in tha medial preoptic area (MPOA). This induction of sexual behavior is not observed when inactive male rats are kindled in the amygdala (AMG). The purpose of the present experiment was to evaluate if the induction of sexual behavior produced by MPOA kindling, has long lasting effects.

Male wistar rats were tested 3 times with receptive females. The animals that did not show any sexual behavior were implanted in the right MPOA. One week after, the animals were retested 3 times with receptive females to assure that they were non-copulators. They received 2 standard kindling stimulus daily and sexual behavior was registered twice a week. Once kindling was fully established, no more stimulus were delivered to the animals and sexual behavior was monitored for 10 months. A non-copulating sham kindling group was observed for the same time. MPOA kindling induced sexual behavior. The rats consistently displayed the bahavior through the 10 month period. Some of the sham kindling group rats occasionally displayed sexual behavior. These results suggest that the neuronal changes produced by kindling induce a permanent change in sexual behavior.

558.8

PATHWAY SPECIFICITY OF ISOPROTERENOL-INDUCED PLASTICITY IN THE DENTATE GYRUS: REPLICATION AND EXTENSION. M. R. Pelletier, R. D. Kirkby and M. E. Corcoran. Dept. of Psychology, U. of Victoria, Victoria, BC, V8W 3P5, Canada.

Previous research has demonstrated that application of β-noradrenergic agonists in vitro can produce long-lasting potentiation (LLP) of field responses evoked in the dentate gyrus (DG) by single-pulse stimulation of the medial perforant path (MPP) and long-lasting depression (LLD) of responses evoked by stimulation of the lateral perforant path (LPP) [Dahl & Sarvey, PNAS, 1989]. We attempted to replicate and extend these important observations.

Transverse hippocampal slices from male hooded rats were placed in an interface chamber. After stable baselines of responses evoked in the molecular layer of DG by stimulation of MPP or LPP were obtained, (-) isoproterenol (ISO) at 1 µM was bath-applied for 30 min, followed by a 30-min wash. High-frequency stimulation (HFS) was then applied to the afferent path. Other slices were subjected to a counterbalance condition, in which HFS was followed by bath-application of ISO.

ISO produced LLP of the MPP-evoked response and LLD of the LPP-evoked response. Subsequent HFS produced a further increase of the MPP-evoked response and recovery to baseline of the LPP-evoked response. In the counterbalance condition, HFS produced LLP of both MPP and LPP-evoked responses. Subsequent application of ISO further potentiated the MPP-evoked response but had minimal effects on the LPP-evoked response.

These results confirm the pathway specificity of the effects of ISO on responses evoked in the DG. They also suggest that the effects of ISO and HFS on responses evoked by MPP stimulation are additive.

558.9

ALTERING THE IMPACT OF EARLY REARING ON THE RAT'S SPATIAL MEMORY WITH PRE- AND POSTNATAL CHOLINE SUPPLEMENTATION. R.C. Tees, E. Mohammadi* and T.J. Adam*. Dept. of Psychology, Univ. of British Columbia, Vancouver, B.C. V6T 1Z4, Canada

Recent work suggests that pre- and postnatal choline chloride dietary supplementation during development results in long-term facilitation of working and reference memory in rats (Meck et al., 1989). Stimulation history has also been reported to have long-term effects on spatial competences in adult animals.

The purpose of the present experiment is to examine the effects of pre- and postnatal supplemental choline on the impact of early rearing regimes including dark rearing (DR), and complex rearing (CR). Acquisition and retention of spatial and nonspatial water-based discriminations in CR and DR rats were examined. Subsequently, these rats were given atropine sulfate and re-evaluated.

Results indicate that both pre- and postnatal choline supplementation enhanced performance on working memory in the spatially oriented task, for rats reared in any environment evaluated. A main effect of enrichment was also obtained. Further, atropine administration was demonstrated to impede the efficacy of working memory in all groups tested. These results suggest that neonatal choline supplementation enhances working memory performance in the adult rat.

558.10

REPEATED INJECTIONS OF SCOPOLAMINE HYDROBROMIDE AMELIORATE THE BEHAVIORAL EFFECTS OF SUBSEQUENT SEPTAL LESIONS. A.M. Schneider*, E. Thomas, W. Martin*, M.G. Folwell*, A. Payne*. Depts. of Psychology, Swarthmore College Swarthmore, PA 19081 and Bryn Mawr College, Bryn Mawr, PA 19010.

The relation between drug tolerance and recovery of function following brain damage was examined. Rats were tested in a standard passive-avoidance two-compartment box. Ordinarily rats will step from a small lighted compartment to a large dark compartment with short latencies. Step-through latencies are increased by either a single injection of scopolamine hydrobromide or bilateral lesions in the septal area. The increased latencies seem to be the result of increased fear of the new compartment and, therefore, appear to be a form of neophobia.

Rats given repeated injections of scopolamine hydrobromide (2 mg/kg), 2 injections per day for 3 days, show tolerance to the neophobia produced by the drug and if the injections are given prior to surgery, show tolerance to the neophobia produced by the lesions. The data are taken as evidence that tolerance to scopolamine and recovery from septal lesions are mediated by the same mechanism, and if that mechanism is in place before the brain is lesioned, the deleterious effects that ordinarily result from the lesion are ameliorated.

558.11

BEHAVIORAL TOLERANCE (BT) TO MORPHINE IN SPINAL RATS. C. Advokat and C. McInnis*. Psychol. Dept., LSU, Baton Rouge, LA. 70803

Behavioral tolerance refers to the fact that many behavioral drug effects are significantly reduced if animals have practiced the response before they receive the drug. We have studied the neural basis of BT by using the spinal, nociceptive tailflick (TF) reflex. TF pretreatment reduced the analgesic effect of morphine and this decrease was retained for at least 1 day after spinalization. To clarify the stimulus-response conditions required for BT, separate groups of intact rats were preexposed to either thermal TF, mechanical tail pinch (TP), or thermal hot plate (HP) nociceptive stimulation, pseudo TF trials or habituation to the experimental context. All rats were spinalized 24 h later and tested with the TF the next day, at 30, 60, 120 and 180 min after 3.0 mg/kg, sc morphine. There was an overall decrease in the antinociceptive effect of morphine in the experimental groups relative to nonpretreated spinal rats, but this was only significant for the TF, TP and HP conditions. The data suggest that BT to morphine, assessed with the TF, in acute spinal rats, requires preexposure to a nociceptive stimulus but not specific pretreatment with the thermal TF test.

Supported by PHS Grant 02845

558.12

EFFECT OF ENRICHED ENVIRONMENT AND MORRIS WATER MAZE TRAINING ON BRAIN CHOLINE ACETYLTRANSFERASE ACTIVITY. G.A. Park, B.A. Pappas, S. Murtha and A. Ally*. Dept. of Psychology, Carleton University, Ottawa, Canada, K1S 5B6.

Weanling Long-Evans rats were raised in an enriched (ENR) or impoverished (IMP, standard caging, two rats per cage) environments for 50 days. Subsequently, half of the rats underwent testing in the Morris Water Maze to assess spatial learning. The maze-trained and the untrained rats were then assayed for choline acetyltransferase (ChAT) activity in frontal and remaining cortex, hippocampus and caudate as determined by *de novo* acetylcholine (Ach) synthesis using tritiated acetyl-coenzyme A as a precursor.

The ENR-reared rats learned the platform location in the maze significantly faster than did the IMP-reared rats. The ENR-reared rats showed significantly elevated ChAT activity in the caudate and this was not further affected by maze training. Maze training did increase ChAT activity in the caudate of IMP-reared rats, however. Maze-training also increased ChAT activity in the frontal cortex and hippocampus, but only for ENR-reared rats. Thus ENR rearing significantly and enduringly augmented Ach synthesis in the caudate. Maze training caused a lesser increase in Ach synthesis in IMP-reared rats. ENR rearing also seemed to prime the hippocampus and frontal cortex such that these areas show increased Ach synthesis after maze experience. Brain Ach synthesis is affected by environmental complexity.

558.13

ABSENCE OF BEHAVIORAL LONG TERM POTENTIATION IN RATS LEARNING A WATER MAZE TASK. **D.P. Cain, E.L. Hargreaves, Z. Dennison, and F. Boon. Dept. Psychology, Univ. of Western Ontario, London, Ont., CANADA, N6A 5C2.**

In an effort to evaluate the occurrence of behavioral LTP we studied the electrophysiological properties of the perforant path-to-dentate gyrus (PP/DG) pathway in rats before and after they learned a water maze task that is known to be disrupted by lesions of this circuit. After implantation of a stimulating electrode into the PP and a recording electrode into the hilus of the DG averaged responses to single pulses applied to the PP yielded baseline input/output (I/O) curves. All stimulation was delivered while the rats were immobile. The rats then learned to escape onto a hidden platform in a Morris water maze. Four blocks of 4 trials were administered over a period of 6 hr. All rats mastered the task as assessed by learning curves and probe trials. Post training I/O curves were identical to baseline I/O curves as assessed through EPSP maximum slope and population-spike peak to tangent amplitude. Trains of high-frequency pulses were applied subsequently through the PP electrode and these induced significant and enduring electrophysiological LTP, indicating that the stimulation/recording arrangements were capable of detecting plasticity in this circuit. We conclude that under the conditions of this study normal spatial learning occurred in the absence of behavioral LTP in the PP/DG circuit. Supported by a grant from NSERC to D.P.C.

559.1

IDENTIFICATION OF THE PATTERN OF PENIS INNERVATION IN THE POND SNAIL *LYMNAEA STAGNALIS*. A.ter Maat, A.W.Pieneman*, Y.A. van Duivenboden* and R.F. Jansen. Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam.

Copulation behavior in the hermaphrodite pond snail *Lymnaea stagnalis* is a 2-4 hr sequence of behavioral acts of variable durations that leads to intromission of the penis and subsequent transfer of semen. The latter behavior has a fixed duration of 36 ± 4 min at 20° C. Male copulation behavior can be induced by isolation of the animals for over two days, in which case copulation is reciprocal, i.e. the animals alternate between male and female roles. The neurons that project in the penis nerve have been identified using cobalt and nickel-lysine backfills. Neurons in three major clusters were stained. Combined intracellular and extracellular recordings confirmed this finding. The clusters are located as follows: 1. the I cluster of the right pedal ganglion; 2. the anterior lobe of the right cerebral ganglion; 3. the ventral lobe of the right cerebral ganglion The neurotransmitter content of these clusters is at least partially known: they contain serotonin, and the peptides APGWamide and FMRFamide, respectively. We are currently investigating the roles of these neurons in penis eversion and retraction.

A pair of interneurons located in the pedal ganglia has been identified. This pair modulates the electrical activity in two of the aforementioned clusters (ventral lobe and I cluster). In addition, they cause strong excitation of the metacerebral giant cells involved in feeding and weak excitation of the caudodorsal cells, a command system for egg laying. Based on these effects, they were termed small wide acting pedal neurons (SWAP neurons).

In order to analyze the functioning of the network outlined above we have started to record from its constituents in the freely behaving animal.

559.2

IN VIVO REGISTRATION OF SPIKING IN THE METACEREBRAL GIANT NEURONS DURING EGG LAYING RELATED BUCCAL RASPING IN THE POND SNAIL, *LYMNAEA STAGNALIS*. R.F.Jansen, A.W.Pieneman* and A. ter Maat. Faculty of Biology, Vrije Universiteit, Amsterdam, The Netherlands.

The egg laying behavior of the pond snail is composed of three phases: Resting, Turning and Oviposition. One of the characteristics of egg laying behavior is the almost total absence of buccal rasping in the 1st, Resting phase (< 50 rasps/10 min.), immediately followed by vigorous rasping (150/10 min.) in the 2nd and 3rd, Turning and Oviposition phases. As shown earlier, this rasping is caused by the coordinated action of feedback from the ovotestis and neuropeptides released from the caudodorsal cells prior to egg laying, and apparently serves to clean the substrate. We therefore investigated the electrical activity of the metacerebral giant cell (MCG), an interneuron that modulates the feeding apparatus, in relation to egg laying. Animals were equipped with fine wire electrodes on one or both the MCG's, and both behavior and electrical activity were recorded. After the experiment, the recorded spiking activity was identified as coming from the MCG by recording synchronous spiking in the contralateral MCG. The dramatic change in the rate of buccal rasping during the transition from Resting to Turning phase behavior seen in normal animals was, surprisingly, not reflected in the rate of spiking of the MCG. After oviposition had ended, however, a significant drop in MCG spiking occurred. This low level of spiking was then maintained for many hours. Since these experiments were done in the absence of food stimuli, we became interested in the interaction between food stimuli and egg laying. In the presence of a chemical stimulus (sucrose, 3.4 gr/l) control animals rasp at high rates (125/10 min) that are maintained for many hours. In egg laying animals, however, the buccal rasping drops to approx. 40/10 min immediately after the projected start of the first, Resting phase of egg laying. It appears that buccal rasping during egg laying is first suppressed, and then increased in the 2nd and 3rd phases. We are currently investigating the role of the MCG in this suppression of rasping in the presence of food stimuli.

559.3

FLUORESCENT DYE LABELING OF STATOLITH AND STATOCONIA IN Aplysia californica. H.A. Pedrozo*, and M.L. Wiederhold. Dept. of Otolaryngology, The University of Texas Health Science Center at San Antonio, TX. 78284-7777.

The gravity receptor organs in Aplysia californica are bilateral paired statocysts. These contain dense statoconia within a fluid filled cyst. The gravitational pull on these calcium rich statoconia causes their interaction with the mechanosensory cells forming the wall of the statocyst. The use of calcium binding fluorescent dyes enables us to more easily determine structural differences between the single statolith with an amorphous interior, present in larval Aplysia and the multiple statoconia with lamellar calcification seen in the post-metamorphic Aplysia 2.0 mm in length or larger. It also allows us to determine the rates of calcium deposition and stone formation. Two fluorescent dyes are employed, tetracycline hydrochloride and xylenol orange. Pluricolor fluorescent labeling is achieved by alternating 24 hr pulsing periods with tetracycline and xylenol orange,with 24 hrs between pulses. The optimum pulsing concentration for tetracycline hydrochloride is 300 mg per liter of sea water for larval Aplysia and 100 mg per liter of sea water for newly metamorphosed Aplysia. For xylenol orange, the optimum concentrationis 20 mg per liter of sea water for both larval and newly metamorphosed Aplysia. Despite structural differences between statolith and statoconia, both can be labeled with the same calcium binding dyes. Supported by NASA Space Biology Program.

559.4

EARTH-STRENGTH MAGNETIC FIELD STIMULI THAT ELICIT SPIKING IN AN IDENTIFIABLE MOLLUSKAN NEURON. Kenneth J. Lohmann and A. O. Dennis Willows. Dept. of Biology, University of North Carolina, Chapel Hill, N.C.; Dept. of Zoology, University of Washington, Seattle, WA 98250.

The marine mollusk *Tritonia diomedea* can orient to the magnetic field of the earth (Lohmann and Willows, *Science*, 235: 331-334 (1987)). Two large, identifiable neurons (L Pe 5 and R Pe 5) in the brain of *Tritonia* respond with enhanced electrical activity to changes in earth-strength magnetic fields (Lohmann et al., in press, *J. Exp. Biol.*). These cells may be components of a neural circuit underlying detection of the geomagnetic field or orientation to it. To study the magnetic field parameters that best elicit responses from L Pe 5 and R Pe 5, we have constructed a computer-controlled coil system consisting of three Rubens cube coils arranged orthogonally; two coil systems control the horizontal component of the magnetic field while a third controls the vertical component. An earth-strength field can therefore be generated in any direction or rotated to a new position at variable rates during experiments. Responses of L Pe 5 to changes in horizontal fields, vertical fields, field intensity, and inclination angle are compared.

559.5

ORIENTATION TO WATER-FLOW IN *TRITONIA*: BRAIN STRUCTURES ASSOCIATED WITH RHEOTAXIS. J.A. Murray, G. Brown, and A.O.D. Willows. Friday Harbor Laboratories, Friday Harbor, WA 98250.

The nudibranch gastropod *Tritonia diomedea* orients to and moves into water-flow (rheotaxis). Field observations indicate that the slugs orient to tidal flow (R=.41, p<.001; n=80). Additionally, flow-tank experiments show that orientation to current reduces hydrodynamic drag. We examined the neural basis of this rheotaxis behavior by recording intracellularly from the brain, in a semi-intact animal preparation. Neurons of the pedal and cerebral ganglia responded to water-flow directed to the oral veil and rhinophore sheaths with bursts of EPSPs and action-potentials (Murray & Willows 1990, SNS Abstracts, 313.3). The activity of the functional motoneuron Pd3 ipsilateral to the flow stimulus was greater than that of its contralateral homologue.

Intact slugs oriented in a sea-water flume with flow rates only 1 cm/s (Rayleigh, p<.001). When Left Cerebral Nerve#1 is cut, the slug continued to orient, and it oriented significantly when all the left cerebral nerves (1-4) were cut. Cutting LPdN3 reduced turning to the left but orientation remained strong. Initial experiments indicated that when all 8 brain nerves (L&RCeN1-4) innervating the head were cut, the slugs' ability to orient to flow was diminished (Rayleigh, .10>p>.05; n=6). Future experiments will determine the respective contributions of each of these cerebral nerves.

We successfully filled the somata of neurons with neurites in each of the brain's nerves using a nickel filling and rubeanic acid developing process which is simpler than cobalt fills with silver intensification. The nerve fills may help us to identify neurons involved in rheotaxis, if we find that certain nerves are more important than others in their contribution to this behavior.

Supported by N.I.H. grant NS22974 to A.O.D.W. and N.I.M.H. fellowship MH10006-02 to G.B..

559.6

PROTEIN SYNTHESIS BY INDIVIDUAL, PUTATIVELY HOMOLOGOUS NEURONS IN DIFFERENT SPECIES OF NUDIBRANCH MOLLUSCS. C.M.F. Lohmann* and A.O.D. Willows. Friday Harbor Laboratories, 620 University Road, Friday Harbor, WA 98250.

Morphological and behavioral considerations suggest that specific neurons in different species of sea slugs may be evolutionarily homologous. We have further investigated this possible homology by using gel electrophoresis to study protein synthetic patterns of several identifiable neurons from four nudibranch species. For example, all nudibranchs that we have examined possess a large, white (under epi-illumination) neuron in the pedal ganglion similar to Pedal 5 of *Tritonia diomedea*. Analysis of Pedal Neuron 5 and its putative homologs shows that all produce one major and several minor bands near 45-55 kD. The results thus indicate that neurons of similar morphology have similar protein banding patterns and support the hypothesis of evolutionary homology. These findings, coupled with existing behavioral and neurophysiological data, imply that 1) similar behavior in different species may be mediated by homologous neurons and 2) differences in behavior may involve different uses of the same neurons.

559.7

A NEW SENSE ORGAN IN CEPHALOPODS: SENSORY HAIR CELLS ON THE NECK OF THE SQUID *LOLLIGUNCULA BREVIS*. T. Preuss* and B.U. Budelmann. Marine Biomed. Inst., Univ. Texas Med. Branch, Galveston, TX 77550.

Decapod cephalopods, such as cuttlefish and squids, are able to move (pitch, roll and yaw) their head relative to the body. However, no data yet exist on possible receptors that detect such movements. Using morphological techniques (LM, TEM, SEM, and cobalt tracing), we describe, for the first time, a receptor organ that possibly serves as a neck proprioceptor organ.

In the squid *Lolliguncula brevis*, epidermal hair cells exist on the dorsal side of the neck underneath the nuchal cartilage, close to the animal's midline on either side (left and right) of the nuchal crest. The cells can be divided into 4 groups, each with 20-30 presumably primary sensory cells. Each cell carries between 30 and 300 kinocilia, arranged in an elongated ciliary group, with a morphological polarization parallel to either the longitudinal or the transverse axis of the animal. The hair cells are innervated by a branch of the postorbital nerve. Cobalt tracing shows that all hair cells project ipsilaterally (and perhaps contralaterally) to one brain area only, the ventral part of the ventral magnocellular lobe. This area receives afferents from the equilibrium receptor systems as well.

In summary, the data indicate that the epidermal neck hair cells are sensory in nature and presumably serve to monitor the position of the head relative to the body.

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559.8

TEMPORAL SELECTIVITY AND RESOLUTION IN THE AUDITORY SYSTEM OF THE GREEN TREEFROG (*HYLA CINEREA*). S.E. Allan* and A.M. Simmons. Psychology Department, Brown University, Providence, RI 02912

Differences in rate of amplitude modulation (AM) carry biologically-relevant information for many species of frogs, and these may be reflected in the temporal resolving power of their central auditory system. Multi- and single-unit activity were recorded in the torus semicircularis (TS) of the green treefrog in response to AM noise stimuli varying in rate and depth of modulation. Selectivity to AM rates and the ability to resolve depth of AM were examined by changes in overall firing rate and synchronization to the modulation frequency. Different types of filter functions for AM were found. These include temporally-tuned units with best AM rates of 50 Hz and 300 Hz, which correspond to the natural AM rates of the species' vocalizations. A characteristic dip in sensitivity at 100 Hz, the AM rate of a sympatric species' call, was also apparent. Comparable threshold functions were found using a psychophysical technique. Temporal modulation transfer functions were low-pass in shape, with thresholds reaching 100% depth at AM rates above 100 Hz. The minimum integration time calculated from these functions ranged from 1.0 - 2.5 ms and the difference limens for detectable amplitude changes at the best AM rates varied between .136 dB - 1.09 dB.

(Supported by NIH, NSF, and DRF)

559.9

AMPLITUDE MODULATION IS AN ATTRACTIVE FEATURE OF *X. LAEVIS* SONG. M.L.Tobias, R.E.Bivins*, S.Nowicki†, D.B.Kelley. Dept. Biol. Sci., Columbia Univ., N.Y., N.Y. 10027, †Dept. Zool., Duke Univ., Durham, N.C. 27706

Male *Xenopus laevis* frogs attract females with rapidly alternating fast and slow trills known as mate calls. The clicks that make up the fast trill portion become progressively louder whereas the clicks of female-typical release calls - ticking - are not amplitude modulated. Sex differences in synaptic efficacy at the laryngeal neuromuscular junction contribute to amplitude modulation by regulating the number of contracting muscle fibers. We thus wished to determine whether amplitude modulation is critical to the attractiveness of male song. An artificial mate call in which the fast trill portion was of uniform amplitude equal to the loudest click was constructed. This modified call was presented to hormone-primed, sexually receptive females in alternation with an unmodified mate call and white noise. The initial angle of turn - towards or away from the speaker- was used to measure the female's phonotaxic response. Receptive females turn towards normal mate calls (positive phonotaxis) and away from white noise (negative phonotaxis). Females are ambivalent to amplitude modified calls; turning towards or away from the call at equal frequencies. The data suggest that amplitude modulation of mate calls - particularly the soft onset of the fast trill - is an important attractive feature for the female. Because amplitude modulation reflects the weak synapses at male neuromuscular junctions, females appear capable of responding appropriately to a sexually dimorphic synaptic property.

Supported by NS 23684 and DC 00402†.

559.10

ACOUSTIC NICHE PARTITIONING BY THREE SPECIES OF NEOTROPICAL FROGS. B.E. McClelland, W. Wilczynski, J.M. Wicks, and A.S. Rand. Dept. of Psychol., Univ. of Texas, Austin, TX, 78712, and Smithsonian Tropical Res. Inst., Balboa, Panama

Three closely related frog species, Hyla ebracatta, H. phlebotes, and H. microcephala, breed sympatrically and can be found in mixed choruses. We investigated acoustic communication in these species in a mixed chorus in Gamboa, Panama. All have high frequency, broad-band advertisement calls, but mean dominant frequency differs among the species: H.e.: 3.2 kHz; H.p.: 3.8 kHz; H.m.: 6.1 kHz. H.e. and H.p. calls also differ in temporal structure; H.p. and H.m. calls are similar in this respect. Mean basilar papilla tuning (determined by midbrain recordings) also differs among the species: H.e.: 2.6 kHz; H.p.: 2.8 kHz; H.m.: 5.3 kHz. Thus these species partition the acoustic channel used for communication in the frequency domain and, in the two species (H.e. and H.p.) where BP tuning predicts reception of both calls, in the temporal domain as well. Morphometric studies indicate volumetric changes in the middle and inner ear cavities coincide with tuning differences. Both are largest in H.e., intermediate in H.p., and smallest in H.m., as would be expected if these features contribute to the resonance of the ear. Additional studies are underway to determine if call differences are reflected in species differences in laryngeal structure. Supported by the Smithsonian Institution and NIMH R01 MH 45350. We thank D.L. Greene for assistance.

559.11

ACOUSTIC MODULATION OF HYPOTHALAMIC FUNCTION IN HYLA CINEREA. J. D. Allison. Dept. of Psychology, Univ. of Texas, Austin, TX, 78712.

Previous studies reveal that the preoptic area (POA) and ventral hypothalamus (VH) of anurans receive input from thalamic and midbrain auditory nuclei. We examined the responses of POA and VH units to acoustic stimuli using standard electrophysiological techniques. Spontaneously active units were isolated and then stimulated for 2.5 min. with eigher conspecific mating calls (MC) or white noise (WN) pulses followed by a 2.5 min. "No stimulus" period. The protocol was repeated. Some cells were presented with both stimulus protocols. PST histograms were generated to examine spike rates and patterns. Differences in spike rates between stimulus conditions were then assessed using the Tukey HSD *a posteriori* test of significance. Thirty-eight POA units were examined, 10 (26.3%) of which increased their firing rate in response to the MC stimulus. Nine (39.1%) of 23 VH units examined increased their firing rate in response to the MC stimulus. Only 1.3% (2/15) of the POA units and 1.1% (1/9) of the VH units responded to the WN stimulus. Thus ε biologically important social cue (the mating call) modulates the activity of some POA and VH neurons. This work was supported by NIMH grant R01 MH45350 to Walter Wilczynski.

559.12

MORPHOLOGICAL CORRELATES OF AUDITORY SENSITIVITY IN ANURAN AMPHIBIANS. **J. H. Fox. Department of Psychology, University of Texas, Austin, TX 78712.**

Comparative studies involving anuran audition are often limited by the paucity of threshold data that have been reported. Certain well documented morphological characteristics may correlate with auditory sensitivity and may therefore be useful as sensitivity indices, enabling comparative work with more diverse taxa. In this study, a database was compiled, and the relationships were examined between snout-vent length (SVL), tympanum area (TA), amphibian and basilar papilla hair cell populations (HCAP and HCBP), and auditory sensitivity measures derived from "cochlear" potential (CP), eighth nerve (8N), and torus semicircularis (TS) audiograms. Sensitivity to white noise was estimated on the basis of reported audiograms and was subdivided into amphibian papilla (AP) and basilar papilla (BP) frequency range components. SVL, TA, and HCAP correlate positively with all sensitivity measures in the AP range. In the BP range, both SVL and TA correlate positively with 8N and TS sensitivity but not with CP sensitivity, and HCBP correlates with no sensitivity measure. Partial correlational analysis indicates that only TA significantly predicts auditory sensitivity, relating more strongly to BP than to AP sensitivity. This relationship probably derives from the direct proportionality between TA and collected acoustic force, also characteristic of other tympanic devices such as microphones. The differential relationship of TA with AP and BP measures is consistent with the differential reliance of these organs on tympanic sound conduction. The correlations involving HCAP, HCBP, and SVL probably derive secondarily from complex interactions with TA.

559.13

SPECTRAL AND TEMPORAL CUES PRODUCED BY THE EXTERNAL EAR OF THE BIG BROWN BAT, Eptesicus fuscus AND THEIR RELEVANCE TO SOUND LOCALIZATION. J.M. Wotton*, T. Haresign and J.A. Simmons. Department of Psychology and Section of Neurobiology Brown University, Providence, RI 02912.

Sound pressure level recordings were made from isolated ears of Eptesicus fuscus to determine the binaural and monaural acoustic cues used in localization. The transfer functions and impulse responses of the external ears were calculated for sound sources located at many different azimuths and elevations. Temporal and spectral cues changed systematically with the position of the sound source. Measurements made after the removal of the tragus show that monaural cues for elevation appear to be produced by pinna-tragus reverberations. The information available to the bat and represented in the transfer functions and impulse responses has been shown to have behavioral relevance for the bat in sound localization experiments.

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559.14

RESPONSE PROPERTIES OF NEURONS IN THE BIG BROWN BAT, Eptesicus fuscus, TO CLOSED FIELD DICHOTIC STIMULATION. T. Haresign, M. Ferragamo*, and J. Simmons. Hunter Laboratory, Brown University, Providence, R.I. 02912

Extracellular recordings were made from the inferior colliculus (IC) and auditory cortex of Eptesicus fuscus. Using a closed field dichotic stimulation system, units were tested to determine their best frequency (BF), latency, interaural intensity difference (IID) tuning, and interaural time difference (ITD) tuning. Units were functionally classified according to their binaural response properties. The most common type of IID tuning among the units tested is EI tuning (contralateral ear excitatory, ipsilateral ear inhibitory). In the IC there is a wide distribution of BF's and latencies, suggesting that one of the functions of the IC may be to act as a latency disperser. Many of the EI units are sharply tuned for frequency and show a sharp boundary between excitation and inhibition in their IID tuning curves. These units should be very sensitive to spectral differences between the ears.

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559.15

A COMPUTER SIMULATION OF THE UNIFIED PERCEPTION OF TIME AND FREQUENCY BY THE BIG BROWN BAT. P.A Saillant*, J.A. Simmons and S.P. Dear. Dept. of Psychology and Section of Neurobiology, Brown University, Providence, RI 02912

A newly developed Spectral Correlation And Transformation algorithm (SCAT) has demonstrated the ability to reproduce the performance of the big brown bat, *Eptesicus fuscus*, in behavioral experiments designed to measure glint resolution, phase sensitivity and amplitude latency tradeoff. The SCAT algorithm is constructed around known physiological properties of inner hair cells, delay-tuned neurons and other components of the bat auditory system, and in addition, incorporates novel, physiologically motivated, processing elements. Like *Eptesicus*, the SCAT algorithm can detect the separation between each pair of glints in targets separated by as little as 4 μs. Jitter experiments in which the phase of the returning echo is inverted by 180^0 show that bats perceive the inverted crosscorrelation function of the emission and echo. This effect is reproduced by SCAT as a result of the temporal properties of delay-tuned FM neurons responding to dominate peaks of the group delays in each filter bank. Amplitude-latency trading experiments, in which the temporal and spectral contributions to complex image formation can be dissociated, have also been replicated by SCAT showing that the novel transforms it employs can help facilitate our understanding of auditory perception in bats. (Work supported by NIMH grant MH18882, ONR grant N00014-89-J-3055 and NIMH grant MH19118)

559.16

HUNTING AND ECHOLOCATION BEHAVIOR IN THE FISHERMEN BAT NOCTILIO LEPORINUS IN THE FIELD. H.-U.Schnitzler, E. Kalko*, I. Kaipf* and A. Grinnell. Dept. Animal Physiology, Univ. of Tübingen, D-7400 Tübingen, Fed. Rep. Germany.

We photographed foraging *Noctilio leporinus* in Costa Rica and recorded their echolocation signals simultaneously. In the flapping search flight, the bats fly about 50 cm over water emitting groups of 2-3 CF and CF-FM pulses of up to 14 ms long. In the extended leg search flight, the bats fly only a few cm over water with their legs straight back. Eventually, they lower their feet to dip into or to rake through the water emitting groups of short CF-FM signals interspersed with 1-3 longer CF and/or CF-FM signals. The bats use three hunting strategies. The pointed dip is directed to a single target to scoop it out of the water. At pointed dips out of the extended leg search flight, the bats do not react to the target in their echolocation behavior. However, at pointed dips out of the flapping search flight, which we evoked with a water jet simulating a jumping fish, the bats reacted with a reduction in pulse duration and interval, finally producing short FM signals. At the directed rake, the bats rake through patches of many jumping fish with no reaction in their echolocation behavior. Therefore we think that they do not localize a single fish, but get enough information to direct their flight to the area with high jumping activity. The random rake strategy is used if there are no jumping fish around. The bats make very long rakes in areas which previously had many jumping fish. As the bats have no way of knowing if fish are still there, we think that they catch fish only by chance. The bats need 50-200 dips or rakes to catch a fish.

(Supported by DFG/SFB 307)

559.17

LEARNED RELEVANCE OF SPECIES-SPECIFIC VOCALIZATIONS AND THEIR ^{14}C-2-DG PATTERNS IN AUDITORY CORTEX IN THE DEGU (OCTODON DEGUS)

S. Braun*, D. Bonke[1], and H. Scheich. Institute of Zoology, TU-Darmstadt and CE Merck[1], D-6100 Darmstadt, Germany

In a Chilenian rodent, the degu, early learning of species-specific vocalizations between mother and offspring was investigated. Degus are highly social and live in large family structured colonies. Degu mothers vocalize while nursing their pups, which are precocial at birth. Behavioral studies of degu pups using a discrimination task revealed an approach to mother calls while pups raised by muted mothers failed to react. This leads to the assumption that the behavioral significance of the calls is learned early during nursing sessions. 2-DG experiments in pups using mother calls and pure tones as stimuli revealed dorso-ventrally oriented bands of high glucose uptake in several areas of auditory cortex. Bands appeared to be wider and more complex in animals stimulated with mother calls or elements of them compared to the same number of bands obtained with pure tones. As the calls showed a complex spectral and temporal pattern results are compatible with a distributed representation of them in tonotopic maps.

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559.18

SELECTIVE RESPONSES OF MACAQUE TEMPORAL NEURONS TO COMPLEX SOCIAL STIMULI. L.A. Brothers & B.D. Ring. UCLA-Sepulveda VA Medical Center & UCLA Brain Research Institute, Sepulveda CA 91343.

The present study extends previous observations in medial amygdala and adjacent cortex of neurons selective for social features. Moving visual and/or auditory stimuli lasting 2s were presented to an alert subject while recording in the region of the right amygdala. Stimuli were drawn from an extensive library of macaque behavior stored on laser disk. Neuronal responses were compared to responses elicited by relevant control stimuli differing from target stimuli along one or more dimensions. Of 533 cells analyzed, 14 showed selective responses to features of presented stimuli, for example, agonistic content. The response of one cell to segments depicting a retreating animal was significantly enhanced by concomitant presentation of the animal's vocalizations. Such neurons may participate in integrations necessary for forming high-level social representations.

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559.19

BEHAVIOR PATTERN ORGANIZATION IN 6 MAMMALIAN SPECIES. G.E. Gerstner, L.J. Goldberg. Section of Oral Biol., Sch. of Dent., Brain Res. Instit., Sch. of Med., UCLA, Los Angeles, CA 90024).

Ethology and behavior ecology studies are often interested in behavioral adaptations; however, they often model behavior as being ideally or optimally adapted. In other words, they rarely consider the existence of neuromuscular physiological components that restrict the evolutionary potential of behaviors. We believe that behaviors are suboptimally adapted as a result of various physiological constraints. The current study was undertaken to provide insight in this regard.

Six giraffes, 4 okapis, 4 roe deer, 6 kangaroos, 4 red pandas, and 3 raccoons) were videotaped for 10 hr periods on 4 separate occasions over a 1.5 yr period. Every behavior pattern occurring during each 10 hr videotaping session was classified in such a way that cross-species comparisons could be made. Behavior pattern durations and occurrences throughout the 10 hr periods were then analyzed to determine what features were similar among all 6 species.

The results indicate that: 1) over 80% of the behavior patterns were similar in all 6 species, 2) of these patterns, their relative frequency of occurrence was similar in all 6 species, 3) a temporal hierarchy of behavior patterns was similar in all 6 species, with spectral peaks occurring near 0.5 s, 3-6 s, 1-2 min, 10-20 min, 1-1.5 hr. The results of this study support the hypothesis that many aspects of behavior organization are conserved in the mammalian lineage, indicating potential physiological restrictions on the evolutionary potential of behavior. We acknowledge the assistance of the San Diego Zoo and Wild Animal Park, Los Angeles Zoo, and Phoenix Zoo staffs. This study was supported by PHS Grant DE00205.

559.20

A MODEL OF NERVOUS SYSTEM FUNCTION DURING CLASSICAL AND INSTRUMENTAL CONDITIONING. A.H. Klopf, J.S. Morgan* and S.E. Weaver*. Wright Laboratory, Wright-Patterson Air Force Base, Ohio 45433-6543.

A computational model of nervous system function during classical and instrumental conditioning is proposed. The model assumes the form of a network of control systems. Each control system is capable of learning and is referred to as an associative control process (ACP). Learning systems consisting of ACP networks, employing the drive-reinforcement learning mechanism (Klopf, 1988) and interacting in a real-time, closed-loop fashion with environments, are capable of being instrumentally conditioned, as demonstrated by means of computer simulations. The simulated systems learn, in multiple-T mazes, to chain responses that eventually lead to primary positive reinforcement. The systems also learn to avoid primary negative reinforcement. The temporal order in which the responses are learned is consistent with that observed in animal learning. Also, consistent with animal learning experimental evidence, the ACP network model accounts for a wide range of classical conditioning phenomena. The ACP network is intended as a model of limbic system, hypothalamic, and sensory-motor function, suggesting a relationship between classical and instrumental conditioning that is consistent with Mowrer's (1960) two-factor theory of learning.

NEUROETHOLOGY: FISH

560.1

Proprioceptive cues necessary for deciphering the mormyrid fish electrosensory world. J.Serrier*, A.Kleiser* and K.Grant. Dept. of Neurophysiol. Sensor., Lab. of Physiol. Nerveuse CNRS F-91198 Gif-sur-Yvette

In mormyrid fish, the electric organ discharge (EOD) is the energy source for the electrosensory system involved in active electrolocation. Current flow through cutaneous electroreceptors will depend on the relative position of the electric organ in the tail and the rest of the body and integration of proprioceptive information is therefore necessary to the electrosensory processing network to compute an accurate central image of the environment. Static body position-related activity has been recorded previously in the *eminentia granularis posterior (EGp)* of the cerebellar caudal lobe (Bell et al., in preparation) and it is probable that integration occurs in this structure which projects via granule cells to the underlying electrosensory lobe.

Recording was carried out in the caudal lobe under metomidate anesthesia which renders the fish insensitive to painful stimuli without supressing the EOD, spinally commanded motor activity (reflex swimming) or electrosensory primary afferent activity. During spontaneous swimming movements it was shown that tonic firing frequency of many *EGp* units is tuned to tail curvature; intracellular biocytin labelling identified these units as fibers which run throughout the *EGp* and which probably originate from the spino-cerebellar tract. In these fibers there seemed to be no relation between the recorded proprioceptive activity and the EOD command. A second class of unit was found close to the border of *EGp,* in the molecular region of the posterior caudal lobe. These were direction sensitive to movement and gave a precise dynamic coding of tail position. Similar units from the opposite side of the brain showed a reciprocal firing pattern. Again there was no relation to the EOD command. However, clear EOD-related activity can be recorded in other neuronal elements in the caudal lobe and it is likely that both types of motor-related information will reach the electrosensory lobe via this relay.

560.2

THE POLARITY PREFERENCE OF TUBEROUS ELECTRORECEPTORS IS AFFECTED BY FREQUENCY TUNING J. R. McKibben and C.D. Hopkins Section of Neurobiology and Beharvior, Cornell University, Ithaca, NY 14853.

Weakly electric fish localize and approach electric organ discharges (EODs) from conspecifics and artificial electrical dipoles by aligning their body axis parallel to the local electric field vector while swimming along curved current lines to reach the source. We are interested in the sensory mechanism behind the **polarity** preference for approaching the "head" electrode of a dipole. Yager and Hopkins (S.N. Abstract 548.2, 1990) already demonstrated that sensory cues about the axis of an electric field are provided by individual electroreceptors positioned over the body surface.

We recorded from burst duraction coders in silenced *Hypopomus sp.* while presenting homogeneous fields from single period sine waves (1 kHz carrier) at 15° intervals around a circle. We determined the number of spikes per stimulus for various amplitudes and directions, and measured polarity sensitivity along the best axis while varying the stimulus carrier frequency from 100 to 6000 Hz. We also measured frequency-tuning curves for these receptors.

With a 1 kHz stimulus, most receptors had asymmetric polar sensitivity plots, nearly all with the strongest response oriented inward, perpendicular to the skin. Fewer units had symmetrical sensitivity plots. When the stimulus frequency was varied, we found two types of units differing in their polarity preference responses. **Type I** units gave strongly asymmetrical responses to carrier sine waves near the best frequency (BF) of the receptor, but more symmetrical responses when stimulated at frequencies above or below the BF. **Type II** units gave symmetrical responses to stimuli near the BF but became weakly asymmetrical at frequencies below the BF. Receptors of both types may reverse asymmetry. The two response types had, respectively, narrow-band and low-pass tuning curves. We simulated these frequency-dependent changes in polarity preference electronically using either a low-pass or a band-pass filter followed by a rectifier. Our data establishes that information is available to the CNS that would permit polarity discrimination.
Supported by NSF grant # BNS 8810080.

560.3

Conditional Oscillatory Discharge in Topographic Maps of the Electrosensory Lateral Line Lobe (ELL). R.W. Turner, J.R. Plant and L. Maler, Dept Anat, Univ Ottawa, Canada K1H 8M5

The weakly electric fish *Apteronotus leptorynchus* generates an electric organ discharge (EOD) to electrolocate objects in their enviroment. The frequency of EOD amplitude modulations (AM) are processed in 3 independent topographic maps (segments) in the ELL. Each segment contains a primary circuit of pyramidal (PC) and granule (GC) cells arranged in a laminar fashion, and 5 GABAergic inhibitory cell types. PCs in each segment respond *in vivo* to a restricted range of AM frequencies (Shumway 1989), increasing from Centromedial segment (CMS,1-8 Hz) to Lateral segment (LS,40-120 Hz). Maler (1988) thus suggested that the ELL acts as an array of temporal filters. In *in vitro* ELL slices, focal ejections of bicuculline (BIC; 50 μM) in only the CMS GC layer evoked oscillations of PC and GC field potentials (10-40 min) restricted to CMS, corresponding to an intracellular 10-20 mV PC membrane oscillation and rhythmic spike discharge (5-6 sec period). Hyperpolarization blocked PC spike discharge and reduced oscillation amplitude without affecting period. Focal ejections of TTX in the GC layer blocked oscillations. Unit recordings reveal that spike frequency and burst duration differs across ELL segments (CMSf = 12 ± 3, *Dur* =1.4 ± .4 s; LSf = 66 ± 29, *Dur* =.61 ± .04 s; n=13). These data suggest that intrinsic segmental mechanisms may contribute to the tuning of PC discharge to AM modulations *in vivo*.

560.4

DEVELOPMENT OF THE MULTIPLE MAPS OF THE ELECTROSENSORY LATERAL LINE LOBE (ELL) IN EIGENMANNIA. S. Viete. SIO, UCSD A-002, La Jolla, CA 92093.

The electrosensory lateral line lobe (ELL) of the weakly electric fish Eigenmannia contains 3 somatotopic maps of tuberous electrosensory input. The multiple maps receive identical primary afferent information as each afferent sends collaterals to all 3 maps, the central medial (CM), the central lateral (CL) and the lateral map (L).

In order to determine when and how the tuberous 3 maps develop, we applied HRP either to the supraorbital ramus (SO-AVN) or the preopercular-mandibular ramus (PM-AVN) of the anterior lateral line nerve (AVN) or the anterior lateral line nerve ganglion itself.

The study revealed that all three maps emerge prior to the onset of the jamming avoidance response (JAR) in juvenile Eigenmannia. The three maps develop successively, from medial to lateral. The primary afferents seem to make contact in one target area, before collaterals branch off to innervate the second and third target area. The central lateral map is the first and only one until the fish has reached a body length of 9 mm. After further growth (9.5 -11.5 mm) the central lateral map develops, and finally the third map is formed at a body length of 12 mm. This developmental succession appears to reflect the evolutionary history of these maps.

560.5

DESCENDING SENSORY INFORMATION PROJECTS HOMOTOPICALLY TO THE ELECTRORECEPTIVE DORSAL OCTAVOLATERALIS NUCLEUS IN SKATES

R. A. Conley and D. Bodznick
Wesleyan University Biology Dept., Middletown, CT 06457

The dorsal granular ridge (DGR) in elasmobranchs is a part of the vestibulolateral cerebellum which projects via parallel fibers to the dorsal octavolateralis nucleus (DON), the primary electrosensory nucleus. Somatotopic representations of electrosensory and propriosensory information in DGR and the topography of the DGR to DON projection suggest that the projection is homotopic. A homotopy has now been demonstrated by activating the parallel fibers with a stimulating electrode placed in DGR. Evoked potential and unit responses were recorded electrophysiologically in DON.

The evoked potential is characterized by a positivity at 4 ms latency followed by a negativity. The positivities are believed to be synaptically induced depolarizations on the dendrites of the DON ascending efferent neurons. Upon parallel fiber activation, typically one or two spikes followed by a long-lasting suppression are elicited in the ascending efferent neurons. Responses to electric fields are reduced in magnitude when presented during this suppression period.

Peak evoked potential amplitudes and unit responses to parallel fiber activation occur only in areas of the DON containing units whose receptive fields correspond with the composite receptive field recorded at the stimulation site in DGR. The data show a homotopic projection of information from DGR onto DON.

560.6

LAMINAR SEGREGATION OF FUNCTION IN THE TORUS SEMICIRULARIS OF WEAKLY ELECTRIC FISH. S.J. Call and G.J. Rose. Dept. of Biology, Univ. of Utah, Salt Lake City, UT 84112.

The processing of sensory information in laminated structures is a common feature of vertebrate central nervous systems. To understand the functional significance of laminar organization, we studied the processing of electrosensory information in the dorsal torus semicircularis of Eigenmannia. This region of the midbrain consists of 12 laminae and approximately 55 neuron types.

We investigated structure-function relations in the torus by labeling physiologically characterized neurons with Lucifer Yellow. Ampullary and tuberous information is processed differentially within the laminae of the torus: neurons of lamina 3 and lamina 5 are predominantly ampullary and tuberous in function, respectively.

Tuberous units respond well to "beats.," i.e., modulations of signal amplitude and phase, that result from the interaction of foreign signals with the fish's own quasi-sinusoidal electric organ discharge. The "beat rate" is proportional to the magnitude of the frequency difference between these two signals. Some units respond well to low beat rates but poorly to high rates (i.e., "low-pass" temporal filtering).

We observed subthreshold fluctuations in the membrane potentials of toral neurons that reflect the patterns of modulations of signal amplitude and phase. Changes in the structure of these psps that accompany changes in the beat rate have provided insight into the mechanism of temporal filtering. Models of these mechanisms will be presented. Supported by grants from NSF and Sloan Foundation.

560.7

LONGITUDINAL TRACKING RESPONSES OF GYMNOTIFORM FISH. G.J. Rose and J.C. Canfield. Dept. of Biology, Univ. of Utah, Salt Lake City, UT 84112.

A variety of behaviors serve to stabilize sensory images on receptor arrays. In one class of these behaviors, global movements of the "surround" elicit compensatory movements of the receptive surface. Previous studies have shown that weakly electric fish are able to track aggregate lateral movements of surrounding objects using exclusively the electric sense.

We now report measurements of longitudinal tracking responses of Eigenmannia and Sternopygus. These movements were elicited by moving a "shuttle" in which the fish was situated. Fish were able to track longitudinal motion of the shuttle using visual or electrosensory cues.

The gain and phase of tracking responses were studied as a function of the magnitude and frequency of shuttle motion. Fish were able to track velocities of shuttle motion of at least 4 cm/s with little decrement in gain.

We delivered signals that excited ampullary receptors, tuberous receptors, or both during longitudinal tracking of various velocities of shuttle motion. The jamming effects of these stimuli will be presented. Supported by grants from NSF and Sloan Foundation.

560.8

A SUBLEMNISCAL PREPACEMAKER NUCLEUS IN GYMNOTIFORM ELECTRIC FISH DEPOLARIZES RELAY CELLS OF THE PACEMAKER NUCLEUS VIA NMDA-TYPE RECEPTORS. C.H.Keller[1], M.Kawasaki[2], W.Heiligenberg, G.Kennedy, W.Metzner. Neurobiol. Unit, Scripps Inst. Oceanog., UCSD, La Jolla, CA 92093; and [1]Inst. of Neurosci., U. of Oregon, Eugene, OR; [2]Dept. Biology, U. of Virginia, Charlottesville, VA.

The medullary pacemaker nucleus of gymnotiform fish is an endogenous oscillator which triggers each electric organ discharge (EOD) by a single command pulse. The pacemaker nucleus consists of electrotonically coupled 'pacemaker' cells, which generate the rhythm, and 'relay' cells, which follow the pacemaker cells and excite the spinal motor neurons of the electric organ. By injecting choleratoxin into the pacemaker nucleus, we have retrogradely labelled a hitherto unknown sublemniscal nucleus which, in addition to the classical diencephalic prepacemaker nucleus, provides input to the pacemaker nucleus.

Stimulation of the sublemniscal nucleus in the genera Sternopygus and Hypopomus causes a sustained depolarization of the relay cells that shuts down the regular electric organ discharges over a period of several seconds. Pressure injection of the NMDA receptor blocker, APV, to the pacemaker nucleus reversibly prevents this response while not affecting other forms of modulations of the pacemaker that are not mediated by NMDA. The same sublemniscal nucleus was also labelled in the genera Eigenmannia and Parapteronotus, but its functional significance has yet to be determined.

560.9

ULTRASTRUCTURAL EVIDENCE OF GABA-ERGIC INHIBITION AND GLUTAMINE-ERGIC EXCITATION IN THE PACEMAKER NUCLEUS OF THE GYMNOTIFORM ELECTRIC FISH, **HYPOPOMUS**. G.Kennedy* and W.Heiligenberg. Neurobiology Unit, Scripps Institution of Oceanography, UCSD, La Jolla, CA 92093

The medullary pacemaker nucleus of Hypopomus is an endogenous oscillator which triggers each electric organ discharge (EOD) by a single command pulse. The pacemaker nucleus consists of electrotonically coupled 'pacemaker' cells, which generate the rhythm, and 'relay' cells, which follow the pacemaker cells and excite the spinal motor neurons of the electric organ. The pacemaker cells receive two separate and independent inputs from the complex of the diencephalic prepacemaker nucleus, a GABA-ergic inhibition and a glutamine-ergic excitation. Whereas the inhibition slows down and, eventually, arrests the pacemaker cycle, the excitation accelerates the pacemaker cycle in a smooth and gradual manner.

We have labelled the two inputs to the pacemaker nucleus anterogradely by injecting HRP to the respective sites of the prepacemaker complex. By using respective immunogold-labelled antibodies and en-grid staining techniques, we then demonstrated GABA and glutamate in labelled synaptic profiles of ultra-thin sections of the pacemaker nucleus. The two types of synapses were interspersed on the surfaces of somata and dendrites of pacemaker cells.

560.10

MIDBRAIN AND DIENCEPHALIC LINKS WITHIN THE NEURONAL NETWORK UNDERLYING THE JAMMING AVOIDANCE RESPONSE IN EIGENMANNIA.

Walter Metzner and Walter Heiligenberg. UCSD A-002, SIO, La Jolla, CA 92093.

Weakly electric fish of the genus Eigenmannia avoid jamming electrosensory stimuli from neighbouring fish by either increasing or decreasing the frequency of their own electric organ discharge (EOD), depending on the sign of the difference to the neighbour's EOD frequency (jamming avoidance response, JAR). The sign of the frequency difference is evaluated by neurons within the deeper laminae of the torus semicircularis dorsalis (Tsd) of the midbrain. Intracellular recording and labelling of neurons of the Tsd show that these sign selective neurons project to those parts of the nucleus electrosensorius complex (nE) that control either the rise (nE↑) or the decrease of the EOD frequency (nE↓). Extracellular injections of cholera toxin into the nE↑ and nE↓, respectively, reveal that these areas project to the anterior thalamus (ATh). It is the ATh, where stimulation with L-glutamic acid causes the most prominent smooth rise in the EOD frequency. Intracellular labelling of neurons of the medial part of the diencephalic prepacemaker nucleus (PPn-G) shows that their dendrites reach into the ATh. The same neurons modulate the frequency of the medullary pacemaker nucleus.

560.11

AFRICAN ELECTRIC FISH, *GYMNARCHUS*, USE THE IDENTICAL COMPUTATIONAL ALGORITHM AS SOUTH AMERICAN ELECTRIC FISH FOR THEIR JAMMING AVOIDANCE RESPONSES. Masashi Kawasaki. Department of Biology, University of Virginia, Charlottesville, VA 22901.

Both African wave-type electric fish, *Gymnarchus*, and South American wave-type electric fish, *Eigenmannia*, gradually shift the frequency of electric organ discharges (EODs) away when they encounter a neighbor of similar discharge frequency. *Gymnarchus* and *Eigenmannia* are quite unrelated belonging to different superorders of fishes and believed to share no common electroreceptive ancestor. The occurrence of this jamming avoidance response (JAR) both in such unrelated phyletic groups invites behavioral and physiological comparison (Bullock et al., 1975). In this study, behavioral tests were performed in curarized *Gymnarchus* to identify basic computational algorithms involved in their JAR.

Fish's own EOD was silenced by curare and a replacement signal, S_1, was offered through a pair of electrodes located in the mouth and at the tail. A signal mimicking neighbor's EOD, S_2, was delivered through the other pair of electrodes straddling the fish. The JAR occurred depending solely upon the frequency differences between S_2 and S_1, and S_1 did not require to be phase-locked to the pacemaker activities which were monitored by a suction electrode attached to the tail, suggesting that the sensory mechanisms for the JAR do not involve the internal reference to the pacemaker activities. When S_1 and S_2 were electronically added and offered through the same pair of electrode, no jamming avoidance response occurred, suggesting that the differential phase comparison is involved. These two computational features for the JAR are identical as those used in *Eigenmannia*.

560.12

SEXUALLY DIMORPHIC DISTRIBUTION OF SUBSTANCE P AND ITS ROLE IN THE REGULATION OF COMMUNICATION IN AN ELECTRIC FISH. M.M. Weld[1], L. Maler[1], R. Quirion[2] and S. Kar[2]. [1]Dept.of Anatomy, University of Ottawa, Ottawa, Ontario, Canada K1H 8M5, and [2]Douglas Hospital Research Centre, Verdun, Quebec, Canada H4H 1R3.

Apteronotus leptorhynchus emits a continuous electric organ discharge (EOD) of constant frequency which is used in electrolocation. The EOD is controlled by the medullary pacemaker nucleus (PM) whose only known input is from the diencephalic prepacemaker nucleus (PPn). The PPn thus controls transient changes in the frequency of the EOD, called "chirps", which are used in communication.

Immunohistochemical studies revealed a major system of substance P-like immunoreactivity (SPli) which originated in the hypothalamus and, in males only, innervated many areas of the diencephalon, including the PPn, its ventral territory (VT, an area containing dendrites of PPn cell bodies), and 2 hypophysiotrophic nuclei. Hypothalamic SPli innervation of the diencephalon was absent in females. Our results suggest that a SP system of hypothalamic origin is involved in regulating both endocrine events and sexual/territorial-related behavior in males. No sexual dimorphism of SPli was found in the telencephalon, mesencephalon or rhombencephalon. Neurokinin 1 and 3 (NK_1 and NK_3) receptors were found in the brain by autoradiography; their distribution showed a very close correlation with that of SPli, in particular the NK_1 receptor, which preferentially binds SP, was found in the VT.

Neuroactive substances were injected into the PPn of curarized, respirated fish to evoke chirps. Glutamate evoked short chirps (8 msec) similar to spontaneous chirps. Substance P-evoked chirps were longer (25 msec) and had a more complex waveform. Glutamate was most effective in the PPn proper. In contrast, SP was most effective in the VT of the PPn, an area with abundant dendrites from PPn cell bodies, dense SPli and NK_1 receptors.

This research was supported by grants from the Medical Research Council of Canada to LM and RQ.

560.13

RETROGRADE TRACING AND IMMUNOHISTO-CHEMISTRY OF ELECTRIC LATERAL LINE LOBE (ELL) NEURONS OF THE TELEOST *GNATHONEMUS PETERSII*, MORMYRIDAE. T. Szabo, J.-P.Denizot*,M. Ravaille-Véron*,C.C. Bell, U. Bonn*. Dept. Neurophysiol. Sens., Lab. Physiol. Nerveuse, C.N.R.S., F-91198 Gif/Yvette Cedex.

Retrograde labeling following mesencephalic injections of HRP showed that only a limited number of ELL neurons are efferent. These are distributed between ganglion and deep fiber layers. Ganglion cells have basal dendrites extending into the granular layer where lateral line nerve afferents terminate, suggesting disynaptic connections between primary afferents and the mesencephalon. Small intralobar injections of HRP demonstrated projections from small cells of the intermediate layer to the molecular layer.

Immunohistochemistry revealed that a large number of ganglion and beard cells are glutamatergic. The somata of ganglion and beard cells were surrounded by GAD-IR terminals and GAD-IR boutons were also distributed along dendritic trees in the molecular layer. Identified efferent cell bodies also bore GAD-IR endings. Small cells in the molecular layer and commissural axons with cells of origin in the deep fiber layer were GABA-IR. GAD-IR terminals were also observed surrounding cell bodies of the ELL nucleus. Granule cells in the ELL dorsal (DLZ) and medial (MZ)zones, but not in the ventrolateral zone (VLZ), and terminals at the ventral border of the molecular layer were labeled with anti-calbindin antibodies. Serotoninergic fibers have also observed previously in all ELL layers.

560.14

EVIDENCE FOR DISTINCT BUT OVERLAPPING POPULATIONS OF COMMISSURAL AND GABA-IMMUNOREACTIVE NEURONS IN THE MEDULLARY ELECTROSENSORY NUCLEUS OF THE LITTLE SKATE, *RAJA ERINACEA*. C.H. Duman and D. Bodznick. Dept. of Biology, Wesleyan University, Middletown, CT 06459.

Activity of neurons of the dorsal octavo-lateralis nucleus (DON), the primary electrosensory nucleus in elasmobranchs, is modulated by electrical interference from the animals own ventilation, but to a much lesser degree than that of the primary afferent fibers. There is physiological evidence that inhibitory commissural connections between the bilateral dorsal nucleii are part of a mechanism for rejecting common-mode noise signals such as ventilatory interference (New and Bodznick, 1990). We have begun anatomical characterization of the commissural pathway. Retrograde transport of horseradish peroxidase or fluorogold injected into the DON resulted in labelling of commissural cells in both central and peripheral zones of the contralateral DON. Double-labelling studies indicate that a population of commissural cells in the central zone labelled by retrograde transport of fluorogold, also show GABA-immunoreactivity. GABA-immunoreactivity was also displayed in non-commissural central zone cells and in stellate cells of the molecular layer of the DON. The doubly labelled cells are likely candidates for mediators of a common-mode noise rejection mechanism in the DON.

560.15

NEUROETHOLOGY AND VISION IN THE BIOLUMINESCENT FISH, PORICHTHYS NOTATUS. A. F. Mensinger and J. F. Case*. Vanderbilt Univ., Nashville TN 37240 and Univ. of California, Santa Barbara, CA 93106.

The midshipman fish, Porichthys notatus, is a nocturnal predator that feeds on luminescent organisms. Fish were presented with photic stimuli characteristic of luminescent prey. Flash intensity, wavelength, duration, pulse frequency and source diameter were regulated.

Fish were attracted to flashes of specific wavelength (470-530 nm), pulse frequency (0.03-0.07 Hz) and duration (250-750 ms). Higher pulse rates (>0.20 Hz) and longer flashes (>2500 ms) evoked negative responses while intermediate values yielded graded reactions. Increasing source diameter while maintaining equal intensity, reduced number of positive responses. Negative stimuli elicited faster responses.

Juvenile P. notatus must acquire exogenous sources of luciferin to remain luminescent. Fish are attracted to photic signals characteristic of these sources and avoid light that may expose themselves to predation.

560.16

CENTRAL NEURAL SUBSTRATES FOR THE CONTROL OF SMOLT TRANSFORMATION IN SALMON (*SALMO SALAR* L.) **P. Ekstrom, T. Ostholm, B. Holmqvist, B. Moverus, L. Ebbesson, and S. Ebbesson***
Dept. of Zoology, Univ. of Lund, Lund, Sweden and Inst. of Marine Science, Univ. of Alaska, Fairbanks, Alaska 99775

Smolt transformation (ST) involves major changes in the physiology and behavior of salmon. During ST the fish change from territorial to schooling behavior, start seaward migration, and form an olfactory memory (imprinting) of their native stream. It is not known how ST is regulated. However, there is evidence that changes in photoperiod are important external cues. To identify the central control systems that may regulate ST, we have traced the neuronal connections of the retina, the optic tectum, the photosensory pineal organ, the olfactory projections and the hypophysiotrophic centers by means of DiI tracing. The results were then correlated with immunocytochemically defined monoaminergic and peptidergic neuronal systems.

The most striking results were (1) the extensive retinohypothalamic projection, (2) the absence of a cyto- or chemoarchitectonically circumscribed nucleus comparable with the suprachiasmatic nucleus in mammals, (3) the extensive pineal projections to the dorsal thalamus and periventricular pretectum, (4) the close association of putatively dopaminergic (DA) neurons with afferent axons from both the retina and pineal organ, (5) DA neurons in the hypothalamic and dorsomedial optic nuclei that project to the optic tectum, (6) a close association of DA neurons in the telencephalon with the olfactory projections. In all, our results indicate a central role for dopamine in the central processing of sensory information known to be essential for several aspects of smolt transformation in salmon.

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560.17

TASTE MEDIATES INNATE FEEDING BEHAVIOR WHILE OLFACTION FACILITATES CONDITIONED DISCRIMINATION OF AMINO ACIDS BY CATFISH. T. Valentincic (University of Ljubljana, Yugoslavia), S. Wegert and J. Caprio (Louisiana State University)

The inhibition of feeding in channel catfish Ictalurus punctatus by "the longterm central escape state" was reduced by maintaining animals individually in aquaria with black gravel, by regular feeding and by the longterm avoiding of visual stimuli which release escape behavior. Naive channel catfish responded to L-alanine and L-arginine (>1μM) and L-proline (>1mM) with the following patterns of feeding behavior: maxillary and mandibular barbel movements, orienting posture, search swimming, bottom and wall searching, turning and snapping. Catfish were subsequently conditioned during 40 trials to swim 90 seconds for a food reward following an application of the amino acid stimulus. The conditioned "search swim" consisted of 40-90 turns greater than 90° compared to 0-40 turns in response to non-conditioned amino acids. Conditioned channel catfish discriminated L-alanine, L-arginine and L-proline from each other and from all non-conditioned amino acids. Following surgical removal of the olfactory organs, animals previously conditioned to 10^{-5}M L-proline did not respond behaviorally to L-proline at concentrations $<10^{-4}$M, whereas strong snapping behavior was triggered by 10^{-3}M L-proline. L-arginine and L-alanine at 10^{-5}M released short-lasting feeding responses in the anosmic catfish; however, even after 45 - 150 re-conditioning trials the anosmic catfish did not show discrimination between the tested amino acids. These results suggest that: a) the conditioning was a result of olfactory input, b) the increased food search in catfish cannot be conditioned by taste alone, and c) the taste mediated responses to amino acids including snapping are most probably innate. Supported by NSF BNS-8819772 and ONR 00014-90-J-1583.

HORMONAL CONTROL OF BEHAVIOR III

561.1

DOCA PRETREATMENT ENHANCES NEURONAL ACTIVITY AND SENSITIVITY TO ANGIOTENSIN II IN THE FOREBRAIN OF RATS. S.N. Thornton and S. Nicolaïdis*. Neurobiologie des Régulations, Collège de France, 11 place Marcelin Berthelot, 75231 Paris CEDEX 05, France.

A rapid and substantive sodium appetite can be initiated in rats that have been pretreated with a low dose of deoxycorticosterone acetate (DOCA) for 3 days, that in itself does not initiate the appetite, and then given an intracerebroventicular injection of a below threshold dose of angiotensin (AII). We have investigated electrophysiologically the effects of these treatments on neurons of the septum and anterior hypothalamus.

Male wistar rats, controls (non treated) or experimentals (pretreated, DOCA 0.5 mg/kg s.c.), were anaesthetised with urethane and the femoral artery catheterised. A 7 barrelled microiontophoretic electrode attached to an extracellular recording electrode was then advanced through the cortex into the anterior ventral hypothalamus where unit activity was recorded.

In the medial septal area, but not in other areas, a greater number of active neurons were recorded in rats pretreated with DOCA than found in the controls (10.2 + 1.1 / electrode decent, n = 19, vs 6.3 + 0.6 / decent, n = 25, P<0.01). In the treated animals Io AII produced a marked enhancement of neuronal activity, something never seen in the controls. Io of aldosterone increased neuronal acitivity of both control and experimental rats but subsequent Io of AII produced marked excitation only in DOCA treated animals. Similar results were obtained in rats treated with DOCA up to 10 days before the electrophysiological recordings.

This long term and persistent DOCA induced enhancement of neuronal activity and excitability to AII could be the mechanism whereby a sodium appetite, induced by the synergistic action of these two hormones, is stimulated. (Supported by MH 43787)

561.2

MEMBRANE-BOUND CORTICOSTEROID RECEPTOR IS COUPLED TO A G-PROTEIN. M. Orchinik, T. F. Murray, and F.L. Moore. Dept. Zoology, Oregon State University, Corvallis, OR 97331-2914.

Steroids hormones alter neuronal activity through intracellular receptor-mediated changes in protein synthesis and through poorly understood interactions with receptors on neuronal membranes. Recently we characterized a high-affinity corticosteroid receptor in synaptic membranes from an amphibian brain, *Taricha granulosa* (Orchinik et al., in press, *Science*). The receptors were localized autoradiographically in the neuropil in discrete brain regions and they appear to mediate rapid behavioral responses to corticosterone (CORT). Since the binding site is not linked to the $GABA_A$/benzodiazepine receptor, we performed radioligand binding studies to determine if the receptor is coupled to guanine nucleotide binding proteins. The specific binding of [^{3}H]CORT in a neuronal membrane preparation was inhibited in a concentration-dependent manner by guanine nucleotides. The rank order potency to inhibit [^{3}H]CORT binding was GTP-gammaS > Gpp(NH)p > GTP > GDP = GMP. Although addition of Mg^{2+} to EDTA-treated membranes did not significantly enhance [^{3}H]CORT binding, Mg^{2+} enhanced the potency of guanine nucleotides to inhibit [^{3}H]CORT binding. These data provide the first evidence for the coupling of steroid receptors to G-proteins; they suggest a transduction mechanism that could account for rapid neuronal responses to corticosteroids.
Supported by NSF grants BNS-8901500 and BNS-8909173.

561.3

THE EFFECTS OF GLUCOCORTICOIDS ON HIPPOCAMPAL PLASTICITY
C. Pavlides, Y. Watanabe & B.S. McEwen. The Rockefeller University, New York.

High glucocorticoids levels are detrimental to hippocampal (HPC) anatomy, physiology and behaviors closely associated with this structure. We investigated the effects of chronic and acute corticosterone (CS) administration on HPC long-term potentiation (LTP). Male rats were injected with CS (40 mg/kg/day, s.c., in sesame oil) or the vehicle for 21 days. Forty eight hours following the last injection the animals were tested for LTP by applying a high frequency stimulus (HFS) to the perforant path (PP) and recording extracellular field potentials in the dentate gyrus granule cell layer. In a second group of animals LTP was tested 1-2 hours following a single injection of CS or the vehicle.

The mean plasma levels of CS were determined to be high 1 hour (68.63 ± 8.24 μg/100ml) following CS injections but returned to normal 48 hours (9.17 ± 2.31 μg/100ml) later, at time of testing. The CS plasma levels of the control animals was determined to be at a low level 1 hour (17.16 ± 6.97 μg/100 ml) and 24 hours (15.18 ± 3.52 μg/100ml, time of testing) after the last vehicle injection.

In vehicle injected controls, HFS of the PP produced a substantial degree of potentiation, as measured by both the slope of the EPSP (18.76 ± 2.04%) and the population spike (100.96 ± 35.16%), in comparison to baseline. In contrast, LTP induction in chronic CS injected animals was significantly ($P < 0.05$) lower than in controls for both the slope (12.78 ± 1.50%) and the population spike (30.10 ± 10.01%) . Similar reductions in LTP induction were seen for the acute CS animals (slope, 4.83 ± 4.92%; spike, 34.93 ± 11.03%, from baseline).

The results indicate that CS may induce both acute and chronic effects on HPC plasticity and that these effects are probably due to different mechanisms.

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561.4

GLUCOSE REVERSES THE BEHAVIORAL EFFECT OF FOOD DEPRIVATION AND ADRENALECTOMY IN THE PORSOLT SWIMMING TEST. D. Jefferys and J.W. Funder. Baker Medical Research Institute, Prahran, Victoria 3181, Australia.

In the Porsolt swimming test rats become progressively immobile over a 15 min test period, and on retest 24 h later are immobile for ~70% of a 5 min retest. Adrenalectomized (ADX) rats acquire the response normally but on retest remain immobile ~35% of the time, an effect reversed by glucocorticoids and kappa–selective opioids. Hypothyroid rats neither acquire nor retain the response, an effect reversed by T_4 or T_3. Rats food deprived for 24 h, like ADX rats, acquire the response but do not retain it on retest. We here present dose response studies on the effect of glucose administration on the behavioral response to food deprivation, ADX and hypothyroidism, and the effect of dexamethasone, ketocyclazocine and T_4 on the response to 24 h food deprivation. Glucose (≥100 mg/kg) reversed the effect of food deprivation, but at 1–1000 mg/kg was without effect in the hypothyroid rat; in contrast, at 1000 mg/kg glucose reversed the effect of ADX up to 1–2 h after initial exposure, but not 1 h prior to retest. Dexamethasone (6 μg/rat), ketocyclazocine (25 μg/rat) or T_4 (15 μg/rat) reversed the effect of food deprivation. The results from the present studies provide further evidence for a critical role of metabolic factors in modulating the acquisition and retention of behavioral responses. The specific site and mode of action of glucose in these circumstances awaits investigation.

Supported by the NHMRC of Australia

561.5

THE RELATIONSHIP BETWEEN HIPPOCAMPAL LESIONS AND GLUCOCORTICOID LEVELS IN AN ANIMAL MODEL OF WANDERING IN ALZHEIMER'S DISEASE. R. Donahue, J.P. Ryan, and R.L. Heintz*. Department of Psychology, State University of New York at Plattsburgh, Plattsburgh, New York 12901.

The focus of the study is to better understand the elevated glucocorticoid levels in Alzheimer's disease. An animal model was used to determine the effect of dorsal hippocampal lesions on neurohormonal and behavioral functions. Thirty-three Long-Evans rats were divided into three groups of twelve: an experimental lesioned group, a control sham-lesioned group and a control non-lesioned group. Experimental lesions were produced by injecting 15 ug/ul of colchicine bilaterally into the dorsal dentate gyrus. Colchicine has been found to mimic some of the neuropathological and neurobehavioral aspects of Alzheimer's disease. The control sham-lesioned group received bilateral injections of saline in the same areas of the brain. The control non-lesioned group received no surgery but underwent anesthetic procedures. Animals were then subjected to behavioral testing using the Morris water maze. Behavioral testing resulted in a significant impairment in spatial memory in the colchicine group in comparison to the control group. Blood samples were taken from all animals before surgery and twice after behavioral testing. All blood samples were taken between 0800 and 1200 hours to avoid changes in circadian glucocorticoid levels and measures were also taken to avoid stress induced glucorticoids. Blood samples were assayed using the HPLC and alterations in glucocorticoid levels were observed. The study suggests a role for the hippocampal modulation of glucocorticoids in Alzheimer's Disease.

561.6

STRESS INDUCED CORTISOL SECRETION INFLUENCES INFORMATION PROCESSING IN MAN. G. Fehm-Wolfsdorf, D. Nagel* and H.L. Fehm*. Depts of Medical Psychology and Internal Medicine, University of Ulm, D 7900 Ulm, Germany.

Glucocorticoids are assumed to exert differential effects on central nervous functions via different receptor types described in rat brain (de Kloet & Reul, 1987). In man we previously studied the influences of hydrocortisone, which increases cortisol levels, as opposed to dexamethasone, which suppresses endogenous cortisol secretion, on sensory functions. In auditory tasks opposite effects of these steroids were most prominent on stapedial reflex: with higher cortisol levels reflex needed more dB to be elicited. In the present experiment we studied this effect during stress induction. In 24 subjects repeated measurements of stapedial reflex and salivary cortisol levels were carried out timed in parallel. Unexpectedly ss were asked to deliver a videotaped speech in bright light which led to a sharp rise of cortisol levels in most of the ss. A second control session followed the identical time course without speech. Significantly more dB were needed to elicit stapedial reflex at the time of cortisol maximum as compared to the minimum after recovery from stress 100 minutes later or to control session. Our results suggest that high glucocorticoid secretion attenuates perceptual abilities.

561.7

AROMATASE ACTIVITY IN PREOPTIC AREA, HYPOTHALAMUS, AND AMYGDALA OF INTACT AND CASTRATE C57BL/6J, DBA/2J, AND B6D2F1 MALE HOUSE MICE. Sinchak, K.[1], C.E. Roselli[2] and L.G. Clemens[1]. [1]Dept. Zoology and Neuroscience Program, Michigan State University, E. Lansing, MI 48824. [2]Dept. of Physiology, Oregon Health Sciences University, Portland, OR 97201.

Most male B6D2F1 hybrid house mice continue to copulate after castration while males of the parental inbred strains (C57BL/6J and DBA/2J) do not. Retention of copulatory behavior after castration in the B6D2F1 male is estrogen dependent, and inhibited by treatment with an aromatase inhibitor (ATD). However we could not distinguish between castrated males that continued to copulate and those that did not based on levels of serum androgens and 17β-estradiol as well as aromatase activity (AA) and estrogen receptor levels in the preoptic area (POA), hypothalamus (HYP), and amygdala (AM) (Soc. Neu. Abst. #308.12, 1990). Because castration abolishes copulatory behavior in the C57BL/6J and DBA/2J strains of mice, but not B6D2F1 males unless treated with ATD, we wished to determine if there are strain differences in AA in the POA, HYP, and AM of intact and castrated C57BL/6J, DBA/2J, and B6D2F1 mice. Adult males were either castrated or sham castrated 18 weeks prior to removal and dissection of the brain for AA assay. Levels of AA in the POA and HYP were greater in intact DBA/2J males than C57BL/6J and B6D2F1 males ($p < 0.05$), but no strain differences were seen in the AM. Compared to intacts, castration reduced AA in each brain region of all three strains of mice ($p < 0.05$). However there were no strain differences in AA in any brain region after castration. In conclusion neural AA differs between strains of intact mice, however, based on this measure we cannot distinguish between strains of mice that differ in the display of copulatory behavior after castration.

561.8

IN VITRO DEVELOPMENTAL CHANGES IN BRAIN AROMATASE OF THE DOVE AND GERBIL. J. B. Hutchison, A. Wozniak, S. D. Holman and R.E.Hutchison. MRC Neuroendocrine Development & Behaviour Group, IAPGR, Babraham, Cambridge, CB2 4AT, UK.

Aromatization of testosterone to estradiol is required for differentiation of brain structure and behavior. In the dove and gerbil aromatase forms estrogen in preoptic cells associated with male sexual behavior. We compared the *in vitro* kinetic properties of the aromatase in the two species. 3H-testosterone and -androstenedione were used as substrates. Adult dove aromatase is > 3 times as active as in the developing (days 1-3 posthatching) POA, and has a Km 10 times lower than the developing enzyme (4 and 40 nM, respectively). Thus, there seem to be two isoenzymes of dove aromatase with different Km values, the more active adult form being only expressed in later life. The kinetics of aromatase activity in the gerbil show a different developmental pattern to the dove, with neonatal preoptic aromatase twice as active as the adult. Km (30 nM) does not change during development , and neonatal preoptic aromatase activity does not show any sex dimorphism. Significant aromatase activity is also present in the neonatal cortex, but is absent from the adult. Preoptic aromatase activity in both developing and adult gerbil is much lower than in the dove. Using 3H-androstenedione as substrate similar Km values were obtained for the aromatase within each species. We conclude that (a) there are species differences in the preoptic aromatase; (b) the substrate affinity of the avian enzyme and possibly its form changes during development. This change is not evident in the mammalian (gerbil) brain.

561.9

ARE BRAIN MECHANISMS OF SEXUAL BEHAVIOR IRREVERSIBLY LATERALIZED BY NEONATAL ANDROGENS IN GERBILS? S. D. Holman and J. B. Hutchison. MRC Neuroendocrine Development & Behaviour Group, IAPGR, Babraham, Cambridge, CB2 4AT, UK.

Androgens are linked to asymmetric brain development in the gerbil. The volume of the left, but not the right, sexually dimorphic area, pars compacta (SDApc) is related to the rate of the male-type upsweep ultrasounds (Holman and Hutchison, Dev. Brain Res., 1991). We compared the effects of neonatal castration (NC, n=8), laryngeal motor nerve denervation (LD, n=25) and sham control (SD, n=18) in adult males on SDApc volume and vocalizations. Left and right SDApc volumes were measured 96 h after surgery. Upsweep vocalization rates (per min) were significantly reduced in the LD group (pretransect.= 44.7±7.2: post-transect.=0.4±0.03), remained constant in the SD group, but were partially demasculinized in NC males (22.5± 6.0). Other sexual behaviors were not changed in LD and SD groups. After transection or sham-treatment the correlation between left SDApc volume and pretransection emission rate was highly significant in both groups (LD, rho=0.64, $p<0.002$: SD, rho=0.68: $p<0.01$), but the right SDApc volume and pretransection emission rates in the LD group also reached significance (rho=0.46, $p<0.05$. SD, rho=0.34, $p>0.1$). A symmetrical relationship was also found in NC males (left, rho=0.68, right, rho=0.71, $p<0.05$). We conclude that (a) the asymmetric relationship between vocalizations and SDApc is eliminated by neonatal castration suggesting a role for gonadal hormones in lateralization during development and (b) brain asymmetry in sexually active males can be modified by de-efferentation showing that brain plasticity is not irreversibly affected by neonatal hormones.

561.10

AGING AND PREOPTIC AREA AROMATASE IMMUNOREACTIVITY IN MALE JAPANESE QUAIL. M.A. Ottinger, N. Thompson, T. Dellovade[+], N. Harada*[|], and E.F. Rissman[+]. Univ. of Maryland, College Park, MD 20742, [+]Univ. of Virginia Charlottesville, VA 22903, and [|]Fujita Health Univ. Toyoake, Japan.

An age-related decline in reproductive, endocrine, and behavioral responses occurs in male Japanese quail. Decreased behavioral activity precedes measurable changes plasma testosterone and estradiol. Changes in hypothalamic catecholamines and reductase activity have been found during aging. This experiment investigates the immunoreactivity (IR) of aromatase enzyme (AR) in the preoptic area of young and aged male quail. Five young (6 months) sexually active and five old (3 yrs) behaviorally inactive males were compared using a polyclonal antibody raised in rabbit against human aromatase enzyme. Colocalization of AR-IR and estrogen receptor IR was examined using a monoclonal antibody (H222 provided by Abbot Labs) raised against purified estrogen receptors (ER) in rat. Results show a correlation in aging males of declining testes size, plasma testosterone, sexual behavior and both quantity of AR positive stained cells and the density of stain within these cells. In addition, in aged animals a larger proportion of AR-IR cells colocalized with ER in aged males compared with young males. Supported by NSF grant BNS 9021226 (EFR) and USDA 88-37242 (MAO).

561.11

RAPID VISUALIZATION OF OCCUPIED ESTROGEN RECEPTORS IN THE RAT BRAIN BY IN VITRO AUTORADIOGRAPHY. M. J. Walters, T. J. Brown, R. Hochberg* and N. J. MacLusky. Dept. Obst. and Gyn., Univ. Toronto, Toronto, ON M5G 1L7 and Yale Univ. Sch. Med., New Haven, CT 06510.

Quantitative receptor autoradiography is a powerful tool for studying steroid hormone binding in the brain, as it permits a precise quantification of radiolabeled hormone uptake combined with a high degree of anatomical resolution. Until now, autoradiographic characterization of estrogen binding in the brain could only be achieved through *in vivo* administration of radiolabeled estrogen prior to removal of the tissue samples for processing. We now report the development of an autoradiographic procedure which utilizes exchange labeling of occupied neural estrogen receptors by ^{125}I-estrogen *in vitro*. Intact adult female rats were injected with a saturating dose of unlabeled estradiol (36 µg/kg) 60 min prior to sacrifice. Thin frozen sections (16-20 µm) were cut through the hypothalamus and preoptic area, thaw-mounted onto microscope slides, and rapidly re-frozen. The sections were then incubated with 2 nM ^{125}I-labeled 11β-methoxy-16α-iodo-estradiol, a synthetic estrogen, for 1-8 hr at 37°C. The sections were washed, dried, and exposed to a sheet of high-resolution Hyperfilm for 24 hr. High levels of binding were evident in the medial and periventricluar preoptic areas, bed nucleus of the stria terminalis, ventromedial nucleus of the hypothalamus, the arcuate-median eminance region, and in the amygdala. Maximal binding was obtained at 2 hr and remained stable for up to 8 hr. Background labeling was low and compared favourably with that obtained with *in vivo* labeling methods. No specific labeling was evident in sections incubated with a 100-fold molar excess of unlabeled estradiol, in sections obtained from ovariectomized females, or in sections incubated at 4 or 25°C. This technique circumvents many of the problems inherent in *in vivo* labeling procedures and permits, for the first time, the selective autoradiographic analysis of occupied estrogen receptors in the brain.

Supported by MRC Canada (N.J.M.) and NIH CA37799 (R.H.).

561.12

IN SITU HYBRIDIZATION FOR ESTROGEN RECEPTOR mRNA IN THE MALE RAT BRAIN FOLLOWING LONG TERM TESTOSTERONE DEPRIVATION AND REPLACEMENT. C.A. Lisciotto and J.I. Morrell. Institute of Animal Behavior, Rutgers Univ., Newark, NJ 07102.

Following long term testosterone (T) deprivation, several days of T exposure is required to restore T-dependent behaviors, including male reproductive behavior. In situ hybridization was used to determine the location and relative amount of estrogen receptor (ER) mRNA in the male rat brain following T deprivation and replacement. Adult male rats were gonadectomized, and 6 weeks later received T or cholesterol via silastic implant, sc. After 3, 10, or 21 days of T exposure, the rats were tested for male reproductive behavior and were sacrificed. ER mRNA was detected by hybridization to an 850 base ^{35}S-labeled cRNA probe (ER cDNA - M. Muramatsu). Hybridization (1 X 10^6 cpm/slide) took place at 60°C for 20 hrs; autoradiographic exposure: 2-6 wks. Robustly labeled ER mRNA containing cells were abundant in the medial preoptic area, bed nucleus of the stria terminalis, arcuate and ventromedial nuclei of the hypothalamus. T exposure altered the relative amounts of ER mRNA. T exposure for 10-21 days was necessary to fully restore male reproductive behavior. We are currently comparing the time course of T-induced alteration in ER mRNA and T-mediated restoration of male reproductive behavior.

561.13

CASTRATION ALTERS TYROSINE HYDROXYLASE IMMUNOREACTIVITY IN THE MEDIAL AMYGDALOID NUCLEUS OF MALE HAMSTERS. S.E. Asmus and S.W. Newman, Department of Anatomy and Cell Biology, University of Michigan, Ann Arbor, MI 48109-0616.

The medial amygdaloid nucleus (Me) processes chemosensory and hormonal information essential for mating behavior in the male Syrian hamster (*Mesocricetus auratus*). With colchicine pretreatment, the hamster Me contains a large population of tyrosine hydroxylase-immunoreactive (TH-IR) neurons, distributed preferentially in the midrostral and caudal regions of this nucleus. To determine whether TH production in these cells is influenced by gonadal steroids, the number of TH-IR neurons was compared in Me of castrated and intact hamsters. At 2, 4, 6 and 12 weeks after castration, age-matched intact and castrated males were injected intraventricularly with colchicine and perfused 48 hrs later with 4% paraformaldehyde. Coronal brain sections (40 um) were incubated with a monoclonal TH antibody (1:10,000; Incstar) which was visualized using the immunoperoxidase technique. The number of TH-IR neurons in midrostral Me declined to 48% of control values 4 weeks after castration. This number returned to control values 12 weeks after castration. The number of TH-IR neurons in caudal Me was similar in castrated and intact animals at all postcastration intervals. These results suggest that circulating testosterone maintains the levels of TH in midrostral Me, a region where lesions eliminate mating behavior. (Supported by NIH, NS 20629 to SWN).

561.14

THE IMPORTANCE OF BRAIN AROMATIZATION FOR SEXUAL DIFFERENTIATION OF ZEBRA FINCH BRAIN. A.P. Arnold & B.A. Schlinger. Department of Psychology and Laboratory of Neuroendocrinology of the Brain Research Institute, UCLA, Los Angeles CA 90024-1563.

The development of extreme sexual dimorphism in brain regions controlling song in zebra finches is thought to be controlled by estrogen secreted by the young male. The evidence favoring this hypothesis is that development of the song system is strongly masculinized by estradiol (E_2) treatment of young females. However, questions persist about the role of estrogen in neural development because E_2 can also have demasculinizing effects in young females, and it is not clear that there are sex differences in plasma levels of estrogen during development. We have re-measured plasma levels of sex steroids during the first 13 days after hatching, and can detect no sex differences in levels of E_2, estrone (E_1), testosterone, androstenedione (A), or dihydrotestosterone.

We also measured aromatase activity (^{3}H-A conversion to ^{3}H-E_1 and ^{3}H-E_2) in various tissues of hatchlings. Aromatase was abundant in ovary but was not detected in testes, adrenals, or other peripheral tissues of males. Aromatase was also found in di- and telencephalon, but there was no sex difference in homogenates of whole brain or in cellular subfractions of telencephalon. The distribution of aromatase suggests that if estrogen is the primary masculinizing agent during development of the vocal control system of males, it is synthesized locally in brain at sites appropriate for action on that neural circuit. In contrast, because the developmental pattern of gonadal aromatase (high in ovary, low in testis) is similar to non-songbirds, demasculinization of copulatory behavior in zebra finches may rely on ovarian estrogen as is assumed to occur in other avian species. Preliminary studies indicate the possible importance of sex differences in estrogen catabolism in the process of sexual differentiation. Supported by NIH grants NS08649, DC00217, and NSF grant BNS9020953.

561.15

THE BRAIN AS A SOURCE OF CIRCULATING ESTROGEN. B.A. Schlinger & A.P. Arnold. Department of Psychology and Brain Research Institute, UCLA, Los Angeles CA 90024-1563.

Local conversion of androgen to estrogen (aromatization) often precedes androgen action in the male brain. While the enzyme aromatase is ordinarily restricted to limbic structures in brain of higher vertebrates, it is also found throughout the telencephalon of zebra finches. Estrogen (E) also circulates at high levels in males of this species. However, when we measured aromatase activity in testes, adrenals, brain and other tissues of adult male zebra finches, activity was found only in brain. Because of the abundance of aromatase in telencephalic microsomes, we have postulated that circulating E in males of this species is derived from brain.

To test this hypothesis, we measured E synthesis *in vivo* by quantifying ^{3}H-E (estrone and estradiol) in the circulation of males and females after injection of ^{3}H-androstenedione (^{3}H-A). Authentic ^{3}H-E was present in jugular vein plasma 5 min after as little as 185 pg ^{3}H-A (1 µl propylene glycol) was injected into the aromatase-rich dorsal telencephalon. Despite the recovery of similar amounts of ^{3}H-A in blood, ^{3}H-E was undetected in jugular plasma after identical ^{3}H-A injections into the cerebellum. ^{3}H-E was also present in plasma collected from the carotid artery 5 min after injection of 1 to 6 ng ^{3}H-A into the ovary but not after injection into the left testis. Finally, 5 min after 50 µl saline containing 6 ng ^{3}H-A was injected into the jugular (near the thorax) of males, ^{3}H-E was present in jugular plasma (collected near the skull) but not in carotid plasma. These results show that plasma androgen can be converted to E by brain and released back into blood. Moreover, the absence of E in male plasma after androgen is injected outside of aromatase-rich brain regions suggests that relatively little E synthesis occurs outside of brain. This control of plasma E by brain synthesis represents a novel neuroendocrine mechanism for regulating body levels of a sex steroid hormone. Support: NSF BNS-9020953 and NIH DC00217.

561.16

NEURAL DISTRIBUTION OF ESTROGEN RECEPTOR AND LHRH IMMUNOREACTIVITY ASSOCIATED WITH PUBERTY IN THE FEMALE MUSK SHREW. T.L.Dellovade and E.F.Rissman NSF Center for Biological Timing, Univ. of Virginia, Charlottesville, VA 22901

Female musk shrews are sexually receptive shortly after weaning (21 days of age) and are fertile by 23-25 days of age. Also, females have adult steroid hormone concentrations in plasma and have completed their reproductive tract development by weaning. To describe the neural mechanisms associated with the onset of puberty in this species we used estrogen receptor (ER) and LHRH immunocytochemistry on brains from 10,20,25 and 40 day old females. We used a rat monoclonal antibody raised to human estrogen receptor, H222 (Abbott Labs) and a rabbit polyclonal antibody to LHRH, LR1 (provided by Dr. Benoit) as primary antibodies. The neural distribution of ER immunoreactivity (IR) in the 25 day old females did not differ from that found at 40 days of age. In addition, there is a similar density of positively stained cells in the preoptic area (POA) and hypothalamic nuclei at all ages. In contrast, the limbic regions, including the lateral septum,bed nucleus of the stria terminalis,and several nuclei of the amygdala,have fewer positively stained cells at 10 and 20 days of age. LHRH-containing cells and fibers extend from the accessory olfactory bulb to the median emminence along the basal regions of the brain at all ages. The medial POA has many LHRH positive fibers at 10 and 20 days of age, but very few positive cell bodies. In contrast,this same area contains many LHRH-IR cells and a less dense fiber network at 25 and 40 days of age. These results indicate that by 25 days of age the distribution of both ER-IR and LHRH-IR is similar to that seen in 40 day old animals. These neuroanatomical findings are consistent with our behavioral and hormonal data. Supported by NSF grant BNS 9021226.

561.17

DISSOCIATION KINETICS OF NEURAL ESTROGEN RECEPTORS. X. Chen* and N. G. Simon. Center for Molecular Bioscience and Biotechnology, Lehigh University, Bethlehem, PA 18015

The biological potency of a steroid-receptor complex may be linked to its dissociation rate (k_{-1}) after transformation to a form capable of binding to DNA. Understanding this process may help elucidate a mechanism contributing to neural target tissue sensitivity or insensitivity to a particular steroid, but to our knowledge such studies have been conducted only with receptor complexes from peripheral tissues. Preliminary experiments showed that factors limiting this work were the relatively low concentration of neural receptors and their instability during warming in vitro. By binding estrogen-estrogen receptor (ER) complexes to hydroxylapatite (HAP) prior to activation, sufficient material was available to quantitate in vitro dissociation kinetics. CF-1 female mice were maintained in accordance with Federal guidelines for animal care. Hypothalamic ER were incubated with 15 nM $[^3H]E_2$ for 6 - 8 hrs at 0°, bound to HAP and activated by warming at 29° for 15 min. The dissociation reaction showed both fast and slow components consistent with peripheral ER studied in a free receptor assay. The k_{-1} for the two components were $5.1 \pm 2.3 \times 10^{-1}$ min^{-1} and $8.6 \pm 1.8 \times 10^{-3}$ min^{-1}. Salt concentration (0.04M or 0.4M KCl) did not significantly affect the dissociation rate of either the fast or slow component, although the 0.4M KCl condition slightly increased the proportion of activated ER.

561.18

ANDROGEN AND ESTROGEN CONCENTRATING NEURONS IN THE MEDIAL PREOPTIC AREA AND MEDIAL AMYGDALOID NUCLEUS OF THE MALE SYRIAN HAMSTER. S.W. Newman, R.I. Wood, J. M. Swann, and R.K. Brabec*. Department of Anatomy and Cell Biology, University of Michigan, Ann Arbor, MI 48109-0616.

The medial preoptic area (MPOA) and medial amygdaloid nucleus (Me) are essential for male sexual behavior in the Syrian hamster. These nuclei integrate chemosensory stimuli and gonadal steroid signals, both of which are required for mating behavior. The objective of this study was to compare the distribution of androgen and estrogen-concentrating neurons in MPOA and Me in the male hamster using steroid autoradiography for estradiol (E2), testosterone (T) and dihydrotestosterone (DHT). Adult males (n=4 per group) received two ip injections of tritiated steroid 6-7 days after castration. The brains were removed and frozen 2h after the first injection, and 6 um frozen sections were mounted onto NTB-2 emulsion-coated slides. The slides were developed with D-19 after exposure for 11-16 months. In both MPOA and Me, neurons were more abundant and more heavily-labeled with E2 treatment than with either T or DHT. Estradiol and DHT-labeled cells were scattered throughout Me, with a high concentration in posterodorsal Me. T-labeled cells were confined to posterodorsal Me. In MPOA, the majority of E2 and DHT-labeled neurons were in the medial preoptic nucleus (MPN) and the preoptic regions of the medial bed nucleus of the stria terminalis. T-labeled cells in MPOA were most prevalent in the MPN. Throughout Me and MPOA, the distribution of E2-labeled cells is more widespread than that of T or DHT. These results provide a detailed comparison of E2, T, and DHT-concentrating neurons in nuclei important to the control of mating behavior. (Supported by NIH-NS-20629)

561.19

LOCALIZATION OF THE CANARY ANDROGEN RECEPTOR mRNA. K.L. Nastiuk and D.F. Clayton. Lab. of Animal Behavior, Rockefeller Univ., New York, NY 10021.

In order to investigate the mechanisms by which gonadal steroids regulate plasticity in the avian song control circuit, we cloned the canary androgen receptor (cAR) and have begun experiments to examine its localization. By in situ hybridization using single-stranded 35S-labelled RNA probes, the receptor message can be detected in the brain but is of very low abundance. The cAR mRNA appears to be relatively enriched in several of the functionally defined song nuclei of the canary brain, including HVc, RA and MAN, which concentrate labeled testosterone (Arnold, JCN 189:421, 1980). We are now using the cAR probe to ask: what cell types in the song circuit express the gene? is there a relationship between cAR gene expression and phases of song development and plasticity? is the cAR gene itself under gonadal steroid regulation (for example, by estrogens which are sufficient to induce "masculinization" of the song circuit in young female zebra finches)?

561.20

ESTROGEN-RECEPTOR IMMUNOREACTIVE NEURONS IN THE FOREBRAIN OF ADULT MALE AND FEMALE FERRETS. S.A. Tobet, M. Basham*, T.O. Fox, & M.J. Baum. Dept. of Biochem., EK Shriver Ctr., Waltham, MA 02254 & Prog. Neurosci., Harvard Medical School, Boston, MA 02115, & Dept. of Biology, Boston University, Boston, MA 02215.

We have previously described estrogen binding neurons in and around the preoptic/anterior hypothalamic area (POA/AH) of male and female adult ferrets using autoradiographic methods (Tobet & Baum, 1991; Dev Brain Res, in press). The present study confirms and extends our observations to estrogen receptor immunoreactive neurons throughout the basal forebrain in the context of regional selectivity and sex differences. Estrogen receptors were visualized using the H222 estrogen receptor antibody (Abbott Laboratories) and Nickel/DAB reaction product (H222ir). One of the most striking features of H222ir cells was the presence of H222ir in neuronal processes. All cells with H222ir in processes also contained H222ir in nuclei, although the converse was not true. In females, increased estradiol levels were associated with decreased immunoreactivity in processes. Cells in the bed nucleus of the stria terminalis and ventrolateral septum were notable for the absence of H222ir in processes. The lateral hypothalamus was rich in H222ir processes in both sexes. More H222ir was noted in the processes of female cells in the dorsal lateral septum and medial POA/AH and at the level of the descending fornix and caudal anterior commissure. The H222ir in neuronal processes in the region comparable to the MN-POA/AH in females presented the appearance of nuclear grouping even though no nucleus is apparent by Nissl stains. The differential dendritic organization of estrogen-binding in multiple brain regions might provide another level of regulation for sex-selective estrogen-dependent functions.

561.21

ARGININE^8-VASOPRESSIN (AVP) POTENTIATES ACUTE ETHANOL-INDUCED MOTOR IMPAIRMENT IN THE RAT. P.H. Wu*, J.F. Liu*, S.J. Mihic*, Y. Israel and H. Kalant*. Department of Pharmacology, Univ. of Toronto, and Addiction Res. Foundation of Ontario, Toronto, Canada M5S 1A8.

Earlier studies have shown clearly that AVP maintains ethanol (EtOH) tolerance after cessation of chronic EtOH treatment. However, the acute interaction of AVP and EtOH has not been well characterized. For that purpose, male Sprague Dawley rats (200 g) were trained on the moving belt test, to a criterion of less than 1.2 sec off belt in a 2 min trial. They were then tested under EtOH (1.5 g/kg IP), and assigned to groups (6 animals/group) matched with respect to their EtOH impairment scores. The EtOH dose-response relationship (range 1.0 - 2.0 g/kg) on the moving belt test was then determined in the same rats, after pretreatment with saline, AVP (10 µg SC or 10 ng ICV), the AVP-V_1 receptor antagonist [Des-Gly^9,d(CH$_2$)$_5$^{1},O-Et-Tyr^2,Val^4,Arg^8]-vasopressin(10 ng ICV), and the V_1 antagonist in combination with AVP (both given ICV, the antagonist 30 min before the AVP). AVP produced a 16% decrease of ED50 for EtOH when given either SC [$F(1)=11.49$, $p<0.002$] or ICV [$F(1)=10.78$, $p<0.002$], without altering blood alcohol levels. Neither AVP nor the V_1 antagonist alone caused any motor impairment. AVP potentiation of EtOH effect was abolished by co-administration of the V_1 antagonist, but the antagonist alone did not attenuate the EtOH effect. Other rats were made EtOH-tolerant by 5 daily injections of either EtOH alone (1.8 g/kg IP) or EtOH (1.5 g/kg IP) + AVP (10 µg SC), each followed by a practice session on the moving belt. In both sets of tolerant animals, AVP potentiation of acute EtOH effects was still seen on day 6 [$F(1)=57.29$, $p=0.000$]. The mechanism of this AVP potentiation of EtOH is unknown, but the failure of the V_1 antagonist alone to alter the effect of EtOH suggests that endogenous AVP is not involved directly in modulating EtOH intoxication. Supported by NIAAA grant #1 R01-AA08212-02.

562.1

CIRCADIAN ACTIVITY AND STARTLE AMPLITUDE IN RATS EXPOSED TO CONSTANT LIGHT: EFFECTS OF OVARIECTOMY AND E2 REPLACEMENT. J. Kaster*, I. R. Cohen and J. Buelke-Sam. Toxicol. Res. Labs, Lilly Res. Laboratories, Eli Lilly & Co., Greenfield, IN 46140.

Estradiol (E2) treatment fails to elicit an LH surge in ovariectomized (OVX) rats following short-term exposure to constant light (LL) (Neurosci. Abst., 16: 1200, 1990). In this study, 55 Fischer 344 rats housed in a 12 hr light: 12 hr dark condition (LD) were assigned to 1 of 3 treatment groups: intact; OVX on Day 0; or OVX on Day 0 and E2 Silastic implant on Day 7. Half of each treatment group was placed into LL on Day 0. Activity under LL or LD was measured on Days 7-9 for 23 hrs/day in SDI figure-8 monitors. Acoustic and tactile startle amplitudes were measured in automated SDI chambers on the morning of Day 10. There were no significant effects of treatment during the first hour or on the pattern of activity over days within either lighting condition. However, LD rats from all groups showed greater habituation across test days during the first hours of testing, as well as greater total activity during the D phase. LL rats showed activity bursts distributed throughout the 23-hr sessions. Startle amplitude of the OVX and OVX+E2 groups was decreased significantly only in the LD rats. Tactile startle was significantly greater than acoustic startle for LL and LD rats from the intact and OVX groups, but not for OVX+E2 rats. Together, these results suggest that OVX, with or without E2 replacement, interacts with lighting condition to alter startle, but does not affect the LD circadian pattern of activity or the disrupted pattern produced by LL.

562.2

N-METHYL ASPARTIC ACID INJECTIONS INTO THE MEDIAL AMYGDALA FACILITATE MATERNAL BEHAVIOR IN RATS. M. Numan, M.J. Numan* and J.B. English*. Dept. of Psychology, Boston College, Chestnut Hill, MA 02167.

The inhibitory role of the medial amygdala (MA) in maternal behavior control was explored. Injections of neurotoxic doses of N-methyl aspartic acid (NMA: 8 ug/ .4 uL) into MA facilitated maternal behavior in nulliparous female rats when pups were presented to them 12 days following the injections. This effect was specific to MA in that NMA injections into the basolateral amygdala were ineffective. Also, the facilitatory effect of NMA injections into the MA was eliminated when ovariectomies were performed at the time of brain surgery. In a subsequent experiment, vaginal smear data indicated that NMA-MA injections induced a pseudopregnant state that lasted about 13 days in females that retained their ovaries. That this pseudopregnant state contributed to the facilitation of maternal behavior was supported by the finding that bromocriptine (a drug that blocks prolactin release) administration suppressed both pseudopregnancy and the facilitation of maternal behavior induced by NMA-MA injections. A final experiment is testing this view further by presenting test pups to females 24 days, rather than 12 days, after NMA-MA injections. It appears that a neuroendocrine mechanism underlies the observed facilitation of maternal behavior.

Supported by a Whitehall Foundation grant.

562.3

DO SEXUALLY MONOMORPHIC PATTERNS OF PLASMA E_2 AND ANDROGENS DURING NESTLING DEVELOPMENT ACCOUNT FOR THE LACK OF A SEX DIFFERENCE IN ADULT SINGING BEHAVIOR IN THE EUROPEAN ROBIN? E.S. Hiatt and H. Schwabl*. *Max-Planck-Institut für Verhaltensphysiologie, Andechs, D-8138, W. Germany; Dept. of Biology, Northeastern Univ., Boston, MA 02130 and Rockefeller Univ. Field Research Center, Millbrook, NY 12545.*

Circulating levels of estradiol-17β (E_2), aromatizable testosterone (T) and non-aromatizable 5-dihydrotestosterone (DHT) were measured in male and female European robin chicks from day 3 to day 19 after hatching. E_2 concentrations were not different between the sexes, but varied significantly with age in males. Levels in males were higher during days 13 and 17 than days 11 and 19, when they were close to basal (50 pg/ml in our RIA system). Levels of T were low though not basal during the first days after hatching in both sexes. They were high at the time of fledging (day 13-15) and decreased by day 19 to basal (50-100 pg/ml). No sexual dimorphism in T levels was apparent. Levels of 5-DHT were very high in both sexes during the early nestling phase and then steadily decreased to basal levels by day 19 in both sexes. These results are consistent with the hypothesis that in species of songbirds in which both sexes sing as adults, as in the robin, levels of sex steroid hormones are elevated and sexually monomorphic during early development, when the neural song control system is organized.

562.4

INFUSIONS OF RAT PROLACTIN AND HUMAN PLACENTAL LACTOGEN INTO THE MEDIAL PREOPTIC AREA STIMULATE MATERNAL BEHAVIOR IN OVARIECTOMIZED, NULLIPAROUS RATS. R.S. Bridges and M. Freemark[1]*. Dept. Comparative Medicine, Tufts University School of Veterinary Medicine, North Grafton, MA 01536 and [1]Dept. Pediatrics, Duke University Medical Center, Durham, NC 27710.

The onset of maternal behavior at parturition is induced in part by the changes in hormone secretion during gestation. In rats, infusions of ovine (o) prolactin (PRL) into either the lateral ventricles or the medial preoptic area (MPOA) of steroid-primed, bromocriptine-treated, nulliparous females stimulates maternal behavior (Proc. Natl. Acad. Sci. USA 87, 8003, 1990). The present study examined the effectiveness of the endogenous lactogen, rat (r) PRL, and a heterologous lactogen, human placental lactogen (hPL), in the neural-hormonal stimulation of maternal care. Adult nulliparous rats were ovariectomized and fitted with bilateral cannulas directed at the MPOA. One week later each rat was implanted s.c. with progesterone- (treatment days 1-11) followed by estradiol-filled (days 11-17) Silastic capsules. Endogenous PRL was suppressed by twice daily injections of bromocriptine (days 11-17). From days 11-13 animals received 5 bilateral infusions of rPRL (40 ng/side), hPL (40 ng/side) or vehicle (0.4 µl). Testing for maternal behavior with foster young was conducted daily from day 12 to 17. Both lactogens stimulated a rapid onset of maternal behavior. Latencies to pup retrieval and full maternal behavior were significantly shorter in the rPRL and hPL groups (0.5-1.5 days) than in vehicle controls (4.0 days). These results demonstrate that rPRL and a heterologous placental lactogen, hPL, stimulate maternal care when infused into the MPOA, and suggest that the behavioral effects of PRL may be shared by other endogenous lactogenic molecules, i.e. placental lactogens. [Supported by NIH Grant HD19789 awarded to RSB]

562.5

EFFECTS OF ENERGY INTAKE, DEMAND, AND PARTITIONING ON NESTBUILDING IN SYRIAN HAMSTERS. A. J. Bhatia, J. E. Schneider*, and G. N. Wade. Neurosci. and Behav. Prog., Depart. of Psychology, Univ. of Mass., Amherst, MA 01003.

Female mammals use a variety of strategies to insure a constant supply of energy for the cellular processes necessary for survival and optimal reproduction. For example, increased nestbuilding in response to cold exposure decreases the energy demands for thermoregulation. We have been studying the effects of food intake and cold ambient temperatures on nestbuilding in Syrian hamsters.

In experiment 1, there was a trend toward lower nestbuilding scores in females fed diets associated with the highest food intake and body weight gain at ambient temperature 22°C. However, when females were housed at 5°C, the differences in nestbuilding among the diet groups were exaggerated and were statistically significant ($p < 0.05$).

In many species, energy partitioning varies with reproductive state. Syrian hamsters lose body fat content without changing food intake during pregnancy. In experiment 2, hamsters showed significant increases in nestbuilding in response to both pregnancy and low ambient temperature (22 vs. 5°C) ($p < 0.05$). In the third experiment, nestbuilding was measured in ovariectomized hamsters implanted with either estradiol (E), progesterone (P), E+P, or empty Silastic capsules. There was a trend toward larger nests with significantly lower body fat contents in hamsters treated with E or with E+P. Taken together, the above results suggest that nestbuilding is increased in Syrian hamsters under environmental and reproductive conditions in which energy demand is high and energy availability low. (Supported by NS10873, AM32976 from NIH, and BNS8719361 from NSF.)

562.6

COCAINE DISRUPTS MATERNAL BEHAVIOR IN LACTATING RATS. C.H. Kinsley, A. Bauer*, M. Beverly*, D. Turco* & J. Wellman*. Department of Psychology, University of Richmond, VA 23173.

Though much attention has been devoted to the behavioral and physiological consequences of cocaine (COC) abuse, little is known regarding the effects on maternal behavior (MB). We examined whether COC affects the initiation (late pregnancy) and/or maintenance (postpartum [PP]) phases of MB. In Exp. 1 COC (5 or 10 mg/kg) or saline was administered on PP day 5 or 6 and MB scored. 5 mg/kg was marginally effective ($p < .1$), whereas 10 mg/kg significantly disrupted full MB. Exp. 2 (with 10 mg/kg) examined specific elements of the disruption and found increases in the latencies to contact, retrieve, lick, group and crouch over pups. In Exp. 3 osmotic mini-pumps containing 20 mg COC/kg/day or saline were implanted sc in day 14 pregnant rats. MB testing was performed on days 1-2 PP together with a T-maze pup-retrieval test on PP days 3-5. COC disrupted MB and performance in the T-maze tests, in general, rendering the females less attentive to young. COC -- perhaps owing to its purported dopamine system activity -- may be affecting prolactin, a hormone which stimulates MB. (Supported by U. Richmond FRC).

562.7

RECOVERY FROM HABENULA LESION-INDUCED DEFICITS IN MATERNAL BEHAVIOR. K.P. Corodimas, J.S. Rosenblatt,* A.D. Mayer* and J.I. Morrell. Inst. Animal Behavior, Rutgers Univ, Newark, NJ 07102.

Previously we demonstrated that bilateral lesions of the habenular complex (Hbc) made on day 12 of pregnancy severely disrupt the hormonal onset of maternal behavior (MB) (retrieving, nursing and nestbuilding) in the rat for 7d. However, by day 8 of testing (13 days after the lesion) the majority of animals in the Hbc lesion group displayed complete MB compared with unoperated controls. These results suggested two hypotheses: either there was a recovery of function during the 13d following the lesion, or pup exposure for 8d stimulated MB despite the lesion. We tested these hypotheses. Bilateral Hbc lesions were made 4 days earlier on day 8 of pregnancy to determine if the passage of time (13d) following the lesion was sufficient for behavioral recovery. All animals were hysterectomized, ovariectomized, and given 20ug/kg estradiol benzoate on day 16 of pregnancy, then tested 48h later with pups for MB. The majority of Hbc-lesioned animals show complete MB by day 3 of testing. Thus, Hbc lesions made earlier in pregnancy result in an earlier recovery in MB. These data suggest that a neural recovery process requiring approximately 13 days may account for the behavioral recovery seen after Hbc lesions. (HD22983 to J.I.M.)

562.8

HORMONAL AND ENVIRONMENTAL INFLUENCES ON MALE INCUBATION IN SEX-ROLE REVERSED SHOREBIRDS. A.J. FIVIZZANI, L.W. ORING* AND M.E. EL HALAWANI* Biology Dept., Univ. of North Dakota, Grand Forks, ND 58202 and Animal Sci. Dept., Univ. of Minnesota, St. Paul, MN 55108.

In a few avian species such as the spotted sandpiper and Wilson's phalarope, males provide all or most of the incubation and brooding of young. Plasma levels of prolactin, the hormone most associated with parental care in birds, is sex-reversed being greater in incubating males than in females. Plasma testosterone declines rapidly at incubation and experimentally maintained high levels interfere with normal incubation behavior. Administration of the androgen blocking agent flutamide, facilitates the onset of incubation. Experimentally switching eggs of know incubation duration to produce longer or shorter than normal incubation periods for particular males indicates that the presence of eggs maintains prolactin beyond the normal time of hatch and the presence of chicks or absence of eggs results in a decline in prolactin before the normal time of hatch. Male incubation appears to be correlated with elevated prolactin, low testosterone and the continued stimulus of eggs in the nest.

562.9

SILASTIC MELATONIN IMPLANTS INDUCE OUT-OF-SEASON SEXUAL BEHAVIOR IN MALE RHESUS MONKEYS. J. G. Herndon, T.H. Robertson*, J.J. Turner* and D.C. Collins*. Yerkes Reg. Primate Res. Ctr. and V.A. Med. Ctr. Emory Univ., Atlanta, Georgia 30322

Seasonally breeding adult male rhesus monkeys (n=8) were moved from outdoor breeding groups to a light-controlled (16L:8D) indoor environment on June 21 (mid non-mating season), and remained on this lighting schedule for 18 months. At the onset of the following mating season, males were randomly divided into two groups and underwent 4 phases of 17 weeks of Melatonin (s.c. Silastic capsule producing 70-80pg/ml serum melatonin) or Control (empty capsule) treatment. For group A, the sequence of control (C) and melatonin (M) treatments in the 4 phases of the study was CMCM; for group B, the sequence was CCMC. The reproductive status of untreated outdoor-housed rhesus monkeys during the four 17-week phases was: (1) early mating season, (2) late mating season - early non-mating season, (3) mid non-mating season, and (4) early mating season. In both groups, phases 1,2, and 4 were characterized respectively by increasing, decreasing and increasing mounting frequency, testosterone level and testis volume. This is similar to the seasonal pattern shown by outdoor-housed animals, and likely represents persistence of an endogenous reproductive rhythm. During phase 3 (when outdoor-housed animals showed no sexual behavior), the group receiving melatonin (group B: CCMC) displayed significant increases in frequencies of mounting. Males not receiving melatonin during phase 3 (group A: CMCM) mounted at a significantly lower rate. Testosterone levels and testis size in the two groups changed with season, but the two groups never differed from each other. The effects of melatonin on sexual behavior thus are independent of changes in testicular function. (NIH grant RR-00165 and Department of Veterans Affairs).

562.10

DIFFERENTIATION OF PERIMENSTRUAL SYMPTOM CATEGORIES AND ENDOCRINE CORRELATES. C.A.Cahill. University of Kansas Medical Center, School of Nursing and School of Medicine Kansas City, KA 66103.

The etiology of dysphoria associated with the last week of the menstrual cycle has puzzled many. Attempts to associate changes in gonadal hormones with symptom severity and type have not demonstrated clear cut associations. Several hypotheses have been offered to explain this apparent contradiction. One possibility is that several different mechanisms are associated with Perimenstrual Symptoms (PS) which may be reflected in different types of symptoms, symptom severity and symptom patterns. In this study, 40 women were selected for study based on symptom type and severity. Twenty women with minimal symptoms and twenty with moderate to severe depression-like symptoms were studied over three consecutive menstrual cycles. Plasma samples were collected twice per week and analyzed for beta-endorphin-like immunoreactivity, cortisol, prolactin and progesterone. During data analysis, six unique patterns of PS were noted. This poster will report attempts to associate hormone levels and patterns with the more rigorous symptom pattern categories.

562.11

BIRTH ORDER AND LEFT-HANDEDNESS: INTERACTION OF PARITY AND SEX ON DIRECTION OF ASYMMETRY IN MICE Robert L. Collins. The Jackson Laboratory, Bar Harbor, ME 04609.

During selective breeding for degree of lateralization for handedness in mice, an interaction of parity and sex emerged for the direction of asymmetry. This interaction affected quantitative and qualitative measures of laterality in males of both HI and LO selected lines. During generations G_8, G_9, and G_{10}, first-litter males were more often left-handed (RPE $\leq$ 25), and second-litter males were more often right-handed (RPE $\geq$ 25): $Pr(RH|L_1) = 0.434$; $Pr(RH|L_2) = 0.557$ ($N_1 = 451$; $N_2 = 210$; $\chi^2 = 8.79$; $p = 0.003$). This interaction was confirmed 5 years later when selection was reimpressed during G_{28}, G_{29}, and G_{30}: $Pr(RH|L_1) = 0.399$; $Pr(RH|L_2) = 0.601$; ($N_1 = 367$; $N_2 = 143$; $\chi^2 = 17.0$; $p = 0.00004$). No changes in frequencies of dextrality were observed for female mice: $Pr(RH|L_1) = 0.480$ and 0.503; $Pr(RH|L_2) = 0.406$ and 0.490, respectively; N's = 585 and 665.

The parity by sex interaction increased as a function of generation number in each selected line. This could be due to cumulative influences of unavoidable inbreeding in finite populations, or to a correlated response to selection for handedness itself—both HI and LO lines necessarily were selected for success in the food reaching task (Collins, In S. D. Glick, *Cerebral Lateralization in Nonhuman Species*, Academic Press, 41–70, 1985).

The male parity effect observed in the selected mouse lines is consistent in type and direction with that suggested by Bakan (*Nature* 229:195, 1971) who reported increased left-handedness in human males of high-risk birth orders (1 and 4+). This finding, however, has proved difficult to replicate (Searleman *et al, Psychol. Bull.* 105:397–405, 1991). Laterality patterns in male offspring of differing birth order may be mediated by hormonal or immunological differences between primiparous and multiparous dams or by differential maternal care. The parity effect also may explain the sporadic and sometimes conflicting reports of nonequiprobably distributed asymmetries in male laboratory animals.

562.12

SEX AND AGE DIFFERENCES IN THE SPONTANEOUS LOCOMOTOR ACTIVITY OF THE MONGOLIAN GERBIL (MERIONES UNGUICULATUS): MULTIVARIATE AND TEMPORAL PATTERNS. **L.A. Mead, E.L. Hargreaves, K.-P. Ossenkopp, and M. Kavaliers. Dept. Psychology and Div. Oral Biology, Univ. Western Ontario, London, Ont., CANADA N6A 5C2.**

A multivariate assessment of the spontaneous locomotor activity of the laboratory bred Mongolian gerbil (Meriones unguiculatus) was obtained using a Digiscan animal activity monitoring system. Motor activity data were collected for 12 consecutive 5 min. samples from each of 42 gerbils obtained from 6 separate litters (aged 24, 39, 62, 116, 150, or 197 days). Variables examined included: total distance travelled (TD), average distance (AD), average speed (AS), number of horizontal movements (NM), time in horizontal movement (MT), time per horizontal movement (TMT), number of vertical movements (NV), time in vertical movement (VT), and time per vertical movement (VMT). Results indicated that across the 12 samples, all activity measures (except AS and TMT) declined for both males and females. Overall differences in locomotor activity between males and females were found over the one hour period on the measures AD and AS. Females exhibited higher levels of activity. Initial sex differences on the measures TD, NV, and VT were strongly reduced after approximately 30 minutes. Age also had an effect on spontaneous activity. All measures of activity increased at the early ages (i.e. for the litters aged 24, 39, and 62 days), and remained relatively constant thereafter (i.e. for the litters aged 116, 150, and 197 days). The activity levels over the one hour period declined more rapidly for the younger litters on the measures of TD, AD, NM, and MT. These results extend previous work examining sex differences in laboratory rats and meadow voles using the Digiscan System.

562.13

ICV PROLACTIN DECREASES INFUNDIBULAR VIP - LIKE IMMUNOREACTIVITY IN INCUBATING DOVES.

C. J. Saldanha and R. Silver. Barnard College of Columbia University, New York, NY. 10027.

Several lines of evidence suggest that vasoactive intestinal polypeptide (VIP) is a prolactin (PRL) releasing factor in birds. Systemic VIP increases, while antibodies to VIP decrease plasma PRL. Additionally, VIP releases PRL from dispersed pituitary cells *in vitro*. Although VIP-like immunoreactive cells are widely distributed in the avian hypothalamus, one group in the infundibulum (INF) increase in diameter during incubation.

To assess whether PRL influences hypothalamic VIP, the effect of icv ovine PRL (o-PRL) was investigated. Breeding doves were given 6 daily injections of o-PRL beginning on incubation day 8 (I 8), while controls received vehicle. Body weight and incubation behavior was monitored during incubation. Following perfusion on I 14, crop weights and gonadal states were measured. The brains were removed, sectioned, and alternate sections were stained for VIP and gonadotropin releasing hormone (GnRH).

O-PRL decreased detectable VIP-like immunoreactive cell number in the INF, but did not change lateral septal VIP or medial preoptic (MPOA) GnRH cell number. O-PRL treated subjects had lower crop weights on I 14, but did not differ from controls in incubation behavior or gonadal state. The results suggest that during incubation, central PRL regulates its own secretion by acting on INF VIP cells. Moreover, the site of feedback is limited to the population of VIP cells that increase in diameter during incubation. Supported by NIMH grant 29380-15.

562.14

EFFECTS OF ESTRADIOL ON EXTINCTION OF CONDITIONED TASTE AVERSIONS: TIME OF ACTION. D.L. Yuan and K.C. Chambers. Department of Psychology, USC, Los Angeles, CA 90089.

Testosterone (T) prolongs extinction of conditioned taste aversions (CTAs) by acting on processes associated with extinction not acquisition. Estradiol (E) increases extinction rate and prevents T from prolonging extinction. The following studies were designed to determine whether E acts during acquisition or extinction. In both studies, a CTA was induced with 0.15M LiCl (10 ml/kg) after access to a 10% sucrose solution and daily extinction trials were initiated 10 or 23 days later. In study 1, the extinction rates of gonadectomized (GX) females given E before and during acquisition and extinction (E/E) were significantly faster than those of GX females given no hormone before and during acquisition and extinction (0/0). The extinction rates of the E/E females and GX females given E before and during acquisition but not extinction (E/0) did not differ and those of the 0/0 females and GX females given E before and during extinction but not acquisition (0/E) did not differ. In study 2, the extinction rates of 0/0 and 0/E GX females were not different. The rates of both of these groups were significantly faster than those of GX females given E during but not before extinction. Thus, E accelerates extinction when present before and during acquisition but prolongs extinction when present during extinction. These results can be accounted for by E toxicity. ONR N00014-89-J-1296

562.15

EFFECTIVENESS OF TESTOSTERONE IN PROLONGING EXTINCTION OF CONDITIONED TASTE AVERSIONS IN FLUID DEPRIVED MALE RATS IS DEPENDENT ON LICL DOSE. E.A. Brownson, C.B. Sengstake,* K.C. Chambers. Neurobiology and Psychology, USC, Los Angeles, CA 90089 and Psychology, PSU, Portland, OR 97207.

Extinction of a conditioned taste aversion (CTA) is modulated by both testosterone (T) and fluid deprivation (FD). Males with normal physiological levels of T exhibit slow extinction rates and gonadectomized males show fast extinction rates. Fluid deprived intact males also exhibit fast extinction rates. The following two studies were designed to determine the relationship between T and FD in Fischer 344 males. In both studies, a CTA was induced in males under a 23 hr FD schedule or under nondeprivation (ND) conditions. The FD and ND males were injected with either a high dose of LiCl (0.30 M, 20 ml/kg) or a low dose (0.15 M, 10 ml/kg). Daily extinction trials began 2 days later. In the first study, blood was drawn from the males prior to the first extinction trial. For both doses of LiCL, the serum T levels in FD and ND males were not different but extinction rates were faster in FD males than ND males. In the second study, FD males were implanted with blank or T-filled capsules. Rates of extinction were faster in blank-treated FD males than ND males. T restored extinction rates to the level found in ND males when the FD males were injected with the high dose of LiCl but not the low dose. These results suggest that FD does not act on a T mechanism to increase extinction rate. ONR N00014-J-1296

562.16

EFFECTS OF GONADECTOMY ON EXTINCTION OF CONDITIONED TASTE AVERSIONS IN FISCHER 344 MALE RATS. K.C. Chambers, E.A. Brownson and D.L. Yuan. Departments of Psychology and Neurobiology, USC, Los Angeles, CA 90089

Extinction of a conditioned taste aversion (CTA) is slower in male rats than females. The extinction rates of Sprague-Dawley (SD) gonadectomized (GX) males are not different than those of females but the rates of Fischer 344 (F344) GX males are slower than those of females. The following experiments were designed to determine whether testosterone (T) plays a role in extinction of CTAs in F344 males. In experiments 1 and 2, a CTA was induced with LiCl (0.15 M, 10 ml/kg) after presentation of a novel 10% sucrose solution and daily extinction trials were initiated 2 days later. In experiment 1, the extinction rates of intact males and GX males were not significantly different. In experiment 2, the extinction rates of untreated GX males were significantly faster than those of GX males implanted with T-filled capsules that produced high physiological levels of T. In experiment 3, the serum T levels of intact F344 and SD males were not significantly different. These results indicate that T prolongs extinction in both F344 amd SD males. However, F344 males appear to be less sensitive to T than SD males. Since GX F344 males but not GX SD males have slower extinction rates than females, perinatal hormones may play a more important role in F344 rats than SD rats. ONR N00014-J-1296

562.17

HORMONAL MODULATION OF THE CUTANEOUS CONTROL OF LORDOSIS IN INFANT AND ADULT RATS.

G.S. Benedict and C.L. Williams. Depts. of Psychology, Columbia University and Barnard College, New York, NY 10027.

Previous work from our laboratory has shown that when 6-day-old rats are stroked on the sides, flanks, and rump they display a lordosis-like response that is facilitated by priming with estradiol benzoate (EB) and progesterone (P). In adult female rats, it is believed that cutaneous input from the anterior curve of the hip is the critical sensory input for the facilitation of lordosis (Kow & Pfaff, 1976). The purpose of this study is to determine the cutaneous control of lordosis in 4- to 6-day-old and adult rats and to determine whether hormone priming influences the specificity of this control. Subjects were denervated on the waist (W), a region containing the anterior curve of the hip, or on the sides (S), a region from axilla to tailbase, or sham denervated. In neonates, denervation of the W and S significantly decreased lordosis of both hormone primed (100 μg EB and 0.5 mg P, administered 40 and 4 hrs prior to testing) and unprimed rats. In contrast, in adult females primed with 100 μg EB and 0.5 mg P, denervation of the W and S had no effect on manually stimulated lordosis. When priming doses of EB were lowered to 5 μg EB and 0.5 mg P, the S-denervated group showed significantly less lordosis than W- or sham-denervated females, which did not differ. When EB priming was given without added P, denervati ons of the W and S were equally effective in reducing lordosis frequency. Results suggest that the waist region is the critical cutaneous input for the facilitation of lordosis in infants and adults. However, the specificity of this input is not under hormonal control in 6-day-old rats and is modulated by EB and P in adult females.

562.18

PROGESTERONE WITHDRAWAL INCREASES ANXIETY IN A RAT MODEL. M.A. Gallo,* J.E. Fletcher and S.S. Smith, Dept. of Anatomy, Inst. for Neurosci., Hahnemann Univ., Philadelphia, PA 19102.

Physiological levels of systemically injected progesterone (P) have been shown to amplify GABA responses of neurons within a model CNS circuit (Smith et al, 1987a,b,c;1989) in a manner similar to the benzodiazepines (BDZ), an effect due to 3αOH-DHP (Harrison et al, 1987), a metabolite formed locally from P. In addition, anxiolytic properties of P have also been demonstrated (Rodriguez-Sierra et al, 1984). The goal of the present study was to test the hypothesis that withdrawal from chronic P treatment could increase anxiety in a rat model using the conditioned defensive burying paradigm. For this paradigm, rats were first habituated to the plexiglass test chamber prior to exposure to an electrified probe (0.05-1 mA from a 350 V source). Once shocked, rats were monitored during subsequent burial of the probe with cage sawdust. Parameters assessed included latency, duration, frequency and pile height of probe burial. Decreases in the latency and increases in the duration of probe burial, compared with control values, are specific as measures of anxiety in that these changes are produced by anxiogenic β-carboline treatment (Tsuda et al, 1989) and reversed by administration of BDZ but not morphine, at analgesic doses (Treit et al, 1981). For the present study, female rats were exposed to 4-day P treatment (500 μgs daily, i.p., in sesame oil) concurrent with 17β-estradiol priming (2 μgs, daily, i.p. in sesame oil) for the first 2 days of the injection schedule. On day 5, withdrawal from chronic P treatment produced increases in anxiety as determined by significant decreases (33%, $P < 0.01$) in latency and increases in duration (55%) of probe burial compared with vehicle-injected controls ($n = 6$, both groups). This anxiogenic effect produced by withdrawal from the steroid could be reversed with subsequent administration of P (500 μgs, i.p.) on day 6 to an estradiol-primed animal. These results suggest that under physiological conditions, "withdrawal" from P during the hormone cycle may increase anxiety. In contrast, combined replacement therapy with estradiol and P is anxiolytic in these animals. The present findings may have relevance for the etiology and treatment of PMS-related anxiety in humans. *(Supp. by NS 25809 to SSS.)*

563.1

EFFECTS OF NEONATAL ADMINISTRATION OF VASOPRESSIN ON CARDIAC AND BEHAVIORAL RESPONSES TO EMOTIONAL STRESS IN ADULT RATS. B.Buwalda*, C.Nyakas*, J.M.Koolhaas* and B.Bohus. (SPON: European Brain and Behaviour Society) Department of Animal Physiology, Centre for Behavioural, Cognitive and Neuro-sciences, University of Groningen, P.O.Box 14, Haren, The Netherlands.

The basis for an adequate neuronal circuit, needed for succesful coping with environmental challenges, is established in the development of inter-neuronal communication. The present study will show the effects of early exposure to the neurohypophyseal peptide arginine-8-vasopressin (AVP) on adult autonomic and behavioral responses to emotional stress. Attention has been also paid to the effect of neonatal exposure to AVP on the modulating properties of vasopressin on the bradycardiac stress response in adulthood. AVP was administered subcutaneously on postnatal days 3-7 in a high (10 μg/100gr bodyweight) and a low dose (1 μg/100gr b.w.) to male Wistar rats. Control animals were untreated or saline injected. Behavioral observations in a novel environment of a complex maze indicated that neonatal administration of AVP increases exploratory behavior in a dose-dependent way. Cardiac monitoring during the conditioned emotional stress of fear of inescapable electric footshock showed that neonatal administration AVP has little effect on bradycardiac stress response. The analysis of cardiac responses also suggested an adult hyposensitivity to AVP in rats treated neonatally with AVP. Furthermore, low doses of AVP were very effective in impairing avoidance behavior in this test situation.

The data indicate that neonatal administration of AVP exerts long-lasting effects upon memory processes related to emotional stress and on the behavioral adaptation to novelty. Neonatal administration of AVP is less effective in changing autonomic stress responses, suggesting a differentiation in time of the critical period of central vasopressinergic systems involved in the regulation of behavior and autonomic functioning.

563.2

NEONATAL STRESS ALTERS DEVELOPMENT OF BRAIN OXYTOCIN RECEPTORS. L.R. Noonan, J.D. Caldwell, L. Li, C.H. Walker, C.A. Pedersen and G.A. Mason. Brain and Development Research Center and Dept. of Psychiatry, School of Medicine, University of North Carolina, Chapel Hill, NC 27599.

A standard neonatal stress procedure consisting of separation and return of rat pups to the mother for brief, daily periods has been shown to permanently diminish endocrine and behavioral stress responses, an effect that may be partially explained by increases in brain glucocorticoid and GABA receptors. We hypothesized that oxytocin (OXT) receptors would be also affected by infant stress because OXT has been reported to modulate stress responses during infancy and adulthood. We separated or did not separate (control condition) Sprague Dawley rat pups for 20 minutes per day, beginning at postnatal day 2 and ending at the time of sacrifice on day 4, 8, 14, or 22. Oxytocin receptor binding was performed using the specific antagonist ^{125}I-OTA. Scatchard analysis indicated that neonatal stress caused a decrease in OXT receptor number (B_{max}), but no changes in affinity (K_D), in hippocampus, cortex and remaining forebrain. There was a 40-60% decrease in OXT receptors in the stress condition at day 4 compared with day 4 controls. The magnitude of this difference decreased across age, although small differences were still evident at day 22 in cortex and remaining forebrain, but not in hippocampus. A developmental decrease in brain OXT receptors produced by infant stress may provide a mechanism by which permanent decreases in stress responsiveness occur.

563.3

TESTOSTERONE-DEPENDENT VASOPRESSIN IN RELATION TO AGGRESSIVE BEHAVIOUR. J.C. Compaan[1], J.M. Koolhaas[1], R.M. Buijs[2], C.W. Pool[2], A.J.H. de Ruiter[1], G.A. van Oortmerssen[1]. (SPON: European Brain and Behaviour Society) [1]Univ. of Groningen, Dept. of Anim. Physiol., P.O.Box 14, 9750 AA Haren, [2]Netherlands Instit. for Brain Res., Meibergdreef 33, 1105 AZ Amsterdam, the Netherlands.

In this study we used mice, genetically selected for aggression. At adult age aggressive males, with a Short Attack Latency (SAL), possess a higher plasma testosterone (T) level compared to males with a Long Attack Latency (LAL). In contrast, at neonatal age, in LAL males a higher T production occurs. In the adult brain, the vasopressinergic (AVP) innervation of the lateral septum (LS) is less abundant in these SAL males. Probably, the neonatal difference in plasma-T level is involved in a differential development of the AVP system in these two selection lines of mice. So far, it is unknown to what extent this system is involved in the differentiation in aggressive behaviour. Therefore adult SAL and LAL males were castrated, and aggression and AVP content were measured 15 weeks later. We found an almost complete reduction in LS innervation of AVP in both selection lines. No difference was found in the nucleus of the solitary tract, a T non-dependent AVP containing nucleus. Surprisingly, only the LAL males show a reduction in aggression. Thus, although AVP in LS is T dependent in both selection lines, only the LAL males are affected in aggression after castration. This suggests a differential involvement of T dependent AVP systems in the organization of aggressive behaviour in the two selection lines of mice.

The investigations were supported by the Foundation for Biological Research (BION-426.061), which is subsidized by the Netherlands Organization for Scientific Research.

563.4

SEXUAL DIMORPHISM IN THE VASOTOCIN SYSTEM OF THE BULLFROG. S.K. Boyd, C.J. Tyler and G.J. DeVries. Department of Biological Sciences, University of Notre Dame, Notre Dame, IN 46556; Neuroscience and Behavior Program, University of Massachusetts, Amherst, MA 01003.

Since vasotocin levels in amphibian brains are influenced by gonadal steroids and differ by sex, we used immunocytochemistry to identify which AVT pathways show sex differences in the bullfrog (Rana catesbeiana). AVT-immunoreactive cells were observed in the septal nucleus, amygdala pars lateralis, magnocellular preoptic area, suprachiasmatic nucleus, hypothalamus, and pretrigeminal nucleus. Immunoreactive fibers were broadly distributed in hypothalamic and extra-hypothalamic areas. The most obvious sex differences were found in the amygdala pars lateralis, which showed significantly more immunoreactive cells and fibers in male than in female bullfrogs. In the suprachiasmatic nucleus, AVT immunoreactive cells were larger in females than in males but did not differ in number. Since the areas that showed sex differences in vasotocin distribution have also been implicated in reproductive behaviors, they may form the neural substrates for the effects of vasotocin on sexually dimorphic behaviors in amphibians.

563.5

SITE-SPECIFIC INJECTIONS OF A SELECTIVE OXYTOCIN RECEPTOR ANTAGONIST AFFECTS FEMALE SEXUAL BEHAVIOR D.M. Witt & T. R. Insel, NIMH, LCS, Section on Comparative Studies of Brain and Behavior, Poolesville, MD 20837.

Previous studies indicate that oxytocin (OT) facilitates female sex behavior and the selective OT antagonist (called OTA) blocks these behavioral responses (Witt & Insel, Endocrinology, June 1991). Although OT receptors have been extensively mapped in the CNS, the specific regions that mediate OT's effect on sexual behavior remain unknown. Brain regions regulating OT-mediated behaviors were determined by site-specific injections of OTA (10 - 100 ng). Injections were administered via bilateral cannulae guides positioned over the medial preoptic area (MPOA) or the ventromedial nucleus (VMN) of the hypothalamus, regions known to mediate sexual behavior. Ovariectomized female rats, primed with estradiol benzoate (sc, 1 μg, for 2 days) and progesterone (sc, 250 μg, for 1 day), received bilateral injections of OTA (5, 25, or 50 ng / side) or artificial CSF (0.5 μl / side). Injections of OTA into the VMN significantly reduced lordosis quotients (LQs), proceptive hopping/ darting and increased rejection behaviors. These behavioral responses occurred at doses considerably lower than those required with icv administration. In contrast, our preliminary studies indicate that OTA injections in the MPOA did not affect LQs, but decreased proceptive behaviors such as physical contact. Results from these site-specific experiments, along with the icv studies, are consistent with the hypothesis that OT plays a physiological role in the expression of female sexual behavior.

563.6

ACTIVATION OF C-*FOS*-LIKE PROTEIN IN PVN OXYTOCIN CELLS DURING MALE SEXUAL BEHAVIOR. T. R. Insel and D.M. Witt, Lab. Clin. Sci., NIMH, Poolesville, MD 20837

The protein product of the c-*fos* oncogene has been previously used to map neuronal activity at the cellular level following seizures, dehydration, stress, and a variety of drug treatments. In the current study, induction of a c-*fos*-like protein was detected immunocytochemically following male sexual behavior to determine neuronal sites of activation associated with mounting and ejaculation. Sexually experienced male Sprague-Dawley rats were studied after (a) ejaculation, (b) intromission and mounting without ejaculation, or (c) exposure to a testing arena vacated by an estrous female. Induction of c-*fos*-like protein was not evident in the medial preoptic area (MPOA) following any of the treatments, although this area has been previously implicated in the mediation of male sex behavior. Treatment effects were noted in the paraventricular nucleus of the hypothalamus (PVN) with increases of 300-500% in the number of parvocellular neurons expressing c-*fos* following either mounting or ejaculation. Significant but less striking increases (60-100%) in c-*fos* expression were noted in magnocellular divisions of the PVN. Double-labelling experiments indicated that approximately 25% of these *fos* - positive cells also expressed oxytocin, but few expressed vasopressin. Ongoing experiments are investigating other neuropeptide phenotypes. These results are generally consistent with other techniques implicating extra-hypothalamic oxytocin projections in the mediation of male sex behavior. The absence of c-*fos* induction in the MPOA does not rule out the possibility that some other transcription factor may be associated with cellular activation in this region.

563.7

TRANSIENT INCREASE IN CENTRAL OXYTOCIN BINDING ASSOCIATED WITH PARTURITION. C.A. Pedersen, C.H. Walker, J.D. Caldwell, G.A. Mason, Dept. of Psychiatry, BDRC and the Neurobiology Curriculum, School of Medicine, The University of North Carolina, Chapel Hill, NC 27599-7250.

Soloff et al, (1979) demonstrated that oxytocin (OT) binding in the uterus increases dramatically a few hrs prior to parturition and then subsides as quickly postpartum. Mayer and Rosenblatt (1984) observed that maternal response to neonates emerges rather abruptly a few hrs before parturition. These observations have led to us hypothesize that a transient rise in central OT binding may also occur at parturition and may be related to the activation of maternal behavior. To test our hypothesis, we employed a radioligand assay to measure OT binding in dissected brain sites in 6 SD rats on days 16-17 of pregnancy, 6 rats in midparturition or less than 2 hrs after delivery of the last pup, and 6 lactating rats 5-7 days postpartum. In the amygdala and the MPOA we measured a significant 20-25% increase in OT binding at midparturition. By 5-7 days postpartum OT binding in these sites returned to the lower levels occurring in late pregnancy. In the VTA, OT binding rose fourfold at parturition compared to late pregnancy. OT binding in the VTA declined almost 50% by 5-7 days postpartum but remained significantly elevated over late pregnancy levels. The MPOA, amygdala and the VTA have all been implicated in the activation of maternal behavior. A transient rise in OT binding in these brain sites at parturition may trigger the onset of maternal behavior even as the transient rise in binding in the uterus facilitates the onset of labor.

563.8

PROGESTERONE - OXYTOCIN INTERACTIONS: EFFECTS ON SEXUAL RECEPTIVITY. J. D. Caldwell, C.H. Walker*, G.A. Mason*, and C.A. Pedersen; BDRC & Dept. of Psychiatry, School of Medicine, Univ. of North Carolina, Chapel Hill, NC 27599-7250

Both the peptide oxytocin (OXT) and the steroid progesterone (P) enhance female sexual receptivity. Here we present evidence that they interact at several levels to achieve this behavioral effect. P treatment changes the pattern of OXT recovery by microdialysis from the medial preoptic area (MPOA) and ventromedial hypothalamus. Estradiol benzoate (EB) treatment increased OXT recovery and resulted in surges of OXT which were lower in P treated animals until four hours after injection when OXT recovery increased dramatically. In OVXed rats given 0.5 μg EB for three days a 500 μg P injection significantly increased the density of OXT receptors in membrane fractions from the MPOA of animals mounted twenty times by males. In OVXed rats given 0.25 μg EB for three days the infusion of 1 μg/side of the OXT antagonist [Pen^1, pMePhe^2, Thr^4, Orn^8]OXT into the MPOA before injection of 250 μg P significantly ($p<.001$) reduced lordosis quotients 4 hours ($t_{25}=3.82$) and 6 hours later ($t_{25}=2.64$; $p<.02$). In adrenalectomized (ADXed)-OVXed rats, given 25% corticosterone implants, infusions of OXT significantly ($p<.01$) increased sexual receptivity ($t_{18}=3.54$). OXT significantly ($p<.05$) facilitated receptivity in sham-ADXed animals ($t_{21}=2.27$) but not in ADXed-OVXed animals receiving cholesterol implants. OXT and P interact to the extent that P affects OXT release patterns and OXT receptor density. Both OXT and P may need to be present for the other to enhance sexual receptivity.

563.9

HOMOZYGOUS BRATTLEBORO RATS DISPLAY ATTENUATED CONDITIONED FREEZING RESPONSES. James D. Stoehr*, Savio Cheng*, Frances McCann and William G. North, Dept. of Physiology, Dartmouth Medical School, Hanover, NH., 03756.

Rats homozygous for hereditary hypothalamic diabetes insipidus (Brattleboro rats) lack measurable levels of AVP in the CNS and peripheral circulation, while rats heterozygous for this condition have a reduced capacity to synthesize AVP that results in levels that are less than half of those found in normal Long-Evans (LE) rats. In the present study we examined the influence of AVP on conditioned freezing behavior to aversive shock treatment by comparing the responses of Brattleboro homozygous (DI) rats, Brattleboro heterozygous (HZ) rats, and LE rats. Animals were placed in a sound-attenuated shock chamber on the training day and given a series of 3 footshocks (1 mA, 0.75 sec, 20 sec inter-shock interval). On the following four days the rats were placed in the chambers where they had received their shock and levels of spontaneous freezing evaluated.

For each of the four days, DI rats displayed significantly less freezing behavior when compared with LE rats and HZ rats. HZ rats displayed trends towards attenuated freezing responses when compared with LE rats. The data then imply that, in rats, a correlation exists between the capacity to produce AVP, and the amount of freezing displayed. These preliminary results suggest that vasopressin may play a significant role in the appropriate autonomic and emotional responses to fearful stimuli.

563.10

OXYTOCIN-INDUCED FACILITATION OF LORDOSIS IS MEDIATED BY THE PELVIC NERVE, K.M.Moody, J.L.Steinman, B.R.Komisaruk and N.T.Adler, Inst. of Neuroscience, Univ. of Penn., Phila.Pa, and Inst. of Animal Behavior, Rutgers Univ. Newark, NJ. Peripheral oxytocin administration increases receptivity in ovariectomized rats. When the uterus is removed, these effects are lost, suggesting that increased uterine contractility is mediating this effect. To determine a mechanism by which oxytocin-induced uterine contractions facilitate lordosis, pelvic, hypogastric and combined neurectomies were performed. Ovariectomized rats received .25ug EB for three days and 500ug P, four hours prior to behavioral testing. Ten minutes before testing, females received either .1cc saline or 2.1ug oxytocin. They were then placed into an arena with a sexually experienced male. Males were allowed to deliver 10 mounts or intromissions and the LQ for each female was determined. One week later, the test was repeated and the drug treatments were reversed. Animals then received either bilateral pelvic, hypogastric or combined neurectomy. After recovery, animals were tested as described. The results indicate that pelvic and combined neurectomies have a significantly greater effect on reducing oxytocin-induced facilitation of behavior than hypoastric neurectomy. This suggests that the pelvic nerve is more important than the hypogastric nerve in mediating the stimulatory effects of oxytocin on receptivity.

563.11

VASOPRESSIN $V1_A$-RECEPTOR BINDING IN RAT AND HAMSTER BRAIN: EFFECT OF GONADAL STEROIDS. A.E.Johnson, S. Audigier*, C.Barberis*, S.Jard*, H.E.Albers, Karolinska Institute, Clin. Res. Ctr., Huddinge Hosp., Huddinge, Sweden; Centre CNRS-INSERM de Pharmacologie-Endocrinologie, Montpellier, France; Depart. of Biology and Psychology, Lab. of Neuroendocrinology and Behavior, Georgia State University, Atlanta, GA.

In rats and hamsters, central vasopressin (VP) transmission is involved in the regulation of a variety of social behaviors that are dependent on circulating gonadal hormones. In rats, testicular steroids modulate VP transmission by altering the synthesis of VP in certain brain regions but does not appear to regulate central VP receptor binding. In hamsters gonadal steroids do not affect VP peptide levels, however their affect on central VP receptors are unknown. To further investigate the role of VP transmission in the regulation of steroid-dependent behavior, the distribution and regulation of central VP-receptors by testicular steroids was studied in rats and hamsters using the highly selective $V1_A$-receptor ligand, ^{125}I-labelled Phaa-D-Tyr(Me)-Phe-Gln-Asn-Arg-Pro-Arg-Tyr-NH_2 (^{125}I-OK). Slide mounted tissue sections were preincubated in 50mM Tris buffer (pH7.4, 15min, 20°C) followed by a 1 hr incubation (20°C) in buffer containing 50pM ^{125}I-OK ± 1μM unlabelled arginine-VP, 5mM $MgCl_2$, 0.1mM bacitracin and 0.1% BSA. The incubation was terminated by washing the sections in ice-cold buffer containing $MgCl_2$. Labelled sections and standards were apposed to film for four days and autoradiograms were analyzed with a computer-assisted densitometer. The results of initial studies revealed binding sites in the expected locations such as the lateral septum, hippocampus, superior colliculus and NTS in rats and hamsters. Additional binding sites were found in the lateral hypothalamus, dorsal raphe, and pineal. The role of testicular hormones in the regulation of ^{125}I-OK binding is currently under investigation.

563.12

ESTROGEN REGULATION OF OXYTOCINERGIC INNERVATION OF THE VENTROMEDIAL NUCLEUS OF THE HYPOTHALAMUS (VMN). L.M. Flanagan, M. Frankfurt and B.S. McEwen, Laboratory of Neuroendocrinology, The Rockefeller University, New York, NY 10021.

Recent evidence suggests that the pituitary hormone oxytocin (OT) acts as a neurotransmitter in the brain to modulate female sexual behavior. Earlier reports from this laboratory demonstrated that OT receptor binding in the VMN is induced by estrogen treatment, and that progesterone causes this binding to spread laterally. In the present series of experiments the OT innervation of the VMN was examined. Using immunocytochemical techniques, oxytocin fibers in the VMN were compared in ovariectomized rats treated with oil or estradiol (10 μg in 100 μl, for 2 days, sc). The number of OT fibers in the VMN was small, whereas the number of fibers passing laterally to the VMN, en route to the pituitary, was relatively large. No differences were observed in the OT fibers within the VMN in the oil-treated versus the estradiol-treated group (n=9 per group). In a second experiment, the retrograde tracer Fluorogold (FG) was injected into the VMN of female rats. Seven days later the rats were perfused, and OT neurons were stained with the immunofluorescent marker Texas Red. Colabelling of OT with FG occurred only occasionally when FG injections were confined to the VMN (n=4) . Taken together, these results suggest that OT fibers do not innervate the VMN itself but rather VMN dendrites that extend laterally towards the OT fiber tract. Thus, the progesterone-induced movement of OT receptors may be the key factor that allows the facilitative effect of OT on sexual behavior. We currently are performing radioimmunoassays of micropunches of the VMN and the area lateral to the VMN to measure OT levels in ovariectomized rats treated with oil or estradiol. Supported by NIH grant NS07080 and NIMH postdoctoral training grant MH15125.

563.13

AVP-IMMUNOREACTIVE PROJECTIONS TO THE MEDIAL PREOPTIC-ANTERIOR HYPOTHALAMIC CONTINUUM (MPOA-AH) IN SYRIAN HAMSTERS. **A. C. Hennessey, D. C. Whitman & H. E. Albers.** Depts. of Biol. & Psych., Georgia State Univ., Atlanta, GA 30303.

Vasopressin, within the MPOA-AH, is critically involved in the neural control of flank marking. Using a retrograde tracing method combined with ICC for AVP, the present study identified afferents of the MPOA-AH that were immunoreactive for AVP. Hamsters (n=6), previously implanted with guide cannula aimed at the MPOA-AH, received a 100 nl injection of a cocktail of fluorescent microspheres and AVP (9 uM). Hamsters flank marked immediately after MPOA-AH microinjection (mean= 83 ± 20.4). Areas found to project to the MPOA-AH include: the median preoptic nucleus, septohypothalamic nuclei, bed nucleus stria terminalis (BNST), paraventricular nucleus (PVN), amygdala, ventromedial hypothalamus, arcuate nucleus, raphe nuclei and periaqueductal gray. Afferents which were also immunoreactive for AVP include: the median preoptic nucleus, BNST, and the PVN. (Supported by NSF BNS-8910863)

563.14

SEPTO-HYPOTHALAMIC ORGANIZATION IN THE CONTROL OF FLANK MARKING BEHAVIOR IN GOLDEN HAMSTERS: EVIDENCE FOR BILATERAL RECRUITMENT OF THE ANTERIOR HYPOTHALAMUS. C.F. Ferris, Y. Delville, M. Potegal, and G.J. DeVries. Dept. of Physiology, Univ. of Mass. Med. Ctr., 55 Lake Avenue N, Worcester, MA 01655, USA.

Microinjection of arginine vasopressin (AVP) into the lateral septum (LS) or anterior hypothalamus (AH) stimulates flank marking , a stereotypic motor behavior in hamsters. The present studies were undertaken to determine which of these two sites is more critical in the organization and expression of AVP-dependent flank marking. Since the LS and the AH have unilateral and reciprocal connections, AVP was injected into the LS in animals with neurotoxic lesions in the ipsilateral AH, or AVP was injected into the AH in animals with ipsilateral lesions in the LS. Lesions in the AH block AVP-stimulated flank marking triggered from the LS but not vice versa. The efficacy of the neurotoxic lesions was assessed by the loss of AVP receptor binding following *in vitro* autoradiography. Furthermore, unilateral lesions of the AH also blocked flank marking stimulated by AVP injections in the contralateral LS, suggesting that flank marking requires the recruitment of neurons from both sides of the AH. The following data support this notion. First, unilateral lesions of the AH block flank marking induced by AVP injection in the contralateral AH. Second, unilateral injection of AVP into the AH stimulates comparable c-*fos* immunolabelling within both sides of the AH. However, anterograde track tracing with Phaseolus vulgaris leukoagglutinin showed very little cross over between both sides of the AH, suggesting the existence of an unknown site providing bilateral recruitment of the AH for the expression of flank marking.

563.15

SEXUALLY DIMORPHIC AND TESTOSTERONE-DEPENDENT VASOPRESSIN BINDING IN THE AREA OF THE VENTROMEDIAL NUCLEUS OF THE HYPOTHALAMUS IN HAMSTERS. Y. Delville and C.F. Ferris. Dept. of Physiology, Univ. of Mass. Med. Ctr., 55 Lake Avenue N, Worcester, MA 01655, USA.

[^{125}I]-[d(CH_2)$_5$, 7-sarcosine, 8-arginine]vasopressin was used to study the neural distribution of vasopressin binding in hamsters. Interestingly, vasopressin binding was observed in the area of the ventromedial nucleus of the hypothalamus (VMHA) and the ventral premammillary nucleus (PMV), apparently overlaying previously described distributions of estrogen receptors in rodents. This binding appears to be specific for V_1 vasopressin receptors. Vasopressin binding was inhibited by incubation with nanomolar concentrations of either vasopressin or a selective V_1 antagonist. In comparison, micromolar concentrations of either a selective V_2 antagonist or oxytocin were necessary to inhibit vasopressin binding in the hypothalamus. However, this distribution of vasopressin binding sites was only observed in males. Females did not have any vasopressin binding within the rostral portion of the VMHA. At more caudal levels, including the PMV, vasopressin binding was present in females, but ostensibly smaller in area. Furthermore, in adult male hamsters, these vasopressin binding sites appear to be testosterone-dependent. Binding in both the VMNA and the PMV disappears within one month after castration, while being maintained in testosterone-treated animals.

The existence of sexually dimorphic and testosterone-dependent vasopressin binding has never been reported in the brain, neither has the existence of binding in the VMHA/PMV. Experiments are currently in progress to test the behavioral significance of these binding sites.

563.16

CRF ENHANCES FEAR AND DEPRESSES BEHAVIORAL ACTIVITY FOLLOWING INFUSION INTO THE PARABRACHIAL NUCLEUS. M.F. Aaron*, C.M. Lorenz*, C.B. Nemeroff, and J.M. Weiss. School of Medicine and Department of Psychiatry, Duke University Medical Center, Durham, NC 27710.

In the experiments reported here, we found that corticotropin-releasing factor (CRF) infused into the parabrachial nucleus (PBN) depressed behavioral activity in a swim test and increased anxiety in a modified Britton open field test where water-deprived animals are tested for their reluctance to drink. Male Sprague-Dawley rats were surgically implanted with guide cannulae 3-4 mm dorsal to the PBN bilaterally (P: -9.3 mm (from bregma), L: -2.3 mm (from midline)). Following 1-2 days post-operative recovery, rats were made water-deprived by limiting access to water (1 hr daily for 3 d) and were then tested in a 36 cm X 36 cm Plexiglas enclosure with a water spout suspended 2 cm above the center of the floor. Following an initial baseline session (10 min) in the enclosure, animals were given a fear conditioning session of grid shock for 30 min (0.8 mA for 2 sec every 2 min). The following morning, rats were injected with CRF (5ng to 500 ng) or vehicle (artificial CSF) in the PBN via 31 gauge needle extending to -7.2 mm (from top of skull) and reexposed to the shock chamber (30 min; 3 shocks given) to increase fear. Animals were then tested again in the enclosure followed by activity testing in a swim tank.

In comparison to CSF treated controls, CRF infused into the PBN significantly decreased the time spent drinking and increased inactivity ("freezing") in the modified open field ($p < 0.05$). In the swim tank, CRF infusion decreased struggling and increased floating. These results suggest that CRF acting in the PBN is anxiogenic and depresses active behavior. (Supported by NIMH MH-42088 and MH-42637).

563.17

BEHAVIORAL AND ENDOCRINE RESPONSES TO SOCIAL CONFLICT STRESS IN RATS: EFFECTS OF A CRF ANTAGONIST, α-HELICAL CRF$_{9-41}$ K.T. Britton[3],E. Merlo Pich[4] ,S.C. Heinrichs[4],K.A. Miczek[1], C. Rivier[2] and G.F. Koob[4]. [1]Tufts University, Dept. of Psychology, [2]Salk Institute, Peptide Biology Laboratory, [3]VA Medical Center,La Jolla and [4]Dept. of Neuropharmacology, Scripps Clinic and Research Foundation, 10666 N. Torrey Pines, La Jolla, CA 92037.

A model of social interaction stress involves the placement of naive intruder rats in the home cage territory of a resident counterpart. The resulting intraspecific aggression produces characteristic defensive behaviors and a reliable increase in plasma ACTH and corticosterone levels in intruder rats during the encounter. In order to characterize the stress-like effect of this resident/intruder interaction, the post-encounter increase in emotionality of intruder rats was studied in the Elevated Plus-Maze, a sensitive measure of the behavioral reactivity to novelty and stress. Social conflict produced a hyperemotional response in intruders by reducing the time spent on exposed relative to enclosed arms of the maze.

Since central nervous system CRF-containing neurons may mediate the stress response induced by social conflict, in the present study we examined the ability of a CRF antagonist, α-helical CRF (9-41), to affect the post-encounter increase in emotionality demonstrated on the Plus-Maze. Following a twenty minute exposure to resident animals, intruder rats received infusions of α-helical CRF(9-41) [5 and 25 μg ICV]. Administration of 25 μg reversed the stress-induced hyperemotionality relative to non-stressed controls. Bilateral, intra-amygdala microinjection of 250 ng, but not 500 ng, was equally effective in blocking the conflict-induced suppression of open arm exploration. These data point to the possible involvement of CRF-containing terminals within the amygdala in mediating the behavioral effects of social interaction stress in the rat.

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563.18

INTERACTION BETWEEN BRAIN CORTICOTROPIN RELEASING HORMONE (CRH) AND BENZODIAZEPINE (BZ) SYSTEMS ON PUNISHED BEHAVIOR. S.F. de Boer, J.L. Katz and R.J. Valentino. Dept. of Mental Health Sciences, Hahnemann Univ., Philadelphia, PA 19102 and NIDA ARC, Baltimore, MD 21224.

Clinical and preclinical findings implicate a link between brain CRH and BZ/GABA-ergic systems in stress and anxiety. This potential interaction was further investigated by comparing CRH, a BZ inverse-agonist (DMCM), and electric-shock as punishers in a modified Geller/Seifter paradigm. Rats were trained on a multiple fixed ratio (FR20) schedule for food reinforcement. Responses in the first component of the schedule were not punished. In the second component, the first response of the FR produced subthreshold shock (0.22 mA) that decreased responding by 10-15%. The components (3 min) alternated 5 times in a session. CRH (0.1-5.6 μg, i.c.v.), DMCM (3-100 μg, i.c.v.) and shock (Δ=0.1-0.7 mA) decreased rates of punished responding at lower doses/intensities than those that decreased rates of nonpunished responding, giving rise to dose/intensity related decrements in suppression ratios (indicating selective proconflict effects). The BZ agonist, chlordiazepoxide (CDP, 10 μg, i.c.v.) had no effect on responding when subthreshold shock was presented. However, CDP antagonized the suppressant effects of equieffective doses/intensities of CRH (1 μg), DMCM (56 μg) and shock (0.75 mA). Pretreatment with the CRH antagonist, α helical CRH$_{9-41}$ (50 μg, i.c.v.) blocked and attenuated, respectively, the suppressant effects of CRH and high intensity shock, but had no effect on the suppression by DMCM. The present results indicate that: 1) CRH, DMCM and shock all serve as punishers; and 2) The BZ agonist, CDP antagonizes all 3 punishers while α helical CRH$_{9-41}$ antagonizes only CRH and shock. Thus, the anxiogenic effects of CRH and shock may share similar mechanisms, while those of DMCM may be different.

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563.19

CRF ALTERS MONOAMINE AND MONOAMINE METABOLITE LEVELS IN DISCRETE MOTOR AREAS IN AMPHIBIAN BRAIN. C.A. Lowry, K.J. Renner, L.M. Laughlin*, and F.L. Moore. Dept. of Zoology, Oregon State University, Corvallis, OR 97331-2914.

Studies using mammals and the amphibian *Taricha granulosa* indicate that CRF may elicit neuroendocrine, autonomic, and behavioral events following exposure to stressful stimuli. Included among the behavioral responses to CRF is an increase in locomotor activity. Evidence suggests that in rodents CRF-induced locomotor activity may be mediated by monoamine systems. The present study investigated in *T. granulosa* the effects of intracerebroventricular injections of CRF on monoamine and monoamine metabolite levels in discrete brain areas.

We characterized the distribution of epinephrine, norepinephrine, dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), 5-hydroxytryptamine (5-HT), and 5-hydroxyindoleacetic acid (5-HIAA) using brain microdissection and HPLC with electrochemical detection. The mean DOPAC/DA ratio was 0.460 $\pm$ 0.050 and the mean 5-HIAA/5-HT ratio was 0.164 $\pm$ 0.017, values considerably lower than those typically observed in mammals. In separate studies we injected control solutions or CRF (25-50 ng), measured locomotor activity 35 min after treatment, then measured monoamine and monoamine metabolite levels in specific brain areas. Compared to control animals, CRF-injected animals displayed increased locomotor activity in an open field and had altered levels of monoamines or monoamine metabolites in two brain areas, the ventral striatum and the periventricular organ (hypothalamic circumventricular organ). In CRF-treated animals the ventral striatum had lower 5-HIAA/5-HT ratios, suggesting that 5-HT utilization was decreased, while the periventricular organ accumulated DA and 5-HT without altering metabolite levels. These data indicate that CRF-induced locomotor activity is associated with changes in the 5-HIAA/5-HT ratio in the ventral striatum and with DA and 5-HT levels in the periventricular organ. Supported by NSF BNS-8909173.

DRUGS OF ABUSE—ETHANOL

564.1

EFFECT OF (+)MK-801 AND KETAMINE ON RAPID TOLERANCE, CROSS-TOLERANCE AND CHRONIC TOLERANCE. J.M. Khanna, J. Weiner*, P.H. Wu* and H. Kalant*. University of Toronto, Toronto, Canada M5S 1A8 and Addiction Research Foundation, Toronto, Canada M5S 2S1

Hypothermia and motor-impairment (tilt-plane test) were used to assess whether N-methyl-D-aspartate (NMDA) receptors play a role in the development of rapid tolerance to ethanol, i.e., tolerance to a second dose of ethanol given 24 hrs after the first dose. Results showed that (+)MK-801 and ketamine blocked the development of rapid tolerance to ethanol on both tests. Similarly, both NMDA antagonists blocked cross-tolerance from ethanol to chlordiazepoxide as well as from chlordiazepoxide to ethanol on both tests. These data suggest that the role of NMDA receptors in ethanol tolerance may be similar to their role in memory and learning, involving a facilitation of transmission in certain synapses. In contrast, both NMDA antagonists failed to block chronic tolerance to ethanol in either test. They did not modify blood alcohol levels in any of the groups, so that the blockade of rapid tolerance cannot be attributed to changes in pharmacokinetics of ethanol. It is possible that NMDA antagonists lose their effectiveness with chronic administration or that the phenomena of rapid tolerance and chronic tolerance have basic differences not previously reported. Supported in part by NIAAA grant No. 1 Rol AA08212-02.

564.2

CHRONIC ETHANOL TREATMENT ALTERS SENSITIVITY TO ACETYLCHOLINE IN RAT HIPPOCAMPUS. B.S. Rothberg and B.E. Hunter, Department of Neuroscience, University of Florida, Gainesville, FL 32610.

Sensitivity of hippocampal field potentials to local (iontophoretic) application of acetylcholine (ACh) was investigated in chronic ethanol treated (CET) and sucrose-fed (control) rats. CET and control rats were fed a liquid diet containing either ethanol or sucrose for 28 weeks. Five to six months after ethanol or sucrose was withdrawn, hippocampal slices were taken and ACh was applied in stratum pyramidale or stratum radiatum of CA1 to observe population spike facilitation or field EPSP inhibition, respectively. An antidromic-orthodromic paired pulse paradigm (PPP) was also used to assess interactions with recurrent inhibition.

Population spikes were facilitated to a considerably lesser extent in CET slices relative to controls, while no treatment differences were observed for dendritic EPSP inhibition. Although preliminary, PPP data suggested that ACh interactions with inhibitory circuitry were also affected. These results suggest a heterogenous effect within CA1 to the chronic actions of ethanol. Since these cholinergic responses have been linked to differing muscarinic receptors, our results may reflect a distinct susceptibility of muscarinic receptor subtypes to the neurotoxic effects of ethanol.
(Supported by the Veterans Administration and NIAAA #AA00200)

564.3

EFFECTS OF CHRONIC ADMINISTRATION AND ABRUPT WITHDRAWAL OF ETHANOL ON CORTICOTROPIN-RELEASING FACTOR (CRF) CONCENTRATIONS IN DISCRETE RAT BRAIN REGIONS. M.A. Vargas , M.J. Owens, C.L. Ehlers and C.B. Nemeroff. Depts Psychiat. & Pharmacol., Duke Univ. Med. Ctr., Durham. NC 27710 and Dept. Neuropharmacol., Res. Inst. Scripps Clin., La Jolla, CA 92037.

Corticotropin-releasing factor (CRF), acting as a neurotransmitter in both hypothalamic and extrahypothalamic areas, is involved in mediating the endocrine, autonomic and behavioral responses to stress. Short- and long-term exposure to alcohol has been demonstrated to activate the hypothalamo-pituitary-adrenal (HPA) axis in both humans and laboratory animals. Because many of the signs and symptoms observed in animals and humans following ethanol withdrawal resemble those of stress, we investigated the effect of chronic ethanol administration (28 days) followed by abrupt ethanol withdrawal on hypothalamic and extrahypothalamic CRF concentrations, and CRF receptor number and affinity. CRF concentrations were not altered by chronic ethanol administration or withdrawal in any of the thirteen brain regions studied. CRF receptor density was significantly increased in the prefrontal cortex, piriform cortex and cerebellum following chronic ethanol administration. CRF receptor number was decreased in the anterior pituitary following ethanol chronic treatment and abrupt withdrawal, indicative of down-regulation of CRF receptors due to increased CRF release into the hypophyseal portal vessels. The present results indicate that ethanol exerts effects on extrahypothalamic CRF neurons. Supported by NIMH MH - 42088.

564.4

CHRONIC ALCOHOL EXPOSURE AFFECTS GABAERGIC INNERVATION IN THE DENTATE MOLECULAR LAYER. E. Fifkova, H. Eason*, and P. Schaner*. Dept. of Psych., Univ. of Colorado, Boulder, CO 80309.

The effect of chronic alcohol exposure and withdrawal from this exposure has been studied in the alcohol-sensitive LS_{IBG} line and alcohol-insensitive SS_{IBG} line (30 mice total). The mice were fed for 4 months either with a control isocaloric liquid diet or with an isocaloric liquid diet containing ethanol (23.5% ethanol derived calories). Some of the ethanol treated mice were withdrawn from the diet for 1 month. Subsequently the mice were prepared for electron microscopy and the GABA terminals were labeled with antiGABA antibodies using a gold probe in a postembedding protocol. GABA terminals make symmetric contacts either on dendrites or dendritic spines of the dentate granule cells. The number of axodendritic and axospinous synapses were counted separately for the proximal third and distal two-thirds of the dentate molecular layer. A gradual loss of axodendritic synapses was seen in the LS mice. A 14.5% loss (statistically nonsignificant) of axodendritic synapses occurred after 4 months of ethanol exposure. This loss continued during the withdrawal period resulting in 38% fewer axodendritic synapses ($p = 0.005$). No changes were observed in the number of axospinous synapses. No loss in either type of contacts was seen in the SS line following ethanol exposure and withdrawal. However, when LS and SS controls were compared, significantly fewer axodendritic synapses (by 29%, $p = 0.033$) were observed in the SS line.

These observations are in line with our previous findings showing a loss of GABAergic basket cells in LS mice following chronic alcohol exposure. This observation could be interpreted as an adaptation of the nervous system to the depressing effect of ethanol. Interestingly, the change starts during ethanol exposure and gets more severe during ethanol withdrawal.

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564.5

ACTIVATION OF BRAIN STRUCTURES DURING ETHANOL WITHDRAWAL: EVIDENCE FOR A ROLE OF ENDOGENOUS GLUTAMATE

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Northern hybridization studies using a ^{32}P-c-*fos* murine DNA probe revealed that peak activation of rat brain cells leading to expression of c-*fos* mRNA occurs at 6-8 hr after the initiation of withdrawal from a 7 day continuous exposure to ethanol vapor. This expression was stereospecifically blocked by a 4 hr pretreatment with the NMDA receptor-associated ion channel blocker, MK-801 (10 mg/kg, *i.p.*). During this period of withdrawal, the concentration of glutamate in the dialysate from a 2 mm microdialysis probe implanted into rat striatum under chloral hydrate anesthesia was estimated by HPLC to be 120 pmol/30 ul vol/20 min. This was a significant ($p<0.01$) 3-fold increase over the control value of 30 pmol/30 ul vol/20 min. No difference was seen in the levels of GABA, glycine, taurine or other detected amino acids. The data suggest a role for glutamate and NMDA receptors during ethanol withdrawal.

564.6

CHRONIC INTERMITTENT ETHANOL IN RATS ALTERS [^{3}H]RO15-4513 BINDING TO DIAZEPAM-INSENSITIVE GABA-RECEPTORS.

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Chronic intermittent ethanol (CIE) administered to rats produces a 'kindling-like' phenomenon in which the PTZ-induced seizure threshold is decreased. A change in PTZ-induced seizure sensitivity suggests that the $GABA_A$ receptor complex is involved in the differences observed between control and CIE animals. $GABA_A$ receptor binding was compared between the two groups of animals using the partial inverse-agonist [^{3}H]Ro15-4513. This ligand was used because of its reported 'alcohol antagonist' activity and its unique binding to both diazepam-sensitive (DZ-S) and diazepam-insensitive (DZ-IS) sites. Crude membrane homogenates were prepared from both the cortex and cerebellum. Several differences in binding were observed between the two groups of animals. In the cerebellum of CIE animals, the binding of Ro15-4513 to DZ-S sites is increased while the binding to DZ-IS sites is decreased, i.e., the ratio of DZ-IS is decreased. Scatchard analysis indicates that the affinity of Ro15-4513 for the DZ-IS sites is decreased two-fold. The ability of diazepam to displace Ro15-4513 binding is also increased in CIE animals. Chronic ethanol thus produces a change in the properties or amount of DZ-IS binding sites for Ro15-4513, possibly mediated by a switch in subunit gene expression.

564.7

ALTERATIONS IN TASTE REACTIVITY TO ALCOHOL IN RATS GIVEN CONTINUOUS ALCOHOL ACCESS FOLLOWED BY ABSTINENCE.

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Taste reactivity tests were used to examine the oro-facial responses of rats (n=16) to the taste of alcohol during four tests: the first test was before alcohol experience; the second test was done after four weeks of continuous access to 10% alcohol (and water); the third test occurred after two more weeks of 10% alcohol access; the fourth test was then done four weeks later, during which time the rats received water only. For each reactivity test, rats were preented with 10%, 20%, 30%, and 40% alcohol and their orofacial reactivity videotaped and then scored for ingestive and aversive responding. Results showed that there was an increase in the number of ingestive responses from test one to test three. The number of ingestive responses recorded during test four, after the alcohol had been removed for four weeks, was almost identical to that of the first exposure. Aversive responses tended to decrease from test one to test three but then increased slightly during test four. It was concluded that the palatability of alcohol increases with continued consumption and then returns to pre-experience levels if rats are without alcohol for a period of time.

Supported by NIAAA grant AA07185

564.8

HEIGHTENED EMOTIONALITY INDUCED BY ETHANOL WITHDRAWAL IS BLOCKED BY INTRA-AMYGDALA INFUSION OF α-HELICAL CRF$_{9-41}$

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A previous study in our laboratory demonstrated that the Elevated Plus-Maze is a sensitive measure of increased reactivity associated with ethanol withdrawal. Withdrawal of ethanol from chronically intoxicated rats reduced exploration of exposed, lighted walkways in favor of enclosed, darkened arms of the maze. This reduction in time spent on the exposed arms reflects a heightened emotionality produced by ethanol withdrawal and was blocked by low doses of a corticotropin-releasing factor antagonist, α-helical CRF(9-41), administered centrally [Baldwin et al, *Psychopharmacology* 103:227-232, 1991]. The specific neuronal substrate for this effect is unknown, however, the amygdala contains a high density of CRF neurons and is integral to emotional reactivity in numerous tasks.

The present study examines the effect of α-helical CRF(9-41) administered into the central nucleus of the amygdala on the ethanol withdrawal-induced increase in emotionality. Following a 17 day period of consumption of ethanol-containing liquid diet (8.5% v/v), bilateral infusion of a-helical CRF(9-41)[250 and 500 ng IC] reversed the hyperemotionality on the Plus-Maze at eight hours post-ethanol exposure. The most effective intra-amygdala dose (250 ng IC) did not affect the behavior of ethanol withdrawn rats when administered intracerebroventricularly (250 ng ICV). Similarly, 250 and 500 ng intra-amygdala doses of CRF antagonist did not affect the Plus-Maze performance of non-dependent rats. These results suggest that a specific, CRF-containing limbic system nucleus may participate in the maladaptive state of behavioral hyperreactivity present during ethanol withdrawal in humans.

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564.9

ONDANSETRON-INDUCED REVERSAL OF ANXIOGENIC BEHAVIORS UPON WITHDRAWAL FROM ETHANOL: ASSESSMENT BY BEHAVIORAL ALTERATIONS IN THE ELEVATED PLUS-MAZE.

P.L. Prather, S.M. Rezazadeh and H. Lal, Department of Pharmacology, Texas College of Osteopathic Medicine, Ft. Worth, TX 76107.

Buspirone, which reduces serotonin (5HT) neurotransmission through $5HT_{1A}$ autoreceptor stimulation, attenuated the anxiogenic behaviors related to ethanol withdrawal as observed by behavioral changes in the elevated plus maze (EPM) (Alcohol, In press). The current study determined whether ondansetron, which decreases 5HT function through antagonism of $5HT_3$ receptors, would also attenuate anxiogenic behaviors observed during withdrawal. Long-Evans hooded rats were fed a nutritionally complete diet that provided adequate ethanol (4.5% w/v) to produce dependence but without any loss in body weight or nutritional deficiency. To cause withdrawal symptoms, the ethanol diet was discontinued after four days. Twelve hours after the last dose of ethanol, separate groups of rats were injected IP with either the vehicle (1 ml/kg) or ondansetron (0.0025, 0.01, 0.04 or 0.16 mg/kg) and placed on the elevated plus-maze 60 min later for a 5 min observation period. Animals experiencing withdrawal and injected only with vehicle, displayed behavior similar to that produced by injection of anxiogenic drugs. There was a marked reduction in % of both the entries onto and the time spent on the open-arms of the EPM. In contrast, ondansetron pretreatment resulted in reversal of the anxiogenic behavior as demonstrated by a dose-dependent increase in open-arm activity back to control values. These data suggest that ondansetron may be efficacious in alleviating an important symptom of ethanol withdrawal. (Supported by Glaxo Group Research).

564.10

EVALUATION OF ANXIOGENIC BEHAVIORS DURING ETHANOL WITHDRAWAL IN THREE STRAINS OF RATS UTILIZING THE ELEVATED PLUS-MAZE.

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Fawn hooded (FH) rats are deficient in brain serotonin function and consume more ethanol (ETOH) than Wistar (W) rats from which they were derived. However, the consequences of these differences on ETOH pharmacology and/or ETOH dependence are not known. Long-Evans (LE) rats show anxiogenic behaviors during ETOH withdrawal (*JPET* 247:508, 1988). This study compared ETOH pharmacology and ETOH withdrawal in FH, W and LE rats. Male rats were fed a nutritionally balanced diet containing 4.5% ETOH (12 g/kg/day) for 2 bouts of 4 d each. They were tested in the elevated plus-maze (EPM) 14 h after the last ETOH dose, following each bout. During ETOH administration, daily consumption of ETOH diet in FH rats was 34% and 23% higher than that consumed by W and LE rats, respectively. FH rats exhibited 83% and 93% lower intoxication scores than W and LE rats, respectively. Upon withdrawal from the first ETOH bout, the % time spent on and entries made onto the open-arms of the EPM were reduced in FH and LE rats but not in W rats as compared to their respective controls. However, after withdrawal from the second bout of ETOH, only LE rats showed reduction in open-arm exploration. In conclusion, FH rats consumed the most ETOH and exhibited the least intoxication. LE rats showed anxiogenic behaviors during withdrawal from either bout of ETOH administration, while W rats did not show any reduction in open-arm activity during withdrawal. In contrast, FH rats showed anxiogenic behaviors only following the first bout. Thus, FH rats displayed greater preference for ETOH while exhibiting reduced sensitivity to ethanol's pharmacological effects. (Supported by grants NIAAA #AA06890-03 & NC-ARA9103.)

564.11

Increased natural killer cell activity and concanavalin A induced lymphocyte proliferation following Chronic alcohol consumption. S. Razani-Boroujerdi, S. M. Smith* and S. Tokuda* Dept. Micro. & Path., Sch. Med., Univ. New Mexico, Albuquerque, NM 87131.

Although alcohol is presumed to be immunosuppresive, there is no agreement regarding its effects on immune responses. The present study examined the effect of chronic alcohol consumption on natural killer cell (NK) activity measured by ^{51}Cr labeled Yac−1 cell lysis and on concanavalin A (Con A) induced lymphocyte proliferation. Male Wistar rats were implanted with gastrostomy catheters which permitted continuous intragastric infusion of liquid diet and ethanol for six weeks. The dose of ethanol was 30% of caloric intake which progressively increased to 40% and resulted in mean blood alcohol levels of 240 mg/100 ml. Control rats received isocaloric dextrose instead of ethanol. Our result showed that: 1) NK activity was 50 to 60 percent increased and, 2) proliferative response with Con A was two to three fold increased in alcoholic rats. Both alterations were statistically significant at $p < 0.05$. The increase in Con A response may suggest an increase in IL−2 production following chronic alcohol consumption and may be a contributing factor in the elevation of NK cytotoxicity. (Support provided by NIH Grant NS 24008).

564.12

TRANSCRANIAL ELECTROSTIMULATION (TES) OF BRAIN OPIOID STRUCTURES (BOS): EXPERIMENTAL TREATMENT OF ALCOHOL WITHDRAWAL SYNDROME (AWS) AND CLINICAL APPLICATION. E.M. Krupitsky*, Ya.S. Katznelson*, V.P. Lebedev, N.V. Flood*, M.A. Patterson*, J.M. Lipton and G.P. Kozlowski. Pavlov Inst. of Physiol., Leningrad, 199034; Neuro Systems Inc., Dallas, TX. 75238 and Dept. Physiol., UT. Southwestern Med. Ctr., Dallas, TX. 75235

Numerous investigations on AWS have demonstrated a functional insufficiency in endogenous opioid systems. Since TES is a regime which selectively activates the BOS, our aim was to investigate the efficacy of non-invasive TES on ameliorating AWS. The method of Erickson et al. (Pharm. Bio. Behav., 13:781, 1980) was used to induce AWS and score withdrawal signs. TES induced an increase in the β-endorphin (BE) level in rat cerebrospinal fluid from 15.93±2.17, to 53.25±6.1 pmol/$_L$ ($p<0.01$) and met-enkephalin from 3.61±1.39 to 7.86±0.94 pmol/ml. Marked therapeutic effect of TES was also demonstrated in this animal model. Clinical double-blind placebo-controlled studies showed that TES of BOS was an effective method in the treatment of AWS in patients. After the TES treatment in AWS patients, the BE concentration in plasma rose from 5.86±0.72 to 10.66±0.65 pmol/L, $p<0.01$. There was a three-fold increase of BE concentration just after one TES session. Small changes in the stimulating parameters results in reduction of both BOS activation and clinical efficacy.

564.13

NORADRENERGIC FUNCTION AND ETHANOL INTOXICATION. C.J. McDougle, J.H. Krystal, L.H. Price, G.R. Heninger, D.S. Charney. Yale Univ. Dept. of Psychiatry, New Haven, CT 06519.

Preclinical and clinical studies have demonstrated that ethanol (ETOH) increases norepinephrine (NE) turnover in the brain. The purpose of this investigation was to assess the effects of the alpha-2 adrenergic receptor antagonist, yohimbine (YOH), on the behavioral manifestations of ETOH intoxication in healthy human subjects. METHODS: 12 subjects participated in 4 double-blind test conditions: placebo ETOH (2.8 mL of 95% ETOH floated on an appropriate mixer p.o.)/placebo YOH (0.45% saline I.V.), ETOH (1.1 mL/kg of 95% ethanol p.o.)/placebo YOH, placebo ETOH/YOH (0.4 mg/kg YOH I.V.), and ETOH/YOH. Fifty minutes after subjects consumed the active or placebo ETOH, they received a 10-minute I.V. infusion of active or placebo YOH. Ratings of intoxication (Sensation Scale (SS)), symptoms of anxiety (Panic Attack Symptom Scale (PASS)), and a subjective feeling of euphoria-high were obtained at baseline and throughout the study. RESULTS: The global Analysis of Variance (ANOVA) of the 4 test conditions revealed significant ETOH X YOH X time interactions for total SS score ($p<0.0001$), total PASS score ($p<0.0001$), and for self-ratings of high ($p<0.0001$). The ANOVA of the ETOH/YOH condition vs the ETOH/placebo YOH condition revealed significant YOH X time interactions for total SS score ($p<0.005$), self-rating of high ($p<0.001$), and a trend for total PASS score ($p<0.17$), with the ETOH/YOH condition being highest on all three variables. CONCLUSIONS: The combination of YOH-induced increases in NE and ETOH produces a significantly greater degree of intoxication and high than ETOH alone. This suggests that central noradrenergic function may contribute significantly to the intoxicating and euphorogenic effects of ETOH in man. Neurochemical data will also be presented.

564.14

GENDER DIFFERENCES IN BLOOD-ETHANOL AND RECALL OF PROSE AFTER ACUTE INTOXICATION. R. M. SHARP*, B. E. Beckwith, and T. V. Petros. Psychol. Dept., Univ. of North Dakota, Box 7187, Grand Forks, ND 58202.

Although many studies have documented the detrimental effect of pre-trial treatment with ethanol on memory in males, few studies have explored the effects of pre-trial treatment on memory in women. Those that have demonstrated greater impairment in memory for women at the same ml/kg dose of ethanol given to males. Male and female subjects who reported being in good health and reported being moderate users of ethanol were given either 1.0 ml/kg of absolute ethanol or the masking solution in a double-blind design. After about 55 mins. they read several expository prose passages and were asked to recall each passage immediately after hearing it. Ethanol significantly impaired recall of the prose passages in males and females. However, the deficit was greater in women than in men. Furthermore, although there was no significant gender difference in peak blood ethanol, women showed a steeper increase in concentration of blood ethanol than men. Recall scores were negatively correlated with concentration of blood ethanol in women but not in men.

564.15

ETHANOL (ETOH) AND REGIONAL BRAIN MONOAMINES IN MICE. M. G. Hadfield and C. Milio*. Medical College of Virginia, VCU, Richmond, VA 23298

We studied acute effects of ETOH (3.5 g/kg p.i. 60 min. post injection) on regional brain monoamine levels in mice and in the respective metabolite/neurotransmitter ratios by HPLC. For the norepinephrine (NE) system, 3-methoxy-4-hydroxyphenylglycol (MHPG) was decreased in the amygdala (AMY) and was increased in the hypothalamus (HT) while the MHPG/NE ratio was increased in the prefrontal cortex (PC) and the HT. For the dopamine (DA) system, DA was decreased in the olfactory tubercle (OT), 3,4-dihydroxyphenylacetic acid (DOPAC) was increased in the PC and septum (SP) and DOPAC/DA was increased in the PC, SP, striatum (ST) and HT. Homovanillic acid (HVA) was increased in the PC and ST while HVA/DA was increased in the same regions plus the olfactory bulbs (OB). 3-methoxytyramine (3MT) was decreased in the OT and ST. The serotonergic system was not altered. The results demonstrate that ETOH produces selective regional changes in catecholamines in mouse brain with a predominant influence on DA systems, and to a lesser extent, on NE activity. The predominant brain structures affected belong to the limbic system. This may help explain why ETOH exerts such a strong influence on emotional behavior and mood.

564.16

BIOPTERIN (BH_4) METABOLISM AND CNS ETHANOL SENSITIVITY IN LONG-SLEEP (LS) AND SHORT-SLEEP (SS) MICE. T. A. French*, J. M. Masserano, M. A. Hossain and N. Weiner. Dept. of Pharmacology, Univ. of Colo. Health Sciences Center, Denver, Colo. 80262.

Catecholamine neuronal systems are involved in mediating differences in CNS ethanol (ETOH) sensitivity between LS (more sensitive) and SS (less sensitive) mice. ETOH (4.2 g/kg at 30-240 min) decreases in vivo tyrosine hydroxylase (TH) activity to a much greater extent in specific brain regions of LS mice than in SS mice. The present study examines the possibility that ETOH-induced (E-I) alterations in the metabolism of the TH cofactor, BH_4, may influence these differences in TH activity and thus relate to ETOH sensitivity. ETOH (4.2 g/kg at 60-120 min) does not result in any changes in BH_4 levels in the cerebellum, hypothalamus or brain stem of either LS or SS mice. However, treatment with BH_4 (10-30 mcg, i.c.v) or synthetic cofactor 6-MPH_4 (100 mg/kg, i.p.) alone will increase TH activity in these areas by up to 50% and, given prior to ETOH, will reduce E-I sleep times in LS mice by 30-40%. This BH_4 or 6-MPH_4 pretreatment is associated with a marked attenuation of the E-I decrease in TH activity in the cerebellum, hypothalamus and brain stem of LS mice. Thus, BH_4 or 6-MPH_4 treatment apparently results in reduced ETOH sensitivity in LS mice by nonspecifically enhancing TH activity rather than by countering any specific ETOH actions to alter brain BH_4 levels. (Supported by NIAAA grant #03527.)

564.17

OPIATE-INDUCED CONDITIONED TASTE AVERSION IN MICE SELECTED FOR DIFFERENTIAL SENSITIVITY TO ETHANOL.
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Mice selectively bred for sensitivity (COLD line) or insensitivity (HOT line) to ethanol-induced hypothermia also display similar sensitivities to opiate-induced hypothermia. In the present experiment, genetic differences in the hedonic (aversive) effects of levorphanol were characterized using taste conditioning. Fluid deprived COLD and HOT mice were given six taste conditioning trials at 48-h intervals. On each trial, access to a saccharin solution was followed by injection of levorphanol (3 mg/kg on trials 1-4, 6 mg/kg on trials 5-6). Saccharin-levorphanol pairing produced aversion that was greater in HOT mice. Rectal temperatures determined before injection and 60 m after injection revealed greater levorphanol-induced hypothermia in COLD mice. These data support previous findings suggesting that genetic selection for sensitivity/insensitivity to ethanol has resulted in alterations in response to opiates. Also, this outcome is consistent with the notion that drug-induced hypothermia is related to drug's hedonic effects.
(Supported by NIAAA grants AA07702, AA07468, AA05828)

564.18

GENETIC SIMILARITIES BETWEEN ETHANOL- AND NICOTINE-INDUCED CONDITIONED TASTE AVERSION.
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Recently, we presented data which demonstrated that genetic differences exist in the acquisition and extinction of nicotine induced conditioned taste aversion. In attempt to ascertain if these differences exist for another drug of abuse, male mice from 3 inbred strains (DBA/2J/lbg, C3H/2lbg and C57BL/6J/lbg) were tested for differences in ethanol-induced conditioned taste aversion. Mice were injected with varying doses of ethanol (between 0 and 3.5 g/kg) after ingestion of a novel saccharin solution. Two bottle choice preference between saccharin and water was then measured over a 14 day time period. Evidence was obtained which suggests that there are genetic differences in the acquisition and extinction of ethanol induced conditioned taste aversion and that these differences are dose dependent. Futhermore, similarities exist among the inbred strains for conditioned taste aversion whether nicotine or ethanol is the unconditional stimulus. This finding may indicate that common genes control the aversive properties of these two agents.
Supported by DA-00116 and AA-06391.

564.19

ALTERATION OF ETHANOL-STIMULATED ACTIVITY BY DOPAMINERGIC COMPOUNDS.
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Selectively bred FAST mice are highly stimulated by ethanol (EtOH), whereas the oppositely selected SLOW mice are much less sensitive to this locomotor stimulating effect. The possibility that dopamine (DA) systems mediate EtOH-induced locomotor stimulation was investigated using DA-ergic compounds. Alpha-methyl-p-tyrosine (AMPT) significantly depleted norepinephrine (NE) and DA levels in both lines; but did not alter locomotor responses to EtOH. Sulpiride, a D_2-specific antagonist, did not alter baseline activity or the activity of animals given EtOH. However, haloperidol, a less specific D_2-antagonist, partially blocked the stimulant response of FAST mice to EtOH. SKF-38393, a D_1-receptor agonist, slightly increased activity in both saline-treated and EtOH-treated FAST mice. The activity of saline-treated SLOW mice was not altered by SKF-38393; but the depressant effect of EtOH seen in the SLOW line was reversed by coadministration of the agonist. The fact that EtOH-stimulated activity was antagonized by haloperidol but not sulpiride suggests that both D_1 and D_2 receptors are involved in this response. Alternatively, it may be that neurotransmitter systems other than DA and NE are involved. Studies to identify the neurochemical substrates responsible for the differential ethanol sensitivity of FAST and SLOW mice are continuing.
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564.20

EVIDENCE THAT G-PROTEINS MODULATE BEHAVIORAL AND PHYSIOLOGICAL ACTIONS OF ETHANOL.
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A number of neurotransmitter receptors (for example, α_2 adrenoceptors, adenosine A_1 and $GABA_B$ receptors) linked to their effector mechanisms by pertussis toxin sensitive guanine-nucleotide binding proteins (G-proteins) have been postulated to modulate of the actions of ethanol. In this study, male NIH Swiss mice were pretreated with pertussis toxin (0.5 and 1.0 µg/animal, i.c.v., 7 days prior to testing). These pretreatments reduced the ataxia induced by a 2.4 g/kg dose of ethanol. Pertussis toxin treated animals also exhibited a diminished hypothermic response to ethanol (2 g/kg), although the toxin treated animals had lower body temperatures prior to ethanol administration compared to sham treated animals. The binding oligomer of pertussis toxin, which lacks the enzymatically active protomer subunit, was inactive. These results suggest that pertussis toxin sensitive G-proteins may modulate some the behavioral and physiological effects of ethanol.

564.21

EFFECT OF ETHANOL AND ATROPINE ON REACTION TIME IN RATS.
Z.M. Post , P.K. Randall and C.K. Erickson . Div. of Pharmacol., Coll. of Pharm., Univ. of TX, Austin, TX 78712.

The purpose of this study was to determine whether striatal cholinergic function is involved in the mechanism by which ethanol (EtOH) slows reaction time (RT) in rats. Male Sprague-Dawley rats were intrastriatally and bilaterally implanted with guide cannulae. Two weeks after surgery, the rats were trained in a RT task to release a bar, as fast as possible, in response to a light and buzzer in order to avoid a mild footshock. In a 6x6 completely balanced Latin Square design, rats received intragastric EtOH (20% w/v in water, 0 or 2.0 g/kg) and intrastriatal atropine (ATR, 0, 0.8, or 8 ug in 1 ul 0.9% saline). RT was recorded in msec every 15 min. for 2 hrs. Placement of cannulae was verified with histology.

EtOH significantly slowed RT ($p < 0.01$). However, the degree to which EtOH slowed RT tended to depend on the dose of ATR given, i.e., EtOH plus 0.8 ug ATR was more effective in slowing RT than EtOH plus saline, and EtOH plus 8 ug ATR slowed RT even more. This dose-response effect was marginally significant as represented by the EtOH by ATR interaction term in the analysis of variance ($p = 0.09$). The effect was further supported in a less conventional followup analysis, in which cumulative frequency distributions formed for each treatment group were compared using nonlinear curve fitting techniques.

The dose of EtOH used in this study was high enough to interfere slightly, but significantly, with the rats' speed of reaction. Treatment with EtOH plus ATR enhanced this interference. It appears that the RT response is mediated in part by the cholinergic function of the striatum, and that EtOH and ATR, alone and in combination, alter cholinergic function, and thus, RT. (Research funds from Univ. of TX, Austin.)

565.1

ALCOHOL PALATABILITY IN RATS AS MEASURED BY TASTE REACTIVITY, LICK RATE, AND CONSUMPTION. N.B. Elder, P.J. Bice and S.W. Kiefer. Department of Psychology, Kansas State University, Manhattan, KS 66506-5302.

The palatability of alcohol for rats was tested using three dependent measures under conditions of mild fluid deprivation: taste reactivity, lick rate, and consumption. Eleven rats received 0.5%, 3%, 6%, 9%, 12%, and 15% (v/v) alcohol in separate tests. Each measure was examined separately and correlations between the measures were calculated to determine their interrelationships. Results showed that ingestive responding was consistent and high for all concentrations; aversive reactivity was consistently low except at the 15% concentration. Lick rates for .5%, 3%, and 6% were essentially identical to water. Rates were lower for the 9% and 12% solution while the 15% solution produced the lowest lick rates. Consumption of the low concentrations was equal to that of water baseline and dropped significantly at the 9% concentration and above. The only consistent correlations found between the separate measures were for lick rate and consumption. Taste reactivity was not significantly correlated with either lick rate or consumption, suggesting that reactivity to alcohol solutions may be reflective of processes different from those that regulate licking behavior or actual consumption.

565.2

BUPRENORPHINE PRODUCES DOSE- AND TIME-DEPENDENT EFFECTS ON ETHANOL SELF-ADMINISTRATION. K.R. Domanque, H.L. June, T.D. Smith, J.T. Dixon, A. Norona[1], M.J. Lewis. Neurobehavioral Laboratory, Howard University, Washington, DC 20059 and OSA, NIAAA, Rockville, MD 20857[1].

Buprenorphine (B), a mixed opioid agonist-antagonist used clinically, has been shown to reduce opioid and cocaine self-administration (SA). Given these effects on such different drugs of abuse, we examined its effects on ethanol (E) SA in freely feeding and drinking animals. Rats learned to consume increasing concentrations of E using a two bottle-limited access paradigm. All animals showed a preference for 11% E(v/v) over water after several weeks of training. Intraperitoneal injections of B (0.03-3.00 mg/kg) given at 1.5 hr prior to E access suppressed drinking greatly. Administration of B 4 hr before E access, however, suppressed drinking only at higher doses (0.30 - 3.00 mg/kg). Low doses (0.03 - 0.15 mg/kg) of B at 4 or 5 hr before E access increased drinking. These data show that B has complex effects on E SA involving several possible neurochemical mechanisms.

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565.3

EFFECTS OF NUCLEUS ACCUMBENS MICROINJECTIONS OF RACLOPRIDE ON ETHANOL SELF-ADMINISTRATION IN THE RAT. H.H. Samson, G.A. Tolliver and M. Haraguchi Alcohol and Drug Abuse Institute, University of Washington, Seattle, WA 98195

Adult, male Long-Evans rats were initiated to lever press with 10% ethanol (v/v) reinforcement using the sucrose-fading technique. Following initiation, bilateral cannula guides directed at the n. accumbens were surgically implanted. Following recovery, the animals received a single bilateral injection once weekly, 10 min prior to the daily 30 min operant session. Doses of 0.1, 0.5 and 1.0ug/brain of the D2 antagonist, raclopride were tested. No animal received more than a total of 5 microinjections.

At the 0.5 and 1.0 doses, raclopride was found to significantly decrease total session responding. No systematic effects were observed at the 0.1 dose. This decrease was the result of termination of the normal pattern of responding sooner in the session, with no marked effect upon momentary response rates. This is similar to the effects observed with systemic administration of haloperidol under the same reinforcement conditions. The decrease in ethanol self-administration resulting from raclopride is clearly different in both pattern and nature when compared to the effects of DA agonists microinjected into the n. accumbens.

565.4

ETHANOL SELF-ADMINISTRATION IN RATS IS MEDIATED BY GABA AND GLUTAMATE MECHANISMS WITHIN THE BASOLATERAL NUCLEUS OF THE AMYGDALA.. S. Rassnick and G. F. Koob. Department of Neuropharmacology, The Res. Instit. of Scripps Clinic, La Jolla, CA 92037.

GABA and glutamate neuronal systems may mediate the reinforcing properties of ethanol (E) since acute E has been shown to facilitate the activity of the GABA/benzodiazepine receptor ionophore complex and to inhibit the activity of the NMDA/glutamate ion channel complex. The basolateral nucleus of the amygdala is part of the limbic system, involved with processing sensory information into emotional behaviors and contains GABA and glutamate. The purpose of this study was to test the effects of GABA and glutamate receptor antagonists into the basolateral nucleus of the amygdala of Wistar rats trained to orally self-administer E. A two-lever free choice task was used: responses at one lever delivered E (10 % w/v) and responses at the other delivered the same quantity of water (30 min daily sessions). Bicuculline methiodide, a competitive GABA antagonist, selectively reduced E reinforced responding, (2.5, 5, 10, & 20 ng; 5 ng as the most effective dose), without altering water responses. A NMDA-selective glutamate antagonist, 2-amino-5-phosphonovaleric acid (APV) also suppressed E responding (1.5, 3, & 6 µg; 1.5 µg as the most effective dose). Doses of 3 and 6 µg produced a trend toward suppressing responding for both E and water. These results contribute further support for GABA and glutamate as neurochemical mediators of the acute reinforcing effects of E and suggests that the underlying neural circuitry includes the amygdala as an anatomical substrate (Supported in part by AA05297, AA06420, and The Alcoholic Beverage Medical Res. Foundation).

565.5

REINFORCING PROPERTY OF ETHANOL: RELATIONSHIP BETWEEN NEUROCHEMICAL RESPONSE AND SELF-ADMINISTRATION. B.A. Blanchard*, C.L. Merski* & S.D. Glick. Department of Pharmacology and Toxicology, Albany Medical College, Albany, NY, 12208.

It has been suggested that the reinforcing properties of ethanol, like other drugs of abuse, are related to dopamine (DA) release in the nucleus accumbens (e.g., DiChiara & Imperato, 1988). Using brain microdialysis, we examined the effect of 0.5 g/kg ethanol (i.p.) on extracellular concentrations of DA, DOPAC and HVA in nucleus accumbens and striatum in adult male and female Long-Evans rats. Among males, ethanol produced an increase in DA release in striatum (approximately 160% of basal release) that persisted for 1 hour, but had no effect in nucleus accumbens. In contrast, females showed increased DA levels in both nucleus accumbens (peaking at 190% of baseline) and in striatum (200% of baseline), with a more prolonged elevation in striatum (1.5 hours). No consistent changes were observed in DOPAC or HVA concentrations in either sex. To examine the relationship between neurochemical response to ethanol and its reinforcing properties, we are currently training these same rats to bar press for oral ethanol reinforcement. To date, it appears that females consume greater amounts of ethanol than males. Since females but not males showed increased DA release in nucleus accumbens following ethanol administration, this suggests that DA release in the nucleus accumbens may indeed be important in mediating the reinforcing properties of ethanol. However, since males do consume ethanol, a role for striatal DA release can not be ruled out.

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565.6

ADRENERGIC DRUGS AND RATS' INTAKE OF ALCOHOLIC BEVERAGE. C.L. HUBBELL, J.D. DELCONTE*, C.A. AMENDOLA*, M.L. NICHOLS* and L.D. Reid. Rensselaer Polytechnic Institute, Troy, NY 12180.

Rats were deprived of fluids daily for 22-h and then given 2-h to take water and a sweetened alcoholic beverage. When intakes of ethanol were stable, dose-response assessments of a number of adrenergic drugs were performed. Following a day when all rats received placebos, there was a day when various groups received one of a number of doses of a drug. Methoxyidazoxan, a specific alpha-2 adrenergic antagonist, and yohimbine, another alpha-2 antagonist, each decreased intakes of ethanol in a dose-related manner without reducing intakes of water. Methoxyidazoxan, in a dose of 3.0 mg/kg, administered interperitoneally, 15 min before the session, produced a marked reduction in intake of alcohol. Clonidine, an alpha-2 agonist, produced a slight increase in intake of ethanol at one dose, but that dose also increased intake of water. Desipramine and nomifensine, which both have adrenergic effects, decreased intake of ethanol at a dose that had minor effects on intake of water. These data, with the exception of that of clonidine, support the idea that adrenergic mechanisms play a role in ethanol's self-administration.

565.7

EFFECTS OF COCAETHYLENE ON ETOH SELF-ADMINISTRATION. R.W. Hughes, H.L. June, H.L. Spurlock, J.T. Dixon, L.H. Hicks, M.J. Lewis. Neurobeh.Lab., Howard Univ.,Washington,DC.

The concurrent abuse of ethanol (E) and cocaine has recently been more frequently reported clinically. This combination has been shown to produce cocaethylene(CE), the ethyl ester of benzoylecgonine. This compound has been found to be self-administered (SA) by primates and to also block reuptake and increase the release of dopamine in rats. The present study examined CE's effects on E SA in freely feeding and drinking rats. Animals learned to consume increasing concentration of E over several weeks using a limited access procedure. All rats showed a preference for 11% E over water. CE (1.0 mg/kg, ip) injection increased E and water consumption. A higher dose (2.5mg/kg, ip) of CE decreased E consumption while not affecting water intake. The effects of CE on E SA appear similar to those of psychomotor stimulants.

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565.8

EFFECTS OF ORAL ETHANOL SELF-ADMINISTRATION AND RO15-4513 ON WHEEL-RUNNING BEHAVIOR. M.A. Bixler, H.L. June, M.J. Lewis. Neurobehavioral Lab., Howard University, Washington, DC 20059.

We have found that ethanol (E) produced dose-related reductions of wheel-running (WR) performance. RO, an inverse BDZ agonist, has been found to antagonize the E-induced suppression of several behaviors. Recently, we have found that RO (.063-3.00 mg/kg, ip) failed to antagonize the depressant actions of experimenter-administered (EA) E (0.75 g/kg, ip) on WR.

The present study examined the actions of orally self-administered E on WR and of RO (2.5 & 5.0 mg/kg) effects on these behavioral responses. Rats who had acquired a preference for E were given 10 min tests of WR following intake of E (11% v/v) during a two-bottle choice of E and water. Unlike with EA injection, oral intake of up to 1.6 g/kg of E did not suppress WR. RO alone and in combination with oral E did substantially reduced WR similar to EA E. These data suggest that route of administration plays an important role in the effects of E on WR. Moreover, RO suppresses WR behavior alone and in combination with E independent of route of administration.

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565.9

EFFECT OF SEROTONIN ANTAGONISTS ON THE DEXFENFLURAMINE (D)-INDUCED ATTENUATION OF ETHANOL INTAKE IN WISTAR RATS. D.M. Tomkins*, E.M. Sellers and G.A. Higgins. Depts. of Pharmacology & Medicine, University of Toronto and Clinical Research & Treatment Institute, Addiction Research Foundation, Toronto, Ontario, Canada M5S 2S1.

Using a computerized drinkometer system, we recently demonstrated that D, a 5-HT releaser and uptake inhibitor, reduces ethanol intake in a dose-related manner in rats allowed continual access to water and 5% ethanol (Higgins et al, JPET, submitted). The aim of the present study was to assess which 5-HT receptor subtypes may be involved in the mediation of this effect. Ethanol-preferring rats receiving saline injections consumed between 2.4-3.3 g/kg ethanol during the 12 h dark phase. D (1 mg/kg s.c.) 1 h prior to lights out produced a marked attenuation in ethanol (-28%, $p < 0.01$) in relation to food (-4%, $p < 0.01$) intake. The reduction in ethanol intake was due to an increase in the latency ($p < 0.01$) and a reduction in the number of drinks ($p < 0.01$); drink size was unaffected. The reduced ethanol intake could be reversed by pre-treatment with either metergoline (1 and 5 mg/kg i.p., $p < 0.01$) or ritanserin (1 and 3 mg/kg, $p < 0.01$) which when given alone had no significant effect on ethanol intake. Ondansetron (0.01, 0.1 and 1 mg/kg) and xylamidine (3 mg/kg) had no significant effect on D-induced reduction in ethanol intake. However, 0.1 mg/kg ondansetron alone significantly reduced ethanol intake by 17% ($p < 0.05$). The reversal of the D-induced reduction of ethanol intake by the non-selective 5-HT antagonist metergoline and the 5-$HT_{1C/2}$ antagonist ritanserin, but not by either ondansetron (5-HT_3 antagonist) or xylamidine (peripheral 5-HT_2 antagonist) suggest a role for central 5-$HT_{1C/2}$ receptors in the mediation of this effect.

565.10

EFFECTS OF ADRENALECTOMY AND SUBSEQUENT CORTICOSTERONE TREATMENT ON ETHANOL CONSUMPTION IN MALE WISTAR RATS. C. Fahlke, J. A. Engel,* C. J. P. Eriksson, E. Hård and B. Söderpalm.Depts. of Psychology, Pharmacology, Psychiatry and Neurochemistry, Univ. of Göteborg, Sweden and Research Lab., ALKO, Helsingfors, Finland.

Stress is accompanied by a rise in the secretion of adrenocortical hormones and it may be considered whether or not these hormones, and especially corticosterone, exert an influence on ethanol consumption. In the present investigation this issue was addressed by studying the effect of adrenalectomy (ADX) and subsequent corticosterone treatment on ethanol intake. During a three week period before ADX the intake of water and 6% (w/v) ethanol was recorded for 60 male rats. Based on their individual preferences for ethanol the rats were partitioned into one ADX group and one sham-operated group. ADX rats received a 0.9% saline solution instead of water and 6% ethanol solution based upon a 0.9% saline solution. Fluid intake was recorded for 8 days after ADX and sham-operation. The ADX and control groups were each divided in two subgroups. One subgroup from ADX rats and one from the sham-operated rats received corticosterone in their fluid bottles (25 mg/1000 ml fluid) according to a method developed by Akana, S. F. et al (Am. J. Physiol. 1985, 249: R527-R532). The remaining two subgroups served as controls for the corticosterone treatment. Fluid intake was recorded for another period of 8 days. The results showed that ADX rats significantly decreased their ethanol intake and preference compared to the sham-operated controls. There was no difference between groups in total fluid intake. Treatment of ADX rats with corticosterone caused a significant increase of ethanol intake and preference compared to the ADX controls. Corticosterone restored the intake of ethanol to a normal level. This suggest that corticosterone is involved in the regulation of ethanol consumption.

565.11

ANGIOTENSIN II AND CAPTOPRIL ENHANCE ETHANOL INTAKE IN RATS. D.A. Fitts. Dept. of Psychology, Univ. of Washington, Seattle, WA 98195.

Chronic infusions of angiotensin II into the lateral ventricles for 7 days increased water intake at 5 and 20 pmol/hr and increased intake of 6% (v/v) ethanol at 20 pmol/hr relative to baseline values and to vehicle-infused controls. Captopril placed into the drinking fluids at 0.1 mg/ml for 4 days significantly increased intake of 4 to 8% ethanol in three of four experiments and never significantly reduced ethanol intake. Functionally identified lesions of the subfornical organ (SFO) totally abolished captopril-enhanced water intake but did not appear to affect the increased intake of 6% ethanol during captopril treatment. The data appear to reflect a general increase in fluid intake rather than a specific appetite for alcohol, such as would be expected with NaCl solutions, but this increase with captopril appears to be independent of the SFO. Our results with angiotensin and captopril were opposite to previously published reports by others, and the data question whether it is appropriate to use converting enzyme inhibitors to treat problem drinking in humans (e.g., Spinosa, G., et al., Alc. Clin. Exp. Res., 12, 65-70, 1988). Supported by NS-22274.

565.12

CCK-8 INHIBITS ETHANOL AND FOOD INTAKE IN RATS SELECTIVELY BRED FOR ETHANOL SENSITIVITY. S.L. Sandoval*, Y.J. Clayborne* and P.J. Kulkosky. Dept. of Psychology, Univ. of Southern Colorado, Pueblo, CO 81001.

Cholecystokinin octapeptide (CCK-8) is a brain-gut neuropeptide shown to inhibit intake of food and alcohol in a variety of species. Recently, Draski and colleagues developed strains of Norway rats differing in sleep time after acute injection of ethanol. We measured the intake of 5% alcohol, food and water in water-deprived rats with high, low, and control alcohol sensitivity (HAS, LAS, and CAS), after ip injection of CCK-8 (0-8 μg/kg). LAS rats consumed significantly more ethanol than HAS or CAS rats, which did not differ reliably. Injection of CCK-8 significantly and equivalently inhibited alcohol and food intake in each group of rats. Water intake following alcohol was unaffected by prior CCK-8 injection. Differences in alcohol sensitivity in these rats cannot be attributed to differential sensitivities to CCK-8. However, differences in alcohol consumption are inversely related to sensitivity to alcohol's anesthetic effect. (Supported by NIH Grant No. RR-08197).

565.13

EFFECTS OF SEROTONERGIC AGENTS ON ETHANOL DRINKING IN THE P LINE OF ALCOHOL-PREFERRING RATS. J.M. Murphy, R.B. Stewart L. Lumeng*, T.-K. Li* and W.J. McBride. Dept. Psychology, Purdue Sch. Sci., IUPUI, Indiana Univ. Sch. Med., Regenstrief Inst. & VAMC, Indianapolis, IN 46202.

Several drugs known to act on CNS serotonin (5HT) systems have been observed to alter alcohol consumption. Compared with rats of the alcohol-nonpreferring NP line, P rats have been shown to have lower levels of 5HT in most forebrain regions and an upregulation of some 5HT receptor subtypes. The present study tested the effects on alcohol consumption of subcutaneous injections of buspirone, a $5HT_{1A}$ agonist (1.0-9.0 mg/kg), and two $5HT_3$ antagonists, MDL72222 and ICS205930 (0.01-3.0 mg/kg). Adult female P rats (n=8 or 9/group) were given ad lib access to food and water, but access to a 10% (v/v) ethanol solution was restricted to 4 h/day. Rats were first habituated to saline injections. Each drug dose was given before alcohol access on 4 consecutive days. Rats were given saline injections and returned to baseline intakes on days between drug doses. Buspirone caused a dose-dependent decrease in alcohol intake, mostly during the first hour of ethanol access ($p<0.001$ compared with saline). MDL72222 and ICS205930 did not significantly alter ethanol intake. The findings indicate a role for $5HT_{1A}$ receptors in alcohol drinking of P rats, but no evidence for the involvement of $5HT_3$ receptors was obtained. (Supported by AA07611 and AA08553)

565.14

REGIONAL DIFFERENCES IN BRAIN CONTENT OF TRH BEFORE AND AFTER HIGH DOSE ETHANOL IN ALCOHOL-PREFERRING (P) AND -NONPREFERRING (NP) RATS. S. Morzorati, S. Keim*, S. Katner*, M.J. Kubek. Depts. Psych., Med. & Anat. & Regenstrief Inst., Indiana Univ. Sch. Med., Indianapolis, IN 46202.

This study was designed to evaluate the endogenous levels of TRH in the brains of P and NP rats at baseline (Exp 1) and after high dose Et (Exp 2). For Exp 1, alcohol-naive rats were sacrificed. For Exp 2, rats lost their righting reflex after a sedating dose of Et (3g/kg). Rats were sacrificed at either a fixed time while still sedated, or when they righted themselves. TRH content was measured in 17 brain regions.

Baseline septal TRH was lower (40%, $P<.05$) in P compared to NP rats. After high dose Et, P rats righted themselves sooner ($P<.0005$) and at higher Blood ACs ($P<.0005$) than NP rats, and TRH content in the medial septum of both lines was higher (50%, $P<.05$) compared to baseline. At the fixed time point, TRH content did not differ from baseline levels. These data support the notion that the medial septum is involved in the analeptic action of TRH. We hypothesize that high Brain AC suppresses central TRH systems. As Brain AC falls, TRH activates septohippocampal neurons to produce arousal. P rats righted themselves sooner because their septal TRH receptors may be more sensitive than those of NP rats. (Supported by AA07611 & NS25661).

565.15

ELECTROPHYSIOLOGICAL MEASURES OF SENSITIVITY TO ETHANOL, MK-801 AND DIAZEPAM IN ALCOHOL -PREFERRING AND NON-PREFERRING RATS. C.L. Ehlers, R. I. Chaplin*, W. Kaneko*, S.L. Lopez*, L. Lumeng and T.K. Li. Dept. Med., Indiana Univ. Sch. Med., and VAMC Indianapolis, IN 46223 and Dept. Neuropharm., Res. Inst. Scripps Clinic, La Jolla, CA 92037

Studies in men at genetically increased risk for the development of alcoholism have demonstrated that they may have a qualitatively different response to acute doses of ethanol. Differential sensitivity to the acute effects of ethanol has also been found in alcohol-preferring (P) and non-preferring (NP) rats. In the present study, the acute response to ethanol, as well as diazepam and the noncompetitive NMDA receptor antagonist MK-801, was assessed in P and NP rats using EEG and auditory event related potentials (ERPs). Nine P and 9 NP rats were implanted with electrodes in cortex (CTX), dorsal hippocampus (DHPC), thalamus (THAL), and amygdala (AMYG). Rats were administered vehicle, ethanol (0.75 mg/kg), diazepam (1.5 mg/kg) and MK-801 (0.1 mg/kg) 20 minutes prior to EEG and ERP recordings. P and NP rats were found to differ on EEG and ERP measures following vehicle administration, in that P rats had more power in the low (1-2 Hz) and high (32-64 Hz) frequency ranges, reduced theta frequency (6-8 Hz) in the EEG, and reduced N1 component amplitudes in CTX and DHPC. In response to ethanol, P rats differed from NPs in that they displayed less EEG slowing and less reduction or increases in ERP component amplitudes. No EEG or ERP differences were found in response to diazepam in P and NP rats. In response to MK-801, P rats did demonstrate less slow waves and less reductions or increases in ERP amplitudes. These studies indicate that in addition to differences in baseline, P rats have reduced electrophysiological effects of both ethanol and MK-801, suggesting that differential response of brain glutaminergic systems may underlie differences in acute sensitivity between P and NP rats. (supported by AA00098, 06059, 07611)

565.16

EEG EFFECTS OF ORAL ETHANOL SELF-ADMINISTRATION IN ALCOHOL-PREFERRING (P) AND -NONPREFERRING (NP) RATS. P. Robledo, L. Lumeng, T-K. Li, and C. L. Ehlers. Dept. of Med. Indiana Univ. Sch. of Med., Indianapolis, IN, and Department of Neuropharmacology, Research Institute of Scripps Clinic, La Jolla CA 92037.

Electrophysiological measures have been shown to differ in human subjects at genetically increased risk for alcoholism both prior to and after challenge doses of ethanol. In the present study EEG was recorded from indwelling electrodes in rats which were selectively bred for alcohol-preferring (P) and non preferring (NP) behaviors during an ethanol self-administration paradigm. Initially, 0.2% saccharin solution was available on a FR1. Ethanol was then added to the saccharin solution in increasing concentrations while saccharin was faded progressively. EEG recordings were obtained in three conditions: 1) 0.2% saccharin 2) 5% ethanol and 3) 10% ethanol. Differences in mean power and peak frequency were statistically compared. P rats were found to have overall increases in EEG spectral power in the theta frequency ranges in all experimental conditions (4-6 Hz, $p<0.006$; 6-8 Hz, $p<0.07$). Ethanol self administration, as compared to saccharin, was found to produce an increase in mean EEG power in both P and NP rats in all frequency bands (4-6 Hz, $p<0.06$; 6-8 Hz, $p<0.002$; 8-16 Hz, $p<0.01$; 16-32 Hz, $p<0.001$), and a decrease in peak frequency in 3 of the bands (4-6 HZ, $p<0.006$, 6-8 Hz, $p<0.002$, 8-16 Hz, $p<0.04$). The pattern of EEG changes observed following ethanol consumption however differed in the P vs. NP rats, and paralleled their consumption patterns. In NP rats, EEG changes were more prominent in the 5% condition, and decreased in the 10% condition where they pressed little for alcohol. In contrast, P rats demonstrated the largest EEG effects in the 10% condition concomitant with increases in their consumption rates. These studies suggest that P rats may need to drink more alcohol to experience the same EEG effects as NP rats. This finding may represent part of the substrate for the difference in alcohol preference between the two rat lines. (supported by AA06059)

565.17

EFFECTS OF AMPHETAMINE AND SCOPOLAMINE ON MOTOR ACTIVITY IN ALCOHOL-PREFERRING (P) AND -NONPREFERRING (NP) RATS. R.B. Stewart, R.N. Russell*, F. Franada* and J.M. Murphy. Dept. Psychology, Purdue Sch. Sci., IUPUI, Indiana Univ. Sch. Med. & Regenstrief Inst., Indianapolis, IN 46202.

Motor activity is increased following low to moderate doses of ethanol in P but not in NP rats. The present study tested P and NP rats for differential sensitivities to pharmacological agents that increase motor activity. Using a counterbalanced within-subject design, P and NP rats (n=16/line) received intraperitoneal injections of saline or amphetamine (0.25-2.0 mg/kg). Two other groups (n=16/line) were injected with saline or scopolamine (0.25-2.0 mg/kg). Rats were tested for one hour after injections in a photocell activity field. Compared with P rats, amphetamine caused a more potent dose-dependent increase in activity for rats of the NP line, with the greatest difference at 1.0 mg/kg ($p<0.001$). In contrast, scopolamine increased activity more in the P rats, with the greatest difference at 0.5 mg/kg ($p<0.005$). Since amphetamine and scopolamine are known to act primarily on CNS dopamine and cholinergic systems, respectively, the differential behavioral excitatory effects of these drugs in the P and NP lines of rats could indicate that selective breeding for alcohol preference and nonpreference may be associated with a functional divergence in brain dopaminergic and cholinergic systems. (Supported by AA07611 and AA08553)

565.18

DENSITIES OF $5\text{-}HT_3$ RECEPTORS IN THE CNS OF ALCOHOL-PREFERRING (P) AND ALCOHOL-NONPREFERRING (NP) LINES OF RATS. W.J. McBride, E. Chernet*, D.T. Wong, D.W. Robertson, L. Lumeng* and T.-K. Li*. Indiana Univ. Sch. of Med., VA Med. Ctr., and Lilly Research Labs., Indianapolis, IN

The densities of $5\text{-}HT_3$ recognition sites labelled with [^{3}H] LY 278584 were determined in the CNS of alcohol-naive, adult male rats (N=4 each) from the alcohol-preferring (P) and non-preferring (NP) lines by quantitative autoradiography. Coronal sections (20 um), prepared from frozen brains, were incubated for 1 hour at room temperature in a Tris-HCl buffer containing 2.0 nM [^{3}H] LY 278584. Among the cortical areas, the highest densities (16-37 fmol/mg prot) were observed in (a) layers 1 and 2 of the medial prefrontal and cingulate, (b) piriform and (c) entorhinal regions. High (18-31 fmol/mg prot) densities were also observed in the amygdala and ventral hippocampus. Low binding (4-9 fmol/mg prot) was observed in the nucleus accumbens. In general, there were no differences in the densities of $5\text{-}HT_3$ sites between P and NP rats. The results do not indicate any abnormality in CNS $5\text{-}HT_3$ receptors which could be associated with high alcohol drinking behavior. (AA08553, AA07462, AA07611)

565.19

RO15-4513 EFFECTS ON ETHANOL-INDUCED LOCOMOTOR ACTIVITY IN ALCOHOL-PREFERRRING AND NON-PREFERRING RATS. H.L.June, and M.J. Lewis, Neurobehav. Lab., Howard Univ., Washington, DC 20059.

Ro15-4513(RO), an inverse BDZ agonist, has been shown to block ethanol self-administration in selectively bred ethanol (E) preferring P and non-preferring NP rats. This compound has also been found to antagonize E-induced locomotor effects in unselected rats. The present study investigated the effects of RO on locomotor activity of P and NP rats.

P rats initially showed higher levels of activity than NP rats during baseline measurement of activity. Injections (ip) of RO (2.5 and 5.0 mg/kg) and E (0.75 g/kg) each were given alone and in combination with each other prior to testing. E alone reduced activity in both P and NP rats, although P rats showed less reduction. RO alone and in combination with E produced greater suppression of locomotor activity in both rats than E alone. These data shows that RO does not antagonize E-induced locomotor depression in P and NP rats. Moreover, they demonstrate the intrinsic depressant effects of the RO and the lack of generality of its effects in blocking E's action.

(Supported in part be AA06263 and RR08016)

565.20

BRAIN AND PITUITARY β-ENDORPHIN SYSTEM OF THE AA AND ANA RATS. J.P. De Waele, K.Kiianmaa* and C. Gianoulakis. Douglas Hospital Research Centre, McGill University, Verdun, Québec; *Research Laboratories, Alko ltd. Helsinki, Finland

Genetically determined differences in the activity of specific components of the endogenous opioid system may be partially responsible for the preference or aversion to ethanol exhibited by selectively bred strains of animals. In the present studies the content of POMC mRNA in the pituitary and hypothalamus as well as of immunoreactive β-endorphin (irβ-EP) in distinct regions of the brain and the pituitary gland of the alcohol-preferring AA and alcohol avoiding ANA rats were measured under basal conditions. The content of irβ-EP was significantly higher in the septum and significantly lower in the amygdala and periaqueductal grey matter of the AA rats, while there was no significant difference among AA and ANA rats in the arcuate nucleus, nucleus accumbens, caudate, hippocampus and cortex. The content of POMC mRNA but not of irβ-EP was significantly higher in the hypothalamus and neuro-intermediate lobe of the AA rats, while in the anterior pituitary the content of both ir-βEP and POMC mRNA were significantly higher in the AA than ANA rats. Thus there are specific differences in the brain and pituitary β-endorphin systems among the AA and ANA rats. The importance of these differences on controlling the voluntary ethanol consumption is under investigation.

565.21

EFFECTS OF TEMPERATURE AND ETHANOL ON GABA-STIMULATED CHLORIDE UPTAKE IN LS AND C57 MICE. D.A. Finn, K.W. Gee, P.J. Syapin, M. Bejanian and R.L. Alkana. Dept. Molec. Pharmacol. Toxicol., Univ. So. Calif., LA, CA 90033.

Offsetting ethanol hypothermia influences behavioral sensitivity to ethanol. The direction of the response depends upon genotype, with ethanol sensitivity increasing in C57 and decreasing in LS mice. Genetic differences also exist in the effects of ethanol on GABA-gated chloride (Cl) channel activity. These differences are consistent with selectively bred differences in behavioral sensitivity to ethanol. Therefore, we hypothesized that changes in behavioral sensitivity to ethanol induced by offsetting hypothermia might be due to an interaction of temperature and ethanol at the GABA$_A$/benzodiazepine receptor complex (GBRC). We found that increasing in vitro temperature from 30 to 38°C eliminated the significant effect of ethanol (25-100 mM) on GABA-stimulated Cl uptake in LS mice, which was present at 30°C. In contrast, there was no significant effect of ethanol (50-200 mM) or temperature (30 & 38°C) on GABA-stimulated Cl uptake in C57 mice. These results suggest that an interaction between temperature and ethanol at the GBRC might explain the negative relationship between temperature and ethanol sensitivity found in LS mice, but it cannot explain the positive relationship between temperature and ethanol sensitivity found in C57 mice. (Supported by USPHS grants AA03972, AA05234, NS25986 & NS24645.)

565.22

PHARMACOLOGICAL AND NEUROCHEMICAL STUDIES IN HOT AND COLD MICE. J.F. Stuart*, J. Dorow*, J.C. Crabbe and D.J. Feller, VA Medical Center and Oregon Health Sciences University, Portland, OR 97201.

Mouse lines are being selected which are sensitive (COLD1,2) and resistant (HOT1,2) to ethanol induced hypothermia. HOT/COLD mice (both replicates) were tested for hypothermic sensitivity to drugs acting through receptor or ion channel specific mechanisms: dexmedotomidine, α2-adrenergic agonist; adenosine agonist; caffeine, adenosine antagonist and phosphodiesterase inhibitor; and diltiazem, calcium channel blocker. COLD mice (both replicate lines) were significantly more sensitive than HOT mice to diltiazem, but not dexmedotomidine. C2 mice were more sensitive to adenosine drugs.than H2 mice, while H1/C1 mice were equally sensitive.The diltiazem data suggests that calcium channel function may be involved in ethanol's effect on body temperature. We previously showed that opiate agonists are more potent in COLD compared to HOT mice. Receptor binding with [3H]DAMGO, a μ-opiate agonist, and morphine inhibition of forskolin stimulated adenylate cyclase were studied in frontal cortex, remainder of cortex, hypothalamus and brain stem from HOT/COLD mice. DAMGO binding was less only in the frontal cortex of COLD compared to HOT mice. However, morphine inhibition of adenylate cyclase was similar in both mouse lines for the frontal cortex and other brain areas. These data suggest that differences between HOT/COLD mice in sensitivity to opiate drugs is not mediated through changes in μ-opiate receptor function.

These studies were supported by Grants AA05828, NIDA Contract 271-87-8120, AA06548 and AA08621.

DRUGS OF ABUSE—COCAINE: PHARMACOLOGY

566.1

MODULATION OF THE DISCRIMINATIVE-STIMULUS EFFECTS OF COCAINE BY MU AND KAPPA OPIOIDS. R.D. Spealman and J. Bergman. Harvard Medical School, New England Regional Primate Research Center, Southborough, MA 01772.

The discriminative-stimulus (DS) effects of cocaine alone and after pretreatment with selected opioids were determined in squirrel monkeys using a two-lever choice procedure. When tested alone, cocaine engendered dose-related increases in cocaine-appropriate responding, reaching a maximum of 97-100% at doses of 0.3 mg/kg or greater. Pretreatment with the mu agonists morphine, levorphanol, and methadone and the partial agonist buprenorphine potentiated the DS effects of cocaine such that the cocaine dose-effect curve was shifted to the left by as much as 30-fold. In contrast, pretreatment with the kappa agonists U50,488 and CI 977 attenuated the DS effects of cocaine, shifting the cocaine dose-effect curve to the right by up to 3-fold. None of the opioids engendered cocaine-appropriate responding in the absence of cocaine. Pretreatment with the opioid antagonists naltrexone and MR2266 did not alter the DS effects of cocaine. Naltrexone did, however, antagonize the cocaine-potentiating effects of both morphine and buprenorphine. The results show that activation of mu and kappa receptors can modulate the DS effects of cocaine in different ways, which may reflect their opposing influences on dopamine release. Supported by DA00088, DA00499, DA03774, MH07658 and RR00188.

566.2

DISCRIMINATIVE STIMULUS PROPERTIES OF COCAINE: LACK OF ANTAGONISM BY BUPRENORPHINE, MORPHINE OR NALTREXONE. P. Doty, A.B. Johnson*, M.J. Picker* and L.A. Dykstra. Dept. of Psychology, Univ. of North Carolina, Chapel Hill, NC 27599.

Recent reports indicate that buprenorphine, an opioid agonist/antagonist, suppresses cocaine self-administration in rhesus monkeys and enhances cocaine's reinforcing effects in a place preference procedure in rats. Given these findings, the present study was conducted to evaluate the effects of buprenorphine on another component of cocaine's action, namely its discriminative stimulus effects. Using a two-lever drug discrimination procedure, rats were trained to discriminate a dose of 10.0 mg/kg cocaine from saline. During substitution tests, both cocaine (1.0-10.0 mg/kg) and d-amphetamine (0.1-3.0 mg/kg) substituted for the cocaine stimulus in a dose-dependent manner. In contrast, buprenorphine (0.03-0.56 mg/kg), morphine (0.3-10.0 mg/kg) and naltrexone (1.0-10.0 mg/kg) failed to substitute for the cocaine stimulus, up to doses that substantially decreased rate of responding. Across the dose range examined, buprenorphine (0.03-0.56 mg/kg) failed to alter the stimulus effects produced by cocaine or d-amphetamine. Morphine (0.3-10.0 mg/kg) and naltrexone (1.0-10.0 mg/kg) also failed to alter cocaine's stimulus effects. Thus, although buprenorphine may alter the reinforcing effects of cocaine, the present study demonstrates that buprenorphine does not alter the stimulus properties of cocaine in rats. (Supported by PHS grants DA 07244 and DA 02749).

566.3

ANXIOGENIC EFFECTS OF COCAINE.

A. Laurel Gorman*, X-M. Yang, Adrian J. Dunn and Nick E. Goeders. Dept. of Pharmacology, Louisiana State University Medical Center, Shreveport, LA 71130-3932.

Cocaine use in humans is sometimes accompanied by feelings of anxiety and panic. Previously, we reported that chronic administration of cocaine (20 mg/kg i.p.) induced anxiety-like behaviors in rats tested in defensive withdrawal. We now report similar effects on defensive withdrawal after the same dose of cocaine given acutely. Acute or chronic cocaine administration both significantly increased plasma concentrations of corticosterone, and decreased catabolite-amine ratios for dopamine and serotonin in brain regions collected immediately after testing for defensive withdrawal. A central mechanism was suggested for the effect of cocaine because i.c.v administration of cocaine (50-200 ng) produced dose-dependent increases in defensive withdrawal behavior.

An anxiogenic explanation is supported by the observation that chlordiazepoxide (5.0 mg/kg, i.p.) pretreatment prevented the acute cocaine-induced increases in defensive withdrawal. Also, acute cocaine (20 mg/kg, i.p.) elicited anxiety-like responses in mice tested in the elevated plus-maze. Drug-induced decreases in the number of entries and the total time spent in the open arms were attenuated by i.p. L-propranolol (2.5 mg/kg) and ketanserin (5.0 mg/kg). These results indicate that cocaine acts by a central mechanism to induce anxiety-like behaviors in rats and mice.

[Supported by grants from NIH (DA06013 and NS27283)]

566.4

EFFECTS OF ALPRAZOLAM ON INTRAVENOUS COCAINE SELF-ADMINISTRATION IN RATS. N.E. Goeders, M.A. McNulty* and G.F. Guerin. Dept. of Pharmacology & Therapeutics, LSU Medical Center, Shreveport, LA 71130.

The following experiments were designed to investigate the effectiveness and specificity of alprazolam on intravenous cocaine self-administration. Alprazolam was investigated since this drug has been proven to be clinically effective in the treatment of anxiety and panic attacks and has been proposed to be useful in the treatment of some types of depression. Cocaine use and withdrawal have also been associated with anxiety, depression and even panic attacks in some cases. Alprazolam was tested under a multiple schedule of intravenous cocaine presentation and food reinforcement. Cocaine (0.5 mg/kg, ip) was available during the first hour of the session under a fixed-ratio 4 schedule of reinforcement. During the second hour, food presentation (45 mg pellets) was available under a discrete-trial fixed-ratio 10 schedule of reinforcement. The animals were pretreated with alprazolam (0, 0.5, 1.0, 2.0 and 4.0 mg/kg, ip) thirty minutes prior to the start of the behavioral session. Initial exposure to alprazolam resulted in non-specific decreases in both cocaine- and food-maintained responding. However, although the animals quickly became tolerant to the effects of the drug on food reinforced responding upon subsequent testing with alprazolam, dose-related decreases in cocaine self-administration were maintained. These data suggest that pharmacological properties inherent to alprazolam (i.e., anxiolytic) specifically altered cocaine reinforcement without affecting responding maintained by food. Experiments are currently in progress to determine the nature of these effects by completing dose-response curves with cocaine and by determining whether the benzodiazepine receptor antagonist, flumazenil (Ro 15-1788), will reverse or attenuate the effects of alprazolam on cocaine self-administration. Supported in part by USPHS Grant DA06013.

566.5

DISCRIMINATIVE STIMULUS EFFECTS OF LOCAL ANESTHETICS IN RATS TRAINED TO DISCRIMINATE BETWEEN COCAINE AND PROCAINE. J.H. Graham*, S.E. Robinson, R.L. Balster, Dept. of Pharmacology and Toxicology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298-0613.

In previous studies, several local anesthetics were shown to have cocaine-like discriminative stimulus effects. For the present study a two-lever drug discrimination procedure was used to train rats (N=6) to discriminate between 10 mg/kg cocaine (associated with one lever)and either 55 mg/kg procaine or saline (associated with the other lever) in order to separate the local anesthetic effects of cocaine from its central stimulatory effects. After the subjects were trained, substitution tests were conducted with cocaine (1.0-30 mg/kg), procaine (6.3-110 mg/kg), dimethocaine (1.0-30 mg/kg), lidocaine (3.7-59 mg/kg) and amphetamine (0.1-3.0 mg/kg). Cocaine, dimethocaine, and amphetamine generalized completely from the training dose of cocaine in a dose-dependent manner. Procaine showed no generalization from cocaine, and lidocaine showed little cocaine-lever responding, even at doses that disrupted behavior. These results provide further evidence that cocaine discrimination is not based on its local anesthetic effects and that dimethocaine, which has high affinity for the cocaine recognition site on the dopamine transporter, has effects more similar to cocaine than procaine. (Supported by NIDA grants DA-00490 and DA-07027 and the Commonwealth of Virginia Center for Drug Abuse Research)

566.6

EVALUATION OF THE EFFECTS OF BUPRENORPHINE IN BOTH A COCAINE SELF-ADMINISTRATION AND A DRUG-DISCRIMINATION PARADIGM. H.O. Pettit, S. Smith*, A.J. Eckstrom*, and G.T. Pryor*, Neuroscience Department., SRI International, Menlo Park, California 94025.

The mixed opioid agonist-antagonist buprenorphine (BUP) can reduce cocaine self-administration in monkeys. Studies were done to help clarify the manner in which buprenorphine can reduce cocaine self-administration behavior. Studies in progress are investigating the ability of BUP to attenuate intravenous cocaine self-administration in rats. Results indicate that a dose of 100 μg/kg (administered i.p. 30 min before testing) significantly attenuates cocaine self-administration. The effects of lower doses are under study. Additional experiments have assessed the effects of BUP on both cocaine and morphine drug-discrimination in rats trained to discriminate drug from saline. BUP (0, 1, 10 and 100 μg/kg, i.p.) substituted for morphine (in morphine trained rats, N=8) but not for cocaine (in cocaine trained rats, N=9). The percentage of animals selecting the drug lever for each dose of BUP was 0, 0, 37 and 87% in morphine rats, and 22, 0, 0, and 11% in cocaine rats. No significant decrease in lever press rates were observed for any dose of BUP. When BUP was combined with the ED_{50} for cocaine or morphine discrimination, a significant increase in the percentage of rats selecting the drug lever was observed in morphine- (N=10), but not cocaine-trained rats (N=13). The percentage of subjects selecting the drug lever for each dose of BUP was 40, 80, 70 and 90% in morphine rats, and 54, 46, 38, and 31% in cocaine rats.The percentage of cocaine trained animals that selected the drug lever did not significantly decrease or increase after BUP administration. When BUP (0.0, 100 and 200 μg/kg) was combined with the training dose of cocaine (5 mg/kg; at which dose approximately 100% responding occurs) no significant decrease in the percentage of rats selecting the drug lever was observed. For each dose of BUP, the percentage of animals selecting the drug lever was 90, 82 and 82%. These results indicate that BUP (1) can attenuate cocaine self-administration in rats; (2) can produce morphine-, but not cocaine-like discrimination effects; and (3) can not attenuate or enhance the discriminative stimulus properties of cocaine.

566.7

CLOZAPINE PRETREATMENT INCREASES BREAKING POINTS ON A PROGRESSIVE RATIO SCHEDULE REINFORCED BY INTRAVENOUS COCAINE IN THE RAT E.A. Loh, T. Fitch*, G. Vickers* & D.C.S. Roberts, Dept. of Psychology Carleton University, Ottawa, Canada, K1S 5B6.

There is substantial evidence to support the hypothesis that dopamine receptor stimulation is essential for psychomotor stimulant reinforcement. Since the atypical neuroleptic, clozapine, is a dopamine antagonist, one would predict it should attenuate the reinforcing effects of cocaine. This was tested by evaluating the effect of clozapine (5-20 mg/kg) on Wistar rats trained to self-administer cocaine (0.6 mg/inj) on a Progressive Ratio schedule of reinforcement.

Paradoxically, clozapine was found to substantially increase breaking points indicating that it in fact potentiated cocaine's reinforcing effects.

Clozapine has actions on other transmitter systems (ie. serotonin) which are likely responsible for this atypical response.

Clearly, all neuroleptics do not block psychomotor stimulant reward.

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566.8

CHRONIC COCAINE ALTERS THE EFFECTS OF STRESS ON LOCOMOTOR ACTIVITY IN MICE. D.J. Mokler and T.K. Sklutas*, Dept. Pharmacol., Univ. of New England, Biddeford, ME.

Interactions have been reported between cocaine and stress. Specifically, stress has been shown to sensitize animals to the motor activating effects of cocaine. Cocaine and stress are proposed to interact with monoaminergic systems. If the behavioral sensitization of cocaine by stress is due to similar mechanisms then cocaine may alter potentiate the behavioral effects of stress. The goal of the present experiment was to determine the effects of chronic cocaine administration on the behavioral effects of chronic stress. Male C57Bl/6J mice were placed on activity moniters for 1 hour a day for 14 days. One-half of the animals were stressed using an acoustic stress consisting of 1 sec of a 105 db, 8 khz tone presented on a variable time 90 sec schedule (0-360 sec range). Three hours following the session half of the animals were injected with 25 mg/kg cocaine HCl or saline. Thus there were four treatment groups: CON - no stress, vehicle; COC - no stress, cocaine; STRESS - stress, vehicle; STRESS-COC - stress, cocaine. CON animals showed a similar level of activity throughout the 14 days of study. STRESSed animals showed an enhanced locomotor activity which varied between 125% (Day 10) and 230% (Day 13) of control. COC animals (cocaine administered 3 hrs after the session) showed an initial increase in activity on days 2-4 which returned to control levels from days 5 through 14. STRESS-COC animals also showed an initial increase in activity on days 1-4 which also returned to control levels for days 5-14. These data do not support a sensitization of the behavioral effects of stress by chronic exposure to cocaine. The early increase in activity in animals exposed to chronic cocaine suggests that cocaine administered 21 hours before the session may have some continued stimulant effects which tolerates by Day 5. Chronic cocaine reverses the behavioral activation of stress. This may relate to the chronic abuse of stimulant drugs to relieve stress.

566.9

DEMONSTRATION OF TOLERANCE TO THE REINFORCING EFFECTS OF COCAINE. M. W. Emmett-Oglesby and J. D. Lane, Department of Pharmacology, TCOM, Fort Worth, TX 76107-2690

Rats were trained to lever-press under a fixed-ratio two schedule of reinforcement, where i.v. cocaine, 0.25 mg/inj, served as the reinforcer. To minimize tolerance during acquisition of responding, total reinforcers were limited to 15 per day (total dose of 15 mg/kg/day). After responding was stable, dose-effect data were obtained, which showed dose-related decreases in the rate of reinforcer intake. Subsequently, rats were treated non-contingently with either saline or cocaine for one week. For this portion of the experiment, subjects were withheld from training, and at 8-hr intervals either saline or cocaine (20 infusions of 1.0 mg/kg) was given. Over the next four days subjects were tested with four doses of cocaine (0.125, 0.25, 0.5 and 1.0 mg/inj). Subjects were then restabilized on the baseline 0.25 mg/inj schedule, and subsequently they were taken through the chronic injection and testing procedure for the other condition (i.e., if treated with saline initially, they now were treated with cocaine). Chronic treatment with cocaine resulted in a roughly two-fold faster intake of cocaine at all doses when compared to the dose-effect curve following chronic treatment with saline. In addition, the lowest dose of cocaine that would maintain self-administration was shifted roughly two-fold to the right. We have previously reported that approximately two-fold tolerance develops to the effects of cocaine used as a discriminative stimulus (e.g., *JPET* **237**:120-125, 1986) when subjects are treated with 20 mg/kg of cocaine every 8-hr for one week. The present data thus expand the overlap of information obtained between the drug-discrimination and drug self-administration paradigms to include tolerance to the reinforcing effects of cocaine. Supported by grants DA RO1-4137 and Texas Advance Technology Research #3711.

566.10

THE PROGRESSIVE RATIO PARADIGM AS A MEASUREMENT OF THE REINFORCEMENT STRENGTH OF COCAINE: AN ANALYSIS OF LONG TERM STABILITY OF BREAKPOINTS AND DOSE/RESPONSE RELATIONS. S. Schenk and R. Peltier, Texas A&M Univ., Dept. Psych., College Station, TX, 77843.

The progressive ratio (PR) paradigm has been applied to the study of the reinforcing effects of intravenously self-administered drugs. Within a test session, the procedure produces an increasing ratio requirement to obtain drug infusions and a "breakpoint", which is the last ratio that is com pleted by the animal. If the assumptions of the paradigm are correct and if long-term stability of breakpoints can be achieved, the procedure offers several advantages in the interpretation of the effects of manipulations on the reinforcement impact of drugs of abuse. We examined the stability of breakpoints for cocaine self-administration over an extended period of several weeks. We also examined dose/response relationships for breakpoints at various cocaine doses in the self-administration procedure (0.25, 0.5 and 1.0 mg/kg/infusion). The results of 70-80 days of testing revealed that breakpoints failed to reach an acceptable level of stability in that they were inconsistent from day to day. In addition, we failed to observe dose dependency either in terms of breakpoints or in terms of number of reinforcements earned. In contrast, when an FR1 schedule of reinforcement was introduced, responding was dose/dependent and reliable. The data argue that the PR procedure, as used under the present circumstances, lacks the internal validity to qualify as an effective measure of the reinforcing impact of cocaine in an intravenous self-administration paradigm.

566.11

EFFECTS OF MAGNESIUM ON RESPONDING MAINTAINED BY COCAINE AND OTHER RESPONSE MAINTAINING STIMULI : SPECIFICITY AND SELECTIVITY OF CHANGES. K.M.Kantak and T.O'Connor*. Lab. of Behav. Neuroscience, Dept. Psychol., Boston University, Boston, MA 02215

In a variety of behavioral experiments, magnesium has been shown to interact with cocaine and other stimulants. Of particular relevance to the present experiments is the recent finding that magnesium maintains responding in cocaine-trained rats. It would be expected, therefore, that injections of magnesium would have rate-altering effects on self-administered cocaine. Five experiments examined the specificity and selectivity of this relationship. Acute and cumulative injections of magnesium (0 - 250 mg/kg s.c.) produced dose-dependent reductions in responding maintained by cocaine (0.1 - 2 mg/kg/infusion). A magnesium-deficient diet produced a dose-dependent increase in cocaine-maintained responding. The acute effects of magnesium injections were specific because food-maintained responding was not affected, however, these effects were not selective because glucose + saccharin-maintained responding was affected in a manner similar to cocaine-maintained responding as was responding maintained by extinction from cocaine. These data indicate that magnesium may be either decreasing responding by increasing the reinforcing properties of cocaine and other highly rewarding substances or suppressing responding by serving as a punishing, aversive or negative stimulus.
Supported by DA04325

566.12

EFFECTS OF REPEATED EXPOSURE TO TRIADIMEFON ON SCHEDULE-CONTROLLED BEHAVIOR IN RATS. A.R. Allen and R.C. MacPhail*, Neurotoxicology Div., US EPA, RTP, NC 27711 and Dept. of Psych., UNC-CH, Chapel Hill, NC 27514.

Triadimefon (TDF), a triazole fungicide, has been found to produce acute stimulant-like effects on schedule-controlled behavior and motor activity. Tolerance to stimulant effects on schedule-controlled behavior frequently develops upon repeated exposures, especially when the initial effect is to decrease reinforcement density. The present study was conducted to determine whether behavioral tolerance would develop when TDF was given repeatedly. Effects of TDF on performance maintained by a multiple fixed-ratio 10 fixed-interval 3-minute (mult FR 10 FI 3-min) schedule were determined in twelve male Long-Evans rats. Rats were then divided into two groups. Eight rats received 30 mg/kg TDF daily for 15 days and then 56 mg/kg TDF daily for the following 15 days. The remaining four rats received vehicle (VEH) daily for 30 days. Effects of 56 mg/kg TDF on response rates, reinforcement rates, and FI index-of-curvature (IOC) values were compared before and after daily TDF or VEH administration. Acute administration of 56 mg/kg TDF produced decreases in FR response and reinforcement rates. Effects of this dosage on FI response rates were variable across rats, although reinforcement rates and IOC were decreased. Daily administration of 56 mg/kg TDF attenuated the decreases in FR rates produced by acute administration of that dosage. Daily administration of 56 mg/kg TDF also attenuated the decrease in FI reinforcement rates and in IOC. In contrast, TDF effects after daily VEH administration differed considerably between rats. These results indicate that tolerance can develop to some of the behavioral effects of TDF.

566.13

DISCRIMINATIVE STIMULUS EFFECTS OF COCAINE IN RATS AND MONKEYS. P. Terry, J.M. Witkin and J.L. Katz. Psychobiology Laboratory, NIDA Addiction Research Center, Box 5180, Baltimore MD 21224.

Drug substitution tests in rats trained to discriminate cocaine (10 mg/kg IP) from saline usually reveal dose-related substitution (often partial) by D1 and D2 dopamine agonists. In monkeys, however, neither subtype agonist has clearly engendered cocaine-appropriate responding. We trained independent groups of rats to discriminate 10 or 3 mg/kg cocaine from saline. At the former dose, structurally diverse D1 and D2 agonists produced 40-80% cocaine-lever responding. D2 agonists were less efficacious at the lower than higher training dose, but D1 agonists were equally or more efficacious. Unlike cocaine, cocaethylene and WIN 35,428, which fully substituted, were equipotent across training doses. In squirrel monkeys trained to discriminate cocaine (0.3 mg/kg, IV) from saline, D2 agonists engendered up to 60% cocaine responding, but D1 agonists to date have shown no substitution at all. These results support a potential species difference in the stimulus effects of cocaine, and demonstrate the importance of training dose to the outcome of substitution tests.

566.14

CAGE CLIMBING IN MICE: STUDIES WITH COCAINE. N. L. Katz, H. Ouranos*, L. R. Moser*, D. J. McGinness Grimes* and R. F. Schlemmer, Jr., Dept. of Pharmacodynamics, University of Illinois at Chicago, Chicago, IL 60612.

When Swiss albino mice are placed in a cylinder surmounted by wire mesh, they will climb the mesh briefly and subsequently descend to the cage floor. Then they will spend the majority of time on the floor, climbing intermittently. This activity constitutes normal exploratory behavior. Administration of the drug apomorphine to mice induces compulsive climbing activity. They climb to the top of the mesh and remain in a vertical position for extended periods. Occasionally, they fall, only to quickly arise and resume climbing. As apomorphine is known to stimulate dopamine receptors, climbing behavior is thought to reflect activation of cerebral dopamine receptors. Previous studies suggested that potent agonist activity at central dopamine receptors was required to elicit cage climbing. In the present study, several manipulations were attempted to determine whether or not cocaine can induce cage climbing in mice. To our knowledge, the effect of cocaine on climbing behavior has not been reported. Single doses of cocaine ranging from 1-60 mg/kg injected subcutaneously failed to induce climbing. However, as little as 3 mg/kg acted as a psychomotor stimulant as revealed by locomotor activity measurements. Various doses of cocaine did not alter climbing elicited by simultaneous administration of threshold doses of apomorphine. Climbing failed to occur when cocaine was given with L-DOPA and the monoamine oxidase inhibitor pargyline. A daily injection of cocaine for 3 consecutive days failed to sensitize mice to climbing when they were challenged with cocaine 1 day later. Apomorphine climbing was not influenced when it was given 3 hr after cocaine, i. e., during cocaine withdrawal. These various manipulations could not achieve the degree of stimulation of dopamine receptors required to induce climbing. Other sensitization schedules may reveal cocaine climbing in mice.

566.15

COCAINE-INDUCED CONDITIONED LOCOMOTION: LACK OF *IN VIVO* EVIDENCE FOR ASSOCIATED CONDITIONED NEUROCHEMICAL EVENTS. E.E. BROWN AND H.C. FIBIGER. Division of Neurological Sciences, Dept. of Psychiatry, University of British Columbia, 2255 Wesbrook Mall, Vancouver, B.C. Canada V6T 1Z3

The potent reinforcing effects of cocaine can readily become associated with salient environmental stimuli, which then acquire secondary reinforcing properties. It has been proposed that the reinforcing properties of these conditional stimuli (CSs) are due to the ability of the CSs to elicit similar neural events as the drug itself. Given the large body of evidence that implicates the mesolimbic dopaminergic pathway in the rewarding properites of cocaine, the present study was designed to determine whether stimuli paired with cocaine elicit similar neurochemical changes as cocaine, as assessed by *in vivo* brain microdialysis.

The administration of cocaine (10 mg/kg for 7 days) in association with a specific environment (CS^+) produced significant conditioned locomotion in that environment. Rats that received cocaine in their home cage (CS^-) did not exhibit increased locomotion on the test day, as compared to subjects that received saline in both settings.

When administered acutely, cocaine (10 mg/kg) produced a potent unconditioned effect on interstitial dopamine (300% of basal values) in the nucleus accumbens. In contrast to the ability of repeated pairing of cocaine with a specific environment to produce conditioned locomotion, there was a lack of evidence for concommitant conditional neurochemical events. Specifically, the modest increase in dopamine (10% above basal values) observed after exposure to the conditional environmental stimuli was equal in the CS^+ and CS^- groups. These data do not support the hypothesis that stimuli paired with cocaine produce their behavioural effects by eliciting similar neurochemical effects as cocaine.

566.16

THE RELATIVE CONTRIBUTION OF PERIPHERAL AND CENTRAL COMPONENTS IN COCAINE-INDUCED PLACE CONDITIONING. S.E. Hemby, G.H. Jones, D.B. Neill, & J.B. Justice, Jr. Departments of Psychology and Chemistry, Emory University, Atlanta, GA 30322.

Place conditioning (PC) induced by intraperitoneal (IP) cocaine is considered to be mediated by a local anesthetic and central dopaminergic component (Spyraki et al. 1982). In the present study, cocaine-methiodide, a quaternary salt of cocaine which shares certain pharmacological properties with cocaine but does not permeate the blood-brain barrier, was compared to cocaine for its ability to induce place conditioning when administered either IP or intracerebroventricularly (ICV). Using a completely balanced PC design, rats treated with IP cocaine (15 mg/kg) exhibited a significant preference for the drug-paired compartment on the test day ($P<0.01$). In contrast, rats receiving either cocaine-methiodide (5, 10, or 20 mg/kg) or procaine (25 or 50 mg/kg) did not exhibit a preference. ICV infusions of cocaine (50 μg/2 μl) or cocaine-methiodide (5 or 10 μg/2 μl) produced significant place conditioning for the drug-paired compartment ($P<0.05$). In a separate experiment, IP injections of cocaine (15 mg/kg) increased extracellular dopamine in the NACC, as measured by microdialysis, in addition to increasing locomotor activity while cocaine-methiodide failed to increase either dopamine or locomotor activity. These results suggest that PC induced by IP cocaine is mediated primarily by a central component. Furthermore, the local anesthetic effects produced by cocaine-methiodide and procaine are not sufficient to induce PC with a balanced design.

566.17

PRAZOSIN AND ONDANSETRON ANTAGONISE COCAINE'S EFFECTS ON FIXED-INTERVAL AND FIXED-RATIO BEHAVIOR. F. van Haaren Dept. of Psychology, University of Florida, Gainesville, FL 32611

Three White Carneaux pigeons pecked a key on a multiple fixed-interval (FI) 2 min, fixed ratio (FR) 30 schedule of reinforcement. Cocaine administration 5 min prior to the start of a session (0.3, 1.0, 1.7, 3.0, 5.6 and 10.0 mg/kg) systematically decreased and eliminated FR rates and FI rates in two of the three subjects. FR rates were more affected by lower doses. Response rates of the third subject were not consistently affected by cocaine. Prazosin (alpha$_1$ antagonist) and, later, ondansetron (GR38032F, 5-HT$_3$ antagonist) were co-administered with doses of cocaine which previously decreased response rates. Both prazosin (1.0 mg/kg) and ondansetron (0.10 and 0.50 mg/kg) antagonized cocaine's rate decreasing effects on the FR and FI schedules and reinstated responding where it had previously been totally suppressed. Prazosin and ondansetron in combination with cocaine decreased FR and FI response rates in the subject whose behavior had not been affected by cocaine alone.

566.18

EFFECTS OF REPEATED COCAINE TREATMENT: BEHAVIORAL REORGANIZATION VS. BEHAVIORAL SENSITIZATION. Ernest N. Damianopoulos and Robert J. Carey VA Medical Center, Syracuse, NY 13210

Rats were given 7 daily cocaine injections (50 mg/kg ip) using a Pavlovian conditioning paradigm. Locomotion behavior in terms of meters traversed and rotation patterns grouped into 4 categories of diameter size were measured during daily 20 min tests. Initially, the paired and unpaired groups did not differ on either behavioral measure. By Day 7, however, the paired group exhibited higher levels of locomotion relative to the unpaired group but this effect only reflected the progressive decline in locomotion (habituation) of the unpaired group. Locomotor rotation pattern analysis showed that both the paired and unpaired treatment groups had a similar distribution of rotation frequency across all four categories of diameter size on Day 1 but by Day 7 this rotation pattern changed over into a highly skewed distribution weighted towards the largest diameter size in the paired animals only. Furthermore, the new pattern was retained after a 7-day drug withdrawal period but was exhibited only under drug test conditions. Altogether, the results suggest a context-specific behavioral reorganization rather than a behavioral sensitization.

566.19

EFFECTS OF CHRONIC COCAINE ON FOOD COMPETITION IN RAT TRIADS. E. EL-AWADY* AND M.C. WILSON. Dept. of Pharmacol., Sch. of Pharm., Univ. of Mississippi, University, MS 38677.

The purpose of the study was to investigate the effects of cocaine(C) on the competitive behavior of fixed triads of male Wistar rats. Within these triads, rats were ranked during the 4th week of housing according to average competition scores for sucrose pellets. Middle ranking rats were treated daily for 2 wk with C (5.0 mg/kg, IP, b.i.d.). Competition was scored 3-5 hr or 5-7 hr after drug administration. C increased competition scores mainly in the early testing period resulting in some rats achieving the dominant status in the triad.

After treatment, 4 days were designated as a withdrawal period, and 3 additional days as a washout period. The middle ranking rats, based on average scores during the washout period, were treated with C (20 mg/kg, IP, b.i.d for 2 wk). This dose failed to affect the competition; however, competition scores of treated animals increased in all triads during the withdrawal period. This increase declined during washout. Therefore, chronic C affected food competition in time- and dose-dependent manners. (Supported by Res. Inst. of Pharm. Sci.)

566.20

CONTINUOUS COCAINE INDUCES PERSISTING BEHAVIORAL AND BIOCHEMICAL CHANGES INVOLVING MUSCARINIC AND BENZODIAZEPINE BINDING SITES. J.W. Lipton[1], M.Waterman[1*], R.W. Olsen[2] and G.D. Ellison[1]. Departments of [1]Psychology & [2]Pharmacology Univ. Calif. Los Angeles, CA 90024.

In attempting to confirm our findings of decreased muscarinic and increased benzodiazepine binding in rat caudate nuclei thirty days after a five day exposure to continuous cocaine, behavioral challenges as well as additional biochemical tests were conducted. Rats were administered continuous cocaine, daily cocaine injections, continuous amphetamine or no drug for 5 days. Forty days after drug exposure, all groups were tested for their responsivity, as measured by automated activity cages, to a low dose of the cholinergic agonist scopolamine. The continuous cocaine group showed a decreased sensitivity to scopolamine as compared to other groups. When tested with a low dose of diazepam, the continuous cocaine group showed the greatest response. However, when observed following an injection of cocaine HCl, the intermittent cocaine group showed the greatest behavioral response. These findings support those of previous studies that indicate the mode of initial cocaine administration is a key factor in the production of persisting changes in behavior. A separate experiment examined the effects of differing exposure lengths of continuous cocaine administration on muscarinic and benzodiazepine binding in the caudate nucleus and hippocampus. Animals were implanted with subcutaneous cocaine pellets for 1, 3 or 5 days, and were sacrificed 12hr, 2 days or 21 days later. Homogenate receptor binding assays were conducted to determine the progressive changes induced by the varying lengths of exposure. Preliminary findings suggest continuous cocaine administration increases benzodiazepine receptor density while decreasing receptor affinity in caudate.

567.1

EFFECTS OF METHYLENEDIOXYMETHAMPHETAMINE (MDMA) AND FENFLURAMINE (FFA) ON MOTOR ACTIVITY, BODY TEMPERATURE AND WEIGHT - CHARACTERISTICS OF ACUTE AND REPEATED DOSING. D.B. Miller, K.F. Jensen and J. P. O'Callaghan, U.S. EPA, Res. Tri. Pk., NC 27711.

Research regarding the possible neurotoxicity of MDMA has caused renewed interest in the properties of another amphetamine derivative, fenfluramine (FFA), an anti-obesity drug. MDMA and FFA affect central and peripheral systems dependent on serotonin (5-HT). Both can reduce brain 5-HT levels for many months. MDMA or FFA (0, 5, 10, or 20mg/kg, s.c.) were used to compare 5-HT related endpoints, locomotor activity, body temperature and weight. LE male rats were dosed at 7 AM and 7 PM for 7 consecutive days. Body weights were obtained daily. At 0.5 hr following the 7 AM dose rectal temperatures were obtained and rats were placed in figure-8 mazes for measurement of locomotor activity. MDMA caused hyperactivity at dosages of 10 and 20 mg/kg. Conversely, FFA only produced dose-related decreases in locomotion. Tolerance to the effects of either MDMA or FFA on activity did not occur with repeated administration. MDMA caused hyperthermia with incomplete tolerance to repeated dosing; temperature was still significantly elevated on the final day of dosing. FFA hypothermia was evident at the end of daily maze testing; partial tolerance occurred to this effect. MDMA and FFA reduced body weight as well as food and water consumption during the period of dosing. Both food and water intake returned to control levels within 24 hrs of the last dose. These data suggest MDMA and FFA have different behavioral profiles. SUPPORTED BY NIDA IAG ND-89-4.

567.2

CHARACTERIZATION OF METHYLENEDIOXYMETHAMPHETAMINE (MDMA) NEUROTOXICITY USING ASSAYS OF SEROTONIN AND GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP): CHANGES IN SPECIFIC NEOCORTICAL REGIONS IMPLICATE SEROTONERGIC AND NON-SEROTONERGIC TARGETS. J.P. O'Callaghan, K. F. Jensen and D.B. Miller. U.S. EPA, Res. Tri. Pk., NC 27711

Enhanced expression of the astrocyte protein, GFAP, reveals sites of chemical-induced injury to the CNS. By assaying the content of GFAP in specific brain regions we found that the neocortex was a major target for MDMA-induced neurotoxicity. Here we compared the effects of MDMA on GFAP and serotonin (5-HT) in five regions of neocortex to further localize sites of damage. (±) MDMA (25-150 mg/kg, s.c.) was administered to rats twice daily for two days. One group of rats received the 5-HT uptake inhibitor, fluoxetine (10 mg/kg), prior to each dose of MDMA. Rats were killed 2 days post dosing. MDMA depleted 5-HT in a fluoxetine-reversible fashion in all cortical regions. Decrements in 5-HT were as great as 70% but the dose-response relationship was U-shaped; in some regions 5-HT was not depleted at the 150 mg/kg dosage. MDMA increased GFAP by as much as 2-fold in specific regions of frontal and posterior cortex; the effect was graded with dose, detectable at 25 mg/kg, and did not require 5-HT depletion. Fluoxetine antagonized the GFAP increase in frontal but not posterior cortex. These data indicate that MDMA damages serotonergic and non-serotonergic targets in rat neocortex.
SUPPORTED BY NIDA IAG ND-89-4

567.3

CHARACTERIZATION OF METHYLENEDIOXYMETHAMPHETAMINE (MDMA) NEUROTOXICITY USING THE DE OLMOS CUPRIC SILVER STAIN AND GFAP IMMUNOHISTOCHEMISTRY: CHANGES IN SPECIFIC NEOCORTICAL REGIONS IMPLICATE SEROTONERGIC AND NON-SEROTONERGIC TARGETS. K. F. Jensen, D.B. Miller, J.K. Olin*, N. Haykal-Coates and J.P. O'Callaghan. U.S. EPA and *ManTech Environmental. Technology Inc., Res. Tri. Pk., NC 27711.

MDMA (0,25,50,75,100,150 mg/kg) was administered to rats twice a day for 2 days and the animals were sacrificed by transcardial perfusion with fixative under pentobarbital anesthesia 48 hrs postdosing. Additional rats receiving 100 mg/kg were sacrificed at various times after the second and fourth dosages. Frozen sections of the brain were stained with a recent modification of the de Olmos cupric silver stain for neuronal degeneration, GFAP immunoreactivity or cresyl violet. As previously reported, these stains revealed evidence of terminal, axonal and perikaryal degeneration in the neocortex. Such degeneration was dose-dependent and detectable after 25 mg/kg. Degeneration was evident as early as 18 hrs after the second dose of 100 mg/kg and persisted for up to 14 days after the fourth dose. The regional pattern of degeneration did not coincide with known patterns of serotonergic innervation and was only partially blocked by fluoxetine implicating serotonergic as well as non-serotonergic targets.
SUPPORTED BY NIDA/EPA IAG ND-89-4

567.4

THE ACUTE AND CHRONIC EFFECTS OF DL-AMPHETAMINE ADMINISTRATION ON BRAIN DNA AND PROTEINS. M. A. Blackshear and B. Quarles*. Dept. of Biological Sciences, Tennessee State University, Nashville, TN. 37209-1561.

The behavioral effects of amphetamine on locomotor activity in crowded and isolated mice have been well documented. This study investigates the effects of amphetamine on brain DNA and proteins after acute and chronic administration. Swiss male mice (26-30g) were used as experimental animals. In the acute studies, the mice received a single injection of 10 mg/kg of dl-amphetamine and were crowded for 30 minutes. In the chronic studies, singly housed mice received daily injections of 10 mg/kg of dl-amphetamine for 14 days. Control animals treated in a similar manner to the drug treated mice received injections of 0.01 ml/gm of physiological saline. DNA was analyzed by phenol-chloroform extraction and the proteins were analyzed by polyacrylamide gel electrophoresis. The administration of a single dose of amphetamine to crowded mice did not appreciably alter whole brain DNA or regional brain proteins. In contrast, chronic amphetamine administration produced differential effects on DNA and proteins. DNA levels were increased in the cortex, but were decreased in the striatum and the midbrain. Brain proteins were unaltered in these regions, except in the midbrain. Repeated amphetamine treatment resulted in a loss of some proteins and an increase in others. The findings suggest that the effects of amphetamine on DNA and proteins may be region specific. Further, these results suggest that the effects of amphetamine are related to exposure time.
(Supported by NIH-RCMI Grant G12RR03033).

567.5

WITHDRAWAL-DEPENDENT EFFECTS OF REPEATED AMPHETAMINE ON CALMODULIN CONTENT AND LOCALIZATION IN RAT BRAIN. M.E. Gnegy, G.H. Keikilani Hewlett* and S.L. YEE.* Department of Pharmacology, University of Michigan Medical School. Ann Arbor, MI 48109-0626.

Repeated amphetamine (AMPH) treatment leads to a behavioral sensitization in rats characterized by increases in stereotyped and rotational behaviors. We have found that repeated AMPH increases the Ca^{2+}-binding protein, calmodulin (CaM) in striatum (Roberts-Lewis et al., Brain Res., 384:383, 1986). To determine whether the AMPH-induced increase in CaM shares known characteristics of AMPH-induced sensitization, CaM was measured by RIA in cytosol and 100,000 x g membrane fractions in rat brain areas at 30 min (30m), 2 days (2d) and 30 days (30d) after cessation from an escalating dose regimen of AMPH (from 1 to 8 mg/kg over 4 weeks) or saline. In the 30m group, there was no significant change in CaM in striatum or limbic forebrain but there was a significant decrease in cytosol fractions of frontal cortex and hippocampus. Measurement of CaM 30 min after acute AMPH (8 mg/kg) showed no change in striatal CaM. By 2d, however, CaM was significantly increased in striatal cytosol (60%) and limbic forebrain (40%) membranes over controls. The increased CaM in these fractions was still present 30d after withdrawal. In the 30d group, however, there was no change in CaM in frontal cortex, hippocampus or cerebellum as compared to controls. In the 30d group, challenge with 1 mg/kg AMPH 30 min before sacrifice, elicited a translocation of CaM from membranes to cytosol in the striatum and limbic forebrain of repeated AMPH-treated but not saline-treated rats. Thus the change in CaM occurs in striatum and limbic forebrain, requires time after treatment to develop and exhibits persistence after withdrawal. These are known characteristics of behavioral sensitization to AMPH suggesting that CaM could play a role in the neurochemical changes underlying behavioral sensitization. Supported by a NIDA grant, DA05066.

567.6

METHAMPHETAMINE NEUROTOXICITY AND STRIATAL GLUTAMATE RELEASE. B.K. Yamamoto and J.F. Nash. Dept. of Psychiatry, Case Western Reserve Univ., Clev., Ohio 44106

Methamphetamine (MA) neurotoxicity is blocked by the excitatory amino acid antagonist MK801 (Sonsalla et al., 1988). The present study examined in vivo the effects of repeated injections of MA or the MA analog, 3,4 methylenedioxymethamphetamine (MDMA) on extracellular levels of glutamate (GLU) and dopamine (DA) in the rat striatum. Male rats were implanted with microdialysis probes into the anteromedial caudate-putamen. Dialysate samples were collected every 30 min. Following a 1.5 hr stable baseline, vehicle or equimole doses of MA (7.5 mg/kg) or MDMA (9.2 mg/kg) were injected i.p. 3 times every 2 hrs into awake rats. Dialysates were analyzed for amino acids and biogenic amines by HPLC/EC. One week later, rats were sacrificed and the caudate dissected and assayed for DA, 5HT and metabolites. MA produced a cumulative elevation in extracellular GLU resulting in a 9-fold increase 2 hrs after the third injection. In contrast, MDMA produced a non-significant 1.8-fold increase. Each injection of MA or MDMA also produced an immediate and reproducible increase and subsequent decrease towards baseline in extracellular DA levels. DA and HVA tissue content were significantly depleted by 32% and 37% respectively, in MA but not MDMA-treated rats. No changes were detected in 5HT or 5HIAA content. These results provide support for a GLU-mediated mechanism of MA neurotoxicity. These data also suggest that, in contrast to MA, MDMA toxicity may be mediated by a mechanism other than through an elevation in extracellular GLU.

567.7

CHRONIC CORTICOSTERONE ADMINISTRATION BLOCKS BEHAVIORAL SENSITIZATION TO AMPHETAMINE. J.R. Pauly, S.R. Robinson and A.C. Collins. Institute for Behavioral Genetics and Dept. of Psychology, U. of Colorado, Boulder, CO 80309.

The biochemical mechanisms that regulate sensitization to amphetamine (AMPH) are not clearly defined although several studies have implicated corticotrophin releasing hormone (CRH) as a possible component of the system. In the present study, female DBA mice were adrenalectomized (ADX) or sham-operated and implanted with 80 mg hormone pellets containing 100% cholesterol, 100% progesterone or a 60% corticosterone/40% cholesterol mixture (CCS60). Following a 3 day recovery period, locomotor activity (number of photocell interrupts in 5 min.) was measured in a circular open field arena, 15 min. following a saline injection. Treatment groups did not differ in activity following the saline challenge. On the 4 subsequent testing days, saline administration was continued for some animals while others received AMPH (5.0 mg/kg). CCS60 animals (SHAM and ADX) were initially more sensitive to AMPH than other treatment groups but did not develop behavioral sensitization. ADX animals were initially insensitive to AMPH but developed sensitization, although the magnitude was not the same as in sham-operated animals. Animals with progesterone implants had patterns of sensitization that were identical to control animals. CRH levels in the CCS60 animals are probably negligible due to enhanced negative feedback by high plasma CCS levels that persist throughout the duration of testing. These data support the hypothesis that drug-induced changes in the hippocampal-pituitary-adrenal-axis may underscore the development of behavioral sensitization to AMPH. Supported by DA-05131 and DA-00116.

567.8

THE NMDA RECEPTOR ANTAGONIST MK-801 BLOCKS THE DEVELOPMENT OF SENSITIZATION TO THE LOCOMOTOR ACTIVATING EFFECTS OF AMPHETAMINE IN THE RAT.
P. Vezina and B. Zurakowski*. Neurosciences, Loeb Research Institute, Ottawa Civic Hospital, Ottawa, Ontario K1Y 4E9 Canada.

The NMDA receptor has been shown to be involved in long-term potentiation and kindling as well as in some types of learning and memory. In the present study, the possibility that this receptor might also play a role in the development of sensitization to the locomotor activating effects of amphetamine was examined. The ability of the NMDA receptor antagonist MK-801 to block its development was assessed. Different groups of rats were injected with either saline or MK-801 (0.3 mg/kg, i.p.) prior to each of five injections of amphetamine (1.0 mg/kg, i.p.) or saline given every third day. On a subsequent test for sensitization, all animals received saline followed by amphetamine (0.5 mg/kg, i.p.). Animals' locomotor activity was monitored following all injections. MK-801 by itself produced dramatic increases in locomotion but, unlike those produced by amphetamine, these did not increase with repeated injections nor did they produce enhanced responding to amphetamine on the test for sensitization. More importantly, animals receiving MK-801 prior to each amphetamine injection showed no evidence of sensitized locomotion on the subsequent test. These results are consistent with those of earlier reports (Karler et al., Life Sci., 45: 599, 1989) and suggest that NMDA receptors play an important role in the development of sensitization to the locomotor effects of amphetamine. They also demonstrate that the repeated elicitation of increased locomotion by itself is not sufficient to produce sensitized locomotor responding.

567.9

ATTENUATION OF THE LOCOMOTOR ACTIVITY AND THE STRIATAL AND NUCLEUS ACCUMBENS DOPAMINERGIC RESPONSES TO AMPHETAMINE BY NALOXONE . M.S. Hooks, D.N.C. Jones, J.B. Justice, Jr., and S.G. Holtzman, Deptartments of Chemistry and Pharmacology, Emory University School of Medicine, Atlanta GA, 30322

Naloxone (NX), an opioid antagonist, has been shown previously to reduce behavioral effects of amphetamine (AMPH) in rats. The neurochemical basis of this interaction is unknown, but could be due to the ability of NX to attenuate the dopaminergic response to AMPH. The present study tested this possibility by determining the effects of NX on 1) stimulation of locomotor activity by AMPH, and 2) AMPH induced release of dopamine (DA) in the striatum (STR) and the nucleus accumbens (NACC) using in vivo microdialysis.

In the first experiment, rats were initially treated with either NX (5 mg/kg) or saline and 30 min later received increasing cumulative doses of AMPH (0.0, 0.1, 0.4, 1.6, and 6.4 mg/kg) at 30 min intervals. Gross locomotor counts following AMPH administration were significantly lower for rats pretreated with NX than for rats pretreated with saline ($p<0.005$). In the second experiment the same drug treatments were given in addition to microdialysis in either the STR or NACC. There was an overall decrease ($p<0.05$) in STR DA concentration between rats treated with NX and those treated with saline. This difference occurred only after AMPH injection and was dose-dependent, ($p<0.0001$). No overall differences were observed in dopaminergic response in the NACC between the two groups, although a Treatment x Time interaction was observed, ($p<0.001$) due to differences between the groups during the later portion of response to 6.4 mg/kg AMPH. There was also a difference in locomotor activity following AMPH treatment between NX and saline treated subjects which were dialyzed, ($p<0.0005$). The STR rats treated with saline showed a larger % increase in DA levels, ($p<0.001$) following AMPH treatment than the NACC rats treated with saline. No differences between STR and NACC dopaminergic responses were observed in NX pre-treated rats.These findings suggest that a decrease in the dopaminergic response to AMPH is the mechanism by which NX attenuates behavioral stimulant effects of AMPH. (Supported in part by grants DA00541 and RSA DA00008).

567.10

REPEATED ADMINISTRATION OF MK-801 PRODUCES SENSITIZATION TO ITS OWN LOCOMOTOR STIMULANT EFFECTS BUT BLOCKS SENSITIZATION TO AMPHETAMINE
M.E. Wolf and M.R. Khansa. Department of Psychiatry, Cellular and Clinical Neurobiology Program, Wayne State Univ. Sch. of Med., Detroit, MI, 48207.

Repeated administration of amphetamine (AMPH) results in an augmentation of the locomotor stimulant effects elicited by subsequent AMPH challenge. The present study examined the possibility that this behavioral sensitization may require stimulation of N-methyl-D-aspartate (NMDA) receptors. Rats received injections of saline, AMPH (1 mg/kg), the NMDA antagonist MK-801 (0.25 mg/kg), or AMPH + MK-801 on days 1-3 and 6-10. Rats were tested for horizontal locomotor activity on several treatment days to verify that a progressive augmentation of responsiveness to AMPH was occurring. On day 14, all rats were challenged with 0.5 mg/kg AMPH and tested for locomotor activity. Sensitization was observed only in the repeated AMPH group, indicating that coadministration of MK-801 blocked the development of sensitization. On day 17, all rats were challenged with 0.25 mg/kg MK-801. The MK-801 and AMPH + MK-801 groups showed robust sensitization, indicating that repeated MK-801 produced sensitization to its own locomotor stimulant effects and that this was not blocked by AMPH coadministration. On day 13, all rats were challenge with saline. The AMPH group showed greater activity than the saline group, suggesting the development of conditioned locomotion. However, conditioned locomotion was blocked by coadministration of MK-801. These results suggest that behavioral sensitization may resemble long-term potentiation, a more thoroughly characterized form of synaptic plasticity, in that both require NMDA receptor stimulation for their induction. *In vivo* microdialysis studies are underway to examine dopamine-glutamate interactions which may account for these findings. Supported by NARSAD and the PMA Foundation.

567.11

INJECTION OF 3,4-METHYLENEDIOXYMETHAMPHETAMINE (MDMA) INTO THE NUCLEUS ACCUMBENS SEPTI (NAS) PRODUCES BEHAVIORAL ACTIVATION IN RATS. C.W. Callaway, M.A. Geyer. Department of Psychiatry, UCSD School of Medicine, La Jolla, CA 92093-0804.

MDMA is a derivative of amphetamine that potently increases release of both serotonin (5-HT) and dopamine (DA). Systemic administration of MDMA to rats produces locomotor hyperactivity that is dependent upon 5-HT release (JPET 254: 454-464). This study examined the contribution of different sites to the behavioral effects of MDMA. Male sprague-dawley rats were surgically fitted with guide tubes for intracranial microinjections. Behavioral testing began at least one week post surgery and employed a behavioral pattern monitor (BPM) in which infrared photobeams tracked the position of the rat inside a 30.5 x 61 cm Plexiglas box. Injection of MDMA (10 or 30 ug in 1 ul) into the NAS produced locomotor hyperactivity lasting 30-40 min. Injections of MDMA (10 ug) into the dorsolateral caudate were ineffective. Although the locomotor activating effects of systemically administered MDMA are antagonized by pretreatment with 5-HT uptake inhibitors, fluoxetine pretreatment (10 mg/kg) did not alter the response to intraaccumbens MDMA. Furthermore, a congener of MDMA that more selectively releases 5-HT, MBDB (3, 10 or 30 ug), did not produce hyperactivity when administered into the NAS. Previous studies found that MBDB and MDMA have similar behavioral effects after systemic administration. These studies thus suggest that MDMA administered into the NAS produces behavioral activation by virtue of the DA releasing properties that MDMA shares with amphetamine and not via the 5-HT releasing properties that MDMA shares with MBDB.

567.12

THE NON-COMPETITIVE NMDA ANTAGONIST, MK801, BLOCKS THE DEVELOPMENT OF CONDITIONED ACTIVITY TO AMPHETAMINE (AMPH). J. Stewart and J.P. Druhan. Center for Studies in Behavioral Neurobiology, Department of Psychology, Concordia University, Montréal, Québec, Canada, H3G1M8.

It has been reported (Karler et al,1989) that preinjections of MK801 block the development of behavioral sensitization to repeated intermittent injections of AMPH, suggesting that NMDA receptor activation is directly involved in the neural changes underlying sensitization. It is known, however, that when preexposure to AMPH is explicitly paired with the testing environment, the expression of sensitization is environment-specific and animals tested under saline show conditioned behavioral activation in that environment. We compared the development of conditioned activity to 1.5 mg/kg AMPH in animals pretreated with either 0.25 mg/kg MK801 or saline prior to activity box injections, using a conditioning procedure in which groups of animals received AMPH in the activity boxes and saline in the home-cage, saline in the activity boxes and AMPH in the home-cage, or saline in both environments. On a test for conditioning, when all animals received only saline in the activity boxes, no evidence for conditioned activity was found in animals previously given AMPH there in the presence of MK801, suggesting either that MK801 blocks the development of conditioned control of sensitization to AMPH and not the development of sensitization, per se, or that MK801 interferes with the unconditioned action of AMPH preventing the development of both conditioning and sensitization.

567.13

AMPA/KAINATE GLUTAMATE RECEPTOR ANTAGONISTS IN THE NUCLEUS ACCUMBENS SELECTIVELY DECREASE THE HYPERMOTILITY RESPONSE TO AMPHETAMINE. D.L. Willins*, L. J. Wallace, M.C. Gerald, and N.J. Uretsky. College of Pharmacy, The Ohio State University, Columbus, OH 43210.

Previous studies show that activation of AMPA and/or kainate (A/K) receptors in the nucleus accumbens (NA) stimulates locomotor activity (LMA). In the present study experiments were conducted in order to determine whether A/K receptors in the NA play a role in the hypermotility response to the psychostimulant drugs, amphetamine, caffeine, and scopolamine. Both DNQX (1 µg/0.5 µl) and GAMS (5 µg/0.5 µl), which are A/K receptor antagonists, injected bilaterally into the NA inhibited the hypermotility responses to D-amphetamine (0.5 mg/kg, s.c.) but not to caffeine (20 mg/kg, s.c.) or scopolamine (0.5 mg/kg, s.c.). In contrast, AFQX, a chemical analog of DNQX which has very low potency in competing for AMPA binding sites, did not inhibit LMA elicited by amphetamine. Neither A/K antagonist significantly affected LMA in control animals. These findings suggest that activation of A/K receptors in the NA is important in amphetamine-stimulated locomotion. Since the response to amphetamine, but not caffeine or scopolamine, is mediated by endogenous dopamine in the NA, the results suggest an association between glutamatergic and dopaminergic transmission at this site.

567.14

EFFECTS OF INTRA-ACCUMBENS INJECTION OF THE GLUTAMATE ANTAGONIST DNQX ON PLACE PREFERENCE AND LOCOMOTOR ACTIVITY INDUCED BY AMPHETAMINE. R.T. Layer, N.J. Uretsky, and L.J. Wallace. College of Pharmacy, Ohio State University, Columbus, OH 43210.

Dopaminergic terminals within the nucleus accumbens (NA) mediate locomotor activity (LMA) and positive reinforcement elicited by amphetamine. Glutamatergic afferents to the NA from limbic structures may also participate in these effects. The purpose of this study was to determine whether an AMPA/kainate receptor antagonist within the NA could inhibit amphetamine-induced LMA or reinforcement as measured by conditioned place preference (CPP). Rats were trained for 8 days, with 4 amphetamine pairings (1 mg/kg, sc) to one compartment and 4 saline pairings to the other. One group of rats received DNQX (1 µg/0.5 µl) in the NA via implanted cannulae at the time of amphetamine injections. Rats were tested for CPP on the 9th day. LMA was monitored on all training and test days. Consistent with data from open field tests, DNQX inhibited amphetamine-induced LMA by 60% during the first training session. However, DNQX reduced LMA by only 20% on the 2nd amphetamine training day and not at all in the final two training sessions. Thus, the interaction between dopaminergic and glutamatergic neurons in the NA may change with exposure to amphetamine. This confounding variable makes interpretation of CPP data difficult, although preliminary data suggest that DNQX treatment inhibits amphetamine-induced CPP.

567.15

MK-801 PARTIALLY ANTAGONIZES THE LOCOMOTOR AND STEREOTYPIC EFFECTS OF METHAMPHETAMINE. G.M. Farfel, N.E. Ferguson and L.S. Seiden. Department of Pharmacological & Physiological Sciences, The University of Chicago, Chicago, IL 60637.

MK-801, a non-competitive NMDA receptor antagonist, has been shown to attenuate both behavioral sensitization (Karler, et al., Life Sci., 45:599-606) and dopaminergic neurotoxicity (Sonsalla, et al., Science, 243:398-400) produced by repeated administration of psychomotor stimulants. To determine whether MK-801 (MK) antagonizes behaviors elicited by a neurotoxic regimen of methamphetamine (MA), rats (n=16/group) were administered 4 injections of either MA (10 mg/kg) or saline (S; 1 ml/kg) i.p., spaced 1 h apart. In addition, half of the rats in each group received MK (2.5 mg/kg) and half received S (1 ml/kg) i.p., 15 min before and 3 h after the first MA or S injection. Two observers scored the animals' behavior 1 h after the first MA or S injection, and 1, 4, 12, 24 and 48 h after the last injections. Differences in behaviors between treatment groups were assessed using Kullback's information statistic. One hour after the first injection, locomotor activity (LMA) was higher in the MK/MA and MK/S animals than in the S/MA and S/S groups, while the S/MA group scored higher in sniffing than the others. Piloerection (PILO) was higher in the S/MA and MK/MA groups than in MK/S and S/S. One hour after the last injection, the S/MA group scored higher in LMA than the others, while the MK/MA group scored highest in splayed body and the MK/S group exhibited significant swaying. PILO was not different in any group at this time point, but the S/MA group exhibited exopthalmia which was significantly different than either S/S or MK/MA. By 4 h, MK/MA animals scored higher than the others in LMA, rearing or falling (from a rearing position), while S/S and S/MA were not moving. At 12, 24 and 48 h, behaviors did not differ between groups. The results indicate that MK-801 attenuates the stereotypic and locomotor behaviors engendered by a neurotoxic regimen of MA. MK-801 also attenuates MA-induced exopthalmia but not piloerection. (Supported by DA-00085.)

567.16

RESPONSE SPECIFICITY IN THE INFLUENCE OF A SHOCK-POSTPONEMENT SCHEDULE ON THE EFFECTS OF *d*-AMPHETAMINE AND COCAINE ON PUNISHED RESPONDING IN SQUIRREL MONKEYS. T. Tatham* and J.E. Barrett. Behavioral Pharmacol. Lab., Uniformed Services University of the Health Sciences, Bethesda, MD 20814.

Squirrel monkeys' low-rate punished lever pressing (food-reinforced fixed-interval schedule with superimposed fixed-ratio shock) is reliably unaffected or decreased by *d*-amphetamine (AMP). However, concurrent or prior exposure to a shock-postponement (avoidance) schedule causes AMP to dramatically increase punished responding, thereby reversing the typical effects of AMP. The present research examined whether exposure to avoidance, per se, reverses AMP's effects or whether the reversal of effects occurs only when both avoidance and punishment are within the same response system (lever-pressing). The present research arranged the contingencies on distinctive manipulanda -- punishment on a lever and avoidance on a pull-chain; the effects of AMP and cocaine were determined before and after exposure to chain-pull avoidance. In contrast to the effects of arranging both contingencies on the same manipulandum, the present procedure produced no appreciable differences in the effects of either drug on punished responding, suggesting that the reversal of cocaine and AMPs' effects is restricted to the response modality in which avoidance is presented. This research was supported by DA 06828.

567.17

A DRUG DISCRIMINATION ANALYSIS OF *l*-DEPRENYL. S. Yasar, C.W. Schindler, C. Cohen*, E.B. Thorndike*, S.R. Goldberg* and I. Szelenyi*#. NIDA Addiction Research Center, Baltimore, MD 21224 and #ASTA Pharma AG, Frankfurt, FRG.

The potential antiparkinsonian agent *l*-deprenyl is a selective inhibitor of B-type monoamine oxidase (MAO). However, *l*-deprenyl is an amphetamine derivative which is metabolized *in vivo* to *l*-amphetamine. As the clinical use of amphetamine-like psychostimulants is limited by development of tolerance and dependence, we tested *l*-deprenyl for amphetamine-like and for cocaine-like discriminative stimulus effects. The discrimininative stimulus properties of *l*-deprenyl were studied in *d*-amphetamine-trained and in cocaine-trained animals using a drug discrimination paradigm (shock discrimination test). We also examined *d*-deprenyl for *d*-amphetamine-like discriminative stimulus effects to determine the stereospecificity of the isomers of deprenyl.

Male Fisher rats were trained under a fixed-ratio 5 (FR 5) schedule of stimulus shock termination to respond on one lever after an i.p. injection of either 1.0 mg/kg *d*-amphetamine or 10.0 mg/kg cocaine and on the other lever after no-drug treatment. *l*-Deprenyl (2.0-17.0 mg/kg) generalized to neither *d*-amphetamine nor to cocaine. In contrast to *l*-deprenyl, *d*-deprenyl, which would be metabolized to *d*-amphetamine, generalized to the *d*-amphetamine stimulus dose-dependently (2.0-10.0 mg/kg). These results suggest that, contrary to a previous report (Psychopharmacology **101**, S40), *l*-deprenyl does not consistently generalize to *d*-amphetamine. Furthermore, in additional experiments, *l*-deprenyl (2.0 mg/kg) given 1 hour before the *d*-amphetamine injection, did not shift the dose-response curve of *d*-amphetamine, further indicating a lack of interaction of these compounds as discriminative stimuli. These data confirm clinical findings, that *l*-deprenyl is free of abuse potential.

567.18

NEURONAL SUBSTRATES OF THE DISCRIMINATIVE STIMULUS EFFECTS OF D-AMPHETAMINE. B. J. Van Groll and J. B. Appel. *Behavioral Pharmacology Laboratory, Department of Psychology, University of South Carolina, Columbia, SC 29208.*

In two experiments, 36 rats were trained to discriminate 1 mg/kg of *d*-amphetamine from saline; they were then given compounds that purportedly act selectively at different NE, DA or 5-HT_3 receptor subtypes, either alone or in combination with the training drug. Adrenergic agonists (clonidine, salbutamol, nisoxetine) did not mimic and NE antagonists (yohimbine, propranolol) did not block the d-amphetamine cue. While several compounds that stimulate postsynaptic DA receptors (metoclopramide, SKF 23390 and quinpirole) did not have amphetamine-like properties, sufficiently high doses of the DA uptake inhibitor GBR 12909 mimicked, and at least one dose of Sch 23390 (0.75 mg/kg) blocked the training stimulus. Two 5-HT_3 antagonists (MDL-72222 and ICS 105-290) did not substitute for, but did potentiate the effects of low doses (0.25-0.4 mg/kg) of *d*-amphetamine; this potentiation was blocked by prazocin. It was concluded that both inhibition of DA uptake and 5-HT_3 receptor mechanisms might be involved in the discriminative stimulus effects of *d*-amphetamine.

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567.19

CARDIOVASCULAR EFFECTS OF METHAMPHETAMINE IN SQUIRREL MONKEYS. C.W. Schindler, J.-W. Zheng*, S. R. Tella* and S.R. Goldberg*. NIDA Addiction Research Center, Baltimore, MD 21224 and Georgetown University School of Medicine, Washington, DC 20007.

The recent discovery of the abuse of methamphetamine (METH) in a smokable form known as "ice", suggests that abuse of this drug may be increasing. As such, it is important to investigate the potential toxic consequences of its abuse. We studied the effects of METH on cardiovascular function in squirrel monkeys, a model we have used extensively to study the cardiovascular effects of cocaine. Squirrel monkeys were implanted with chronic arterial and venous catheters and the effects of METH were studied while the animals were conscious and seated in a standard squirrel monkey restraining chair. METH (0.1-3.0 mg/kg, i.v.) produced a dose-dependent increase in blood pressure. Its effects on heart rate were more complex, with lower doses (0.1-0.3 mg/kg) producing increases in heart rate and higher doses (1.0-3.0 mg/kg) producing decreases. To determine the pharmacological mechanisms involved in METH's effects, we tested a number of drugs as pretreatments to an injection of 0.2 mg/kg METH. This dose produced a maximal heart rate increase. The alpha-1 antagonist prazocin (0.1 mg/kg, i.v.) completely antagonized the effects of METH on blood pressure, while the beta antagonist propranolol (3.0 mg/kg, i.v.) completely antagonized the tachycardiac effect of METH. Propranolol also appeared to potentiate the effects of METH on blood pressure by prolonging its effect. These effects are similar to those observed previously with prazocin and propranolol when tested against cocaine (FASEB J. 5, A1587). Unlike its effects against cocaine (Life Sci. 48, 1547), however, the dopaminergic antagonist haloperidol (0.03 mg/kg, i.m.) antagonized both the pressor and tachycardiac effects of METH. Thus, while METH and cocaine produce similar effects on cardiovascular function, they may be mediated through different mechanisms, with dopaminergic mechanisms being more important for METH than for cocaine.

DRUGS OF ABUSE—OPIOIDS

568.1

ETHANOL REGULATES TYROSINE HYDROXYLASE GENE EXPRESSION IN N1E-115 NEUROBLASTOMA CELLS. Greg G. Gayer*†, Adrienne Gordon†¶ and Michael F. Miles¶ Ernest Gallo Research Center and, Departments of †Pharmacology and ¶Neurology, Univ. of Calif., San Francisco, CA 94143. Previous studies show that changes in catecholoamines contribute to neurophysiologic effects of ethanol. We have therefore studied ethanol-induced changes in tyrosine hydroxylase (TH), the rate-limiting enzyme for catecholamine biosynthesis, in N1E-115 neuroblastoma cells. Northern and Western blot analyses showed that a 3 day treatment with 25-200 mM ethanol caused a dose-dependent increase in TH-mRNA and protein levels in N1E-115 cells. Subclones of N1E-115 stably transfected with a plasmid containing the TH promoter fused to a chloramphenicol acetyltransferase (CAT) reporter gene showed ethanol-induced increases in CAT activity. These data suggests that ethanol modulates TH gene transcription. Simultaneous treatment of transfected cells with 100 mM ethanol and either 1 mM prostaglandin E_1, 10 mM (-)-N^6-(R-phenylisopropyl)-adenosine, or 0.5 mM 8-bromo-cAMP, potentiated TH-promoter activity when compared to treatment with the respective agents without ethanol. These results suggest that chronic ethanol has a prominent effect on both basal and cAMP-regulated TH expression. Using deletion analysis of the cis-acting regulatory sequences of the TH-promoter, the regions responsible for both basal and cAMP-mediated ethanol induction were characterized. Ethanol-induced changes in TH expression may be a critical aspect of central nervous system adaptation to chronic ethanol exposure.

568.2

LONG TERM EFFECT OF PRENATAL METHADONE EXPOSURE ON NEUROPEPTIDE EXPRESSION IN RAT HYPOTHALAMUS. J. W. Nemitz. W VA School of Osteopathic Medicine, Lewisburg, W VA, 24901.

Previous work provided evidence that prenatal methadone exposure results in a decrease in mRNA expression for proenkephalin (ENK), oxytocin (OT), and arginine-vasopressin (AVP) in hypothalamic nuclei of the neonatal rat. The present study examined the mRNA expression of these neuropeptides in hypothalamic nuclei of older rats that were prenatally exposed to methadone.

Adult, female, Sprague-Dawley rats made physically dependent to methadone (9mg/kg/day) by an osmotic minipump (Alzet), were mated and the pregnancies brought to term. Offspring were sacrificed by decapitation between ages six months to one year after birth. Frozen serial coronal sections (20 um) obtained at the level of the hypothalamus were processed for in situ hybridization using ^{35}S-oligonucleotide probes (DuPont). Results indicate that OT and AVP mRNA levels are increased in the retrochiasmatic supraoptic nucleus (RSO) of the hypothalamus of prenatally methadone exposed rats as compared to controls. Differences were not observed in the paraventricular (PV) and supraoptic (SO) nuclei. Results for ENK mRNA expression will also be presented. These data suggest that prenatal methadone exposure can result in selective long lasting changes in neuropeptide expression for a hypothalamic nucleus. Supported by WVSOM funds.

568.3

mRNA EXPRESSION PROFILES AFTER OPIATE TREATMENT IN CELL CULTURE & THE RAT CNS. SA Mackler & JH Eberwine. Dept of Pharmacology, Univ of Pa School of Medicine, Phila PA 19104

Opiate use alters the amounts of several proteins. The present studies use the *in vitro* amplification of RNA from limited amounts of tissue (aRNA;PNAS87:1663) to define the composite of relative changes in mRNA abundance which result from opiate stimulation or withdrawal of stimulation.

NG108-15 cells were exposed to 10nM DADLE or 10nM DADLE followed by 100uM naloxone for varying time periods to achieve maximal delta opiate receptor down- and up-regulation, respectively. PolyA+ RNA was isolated and synthesized into 32-P labeled aRNA. This was hybridized to blots containing several cDNA clones and comparisons made among the individual autoradiographic bands. Both receptor stimulation & withdrawal of stimulation resulted in increased signals for the proto-oncogenes fos & jun and decreased signals for K+ channels. Naloxone increased the signal for the Na+ channel & DADLE use increased the signal for the G protein stimulatory alpha subunit.

Male adult rats received subcutaneous pellet administration of either morphine or a placebo for three days. Coronal sections of the striatum underwent *in situ* transcription of the polyA+ RNAs, which were processed for aRNA synthesis. These aRNAs were also used to screen similar cDNA-containing gel blots. Morphine treatment again decreased the signals for the K+ channels & increased the signals for fos, jun, & Gs alpha. There was also an increased signal for the Na+ channel.

These studies demonstrate several changes in gene expression as a consequence of opiate use and provide hypotheses for testing the molecular mechanisms of addiction.

568.4

EFFECTS OF PRENATAL MORPHINE ON ADULT SEXUAL BEHAVIOR AND ON BRAIN CATECHOLAMINES IN RATS. Ilona Vathy. Dept. Psychiatry, Albert Einstein Coll. Medicine, Bronx, NY 10461.

Female rats exposed to morphine sulphate (MS) in utero (5-10 mg/kg twice a day on days of 11-18 of gestation) were substantially inhibited in their adult sexual behavior when compared to saline-exposed (S) controls. In contrast, males exposed prenatally to MS had shorter post-ejaculatory intromission latencies and exhibited increased mounting and intromitting activity relative to controls. Examination of the catecholamine content in the preoptic area (POA) and in the hypothalamus (HYP) also revealed that prenatal MS treatment affects males and females differently. Norepinephrine (NE) and dopamine (DA) content in the HYP of S and MS-treated females were essentially identical; however, NE and DA levels in the POA of MS-treated females were decreased about 60%. NE content in the HYP of MS-treated males was increased about 50% relative to that in S-exposed animals while the DA content was not affected. In addition, the DA content was decreased (30%) and the NE content increased (30%) in the POA of MS males when compared to controls. These results suggest that prenatal MS exposure, which differentially affects adult male and female sexual behavior, also alters the content of catecholamines in the HYP and POA in a sexually dimorphic fashion. Supported by NIDA DA 05833.

568.5

CP55940 HAS THC-LIKE DISCRIMINATIVE STIMULUS EFFECTS IN RATS. L.H. Gold, R.L. Barrett, R.L. Balster and B.R. Martin*. Dept. of Pharmacology and Toxicology, Medical College of Virginia, Richmond, VA 23298-0613.

CP55940, a phenyl-cyclohexanol derivative, is a potent bicyclic analog of Δ9-tetrahydrocannabinol (THC) which has been used as a probe for a cannabinoid recognition site in neural tissue. In the present study, CP55940 was evaluated in rats trained to discriminate THC (3.0 mg/kg, ip) from vehicle in a two-lever operant procedure. In addition, catalepsy was measured using an adaptation of the mouse ring-test. Dose-effect relationships were established for the training drug (THC 0.01-10.0 mg/kg, ip) and CP55940 (0.003-0.3 mg/kg, ip) at 30 and 90 min after injection. CP55940 substituted completely for THC at both time periods at a dose (0.1 mg/kg) that had minimal effects on rate of responding. CP55940 was about 10-30-fold more potent than THC, consistent with its greater affinity for the cannabinoid site. Doses of THC (>3.0 mg/kg) and CP55940 (>0.1 mg/kg) that reduced response rates by greater than 50% were associated with substantial increases in immobility. A study of the duration of drug effects revealed that both CP55940 (0.1 mg/kg) and THC (3.0 mg/kg) produced diminished levels of THC-lever responding by 360 min post-injection. These results support the use of this potent cannabinoid as a probe for the neural substrate mediating the behavioral responses to THC.
(Supported by NIDA grants DA-03672 and DA-07027)

568.6

Pharmacological Characterization of Stably Expressed Rat and Human Marijihuana Receptor Clones

*Christian C. Felder, *Jeffrey S. Veluz, *Holly L. Williams, Ted B. Usdin, *Lisa A. Matsuda, Laboratory of Cell Biology, National Institute of Mental Health, Bethesda, MD 20892

Both rat and human cannabinoid receptor clones were stably expressed in mammalian fibroblast cells. Activation of the cloned receptor with various natural and synthetic cannabinoid ligands demonstrated rank order potency and efficacy for the inhibition of forskolin-stimulated cAMP accumulation similar to previously published data using cells containing native cannabinoid receptors. Other second messenger pathways were tested for possible cannabinoid receptor-mediated activation. Cannabinoid receptor activation stimulated calcium influx independent of IP_3 and intracellular calcium release. Potent cannabinoid agonists, CP55,940 and HU-210 (1,1-dimethylheptyl-7-hydroxy-delta-6-tetrahydrocannabinol), and the inactive enantiomer HU-211, stimulated arachidonic acid release at concentrations above 10uM in cells expressing the cannabinoid receptor and in untransfected control cells. These results suggest that cannabinoid ligands stimulate arachidonic acid release through a non-receptor mediated mechanism and only at elevated concentrations. No functional difference was observed between the rat and human cannabinoid receptor for the second messenger pathways tested.

568.7

TETRAHYDROCANNABINOL EFFECTS ON BRAIN STIMULATION REWARD AFTER SUBACUTE HALOPERIDOL OR AMPHETAMINE. M. Borrero* and B. Hine. U. of Puerto Rico Sch. Pharm., San Juan, PR 00936.

Using a brain stimulation reward (BSR) procedure that has demonstrated threshold decreases and response rate increases after low morphine doses (Psychopharm. 102:309, 1990), we have found non-uniform changes in BSR thresholds after low doses of Δ^9-tetrahydrocannabinol (THC) in Lewis and SD rats (The FASEB Journal 5: A703, 1991). Since the BSR threshold lowering effect of THC only in Lewis rats, reported by Gardner, has been related to facilitation of mesolimbic dopamine (DA) release by THC (Psychopharm. 96: 142, 1988; 102: 156, 1990), we tested the hypothesis that THC would lower BSR thresholds, and/or increase response rates, in SD rats that were sensitized to DA agonists. Twelve days after withdrawal from osmotic pump infusion of haloperidol (0.4 mg/rat/day for 14 days), significant increases in BSR rates occurred in male SD rats, and this treatment attenuated the threshold increases seen after 0.5 and 1.0 mg/kg THC doses; THC-induced threshold decreases were not seen at other doses, nor did they occur reliably in female SD rats receiving similar THC challenge doses 11 days after withdrawal from a week of daily d-amphetamine sulfate injections, a procedure reported to increase mesolimbic DA utilization in female rats (Robinson: Brain Res. 343: 374, 1985). The basis for the reported strain-specific BSR threshold lowering effect of THC is not apparent and requires further investigation to clarify the conditions that permit unequivocal designation of THC as a rewarding drug. Supported by S06RR8224.

568.8

EFFECTS OF PEDUNCULOPONTINE TEGMENTAL NUCLEUS LESIONS ON MORPHINE INDUCED CONDITIONED PLACE PREFERENCE AND ANALGESIA IN THE FORMALIN TEST. M.C. Olmstead and K.B.J. Franklin. Dept. Psychology, McGill University, Montreal, Canada, H3A 1B1

The pedunculopontine tegmental nucleus (PPTg) receives projections from limbic structures associated with reward systems, and it has been suggested that the region is a critical site in neural systems subserving the rewarding effects of morphine. There is also evidence that analgesia in the formalin test depends on the neural systems that mediate the rewarding properties of drugs. Bechara and Van der Kooy (1989) demonstrated that PPTg lesions block the rewarding properties of morphine: we predicted a correlation between attenuation of morphine induced reward and analgesia in these animals. We have confirmed that excitotoxin lesions of the PPTg block development of conditioned place preference to morphine. In the same animals, morphine induced analgesia was reduced, but not eliminated. The reduction of reward and/or analgesia was not significantly correlated with the loss of cholinergic cells in the PPTg, suggesting that the neural substrates mediating morphine reward and analgesia involve the PPTg, but are not dependent on the cholinergic cells in the region. Further studies demonstrated that PPTg lesioned animals display reduced pain scores when tested in the absence of analgesics. During behavioral testing and handling, we observed that sham and lesioned animals differ in their motor abilities. For example, sham and lesioned animals differed in measurements of spontaneous motor activity and catalepsy in both morphine and drug free trials. Furthermore, neurological tests revealed that PPTg lesioned animals exhibit motor abnormalities, including problems with postural support and muscle tone. We believe that the hyperactivity and motor abnormalities that PPTg lesioned animals exhibit may interfere with the expression of pain in the formalin test, resulting in reduced pain scores compared to shams.

568.9

MORPHINE REDUCES THE AVERSIVENESS OF NOVEL QUININE SOLUTION. L.A. Parker. Department of Psychology, Wilfrid Laurier University, Waterloo, Ontario, Canada, N2L 3C5.

The ability of morphine (2 mg/kg, sc) to modify the palatability of .05% quinine solution and 20% sucrose solution was assessed in Sprague-Dawley rats using the Taste Reactivity (TR) test. The TR test is a direct measure of the palatability of tastants. Morphine suppressed the aversive TR responses of chin rubbing, gaping and paw treading elicited by quinine on the first 10 minute test trial without modifying general activity level (horizontal and vertical). The effects of morphine on the palatability of sucrose solution were minimal.

568.10

ANABOLIC STEROIDS DISRUPT LATENT INHIBITION. M.J. Kelley[1]*, R.B. Messing[1,2], E. Nwokolo[1] and S.B. Sparber[1]. [1]Pharmacology Dept., [1]Univ. of Minnesota, [2]Minn. Dept. Health, Mpls. MN 55455.

Latent inhibition (LI) was studied in male rats by exposures to a Skinner box prior to administering grid shock in the box. Control rats were exposed to a nesting cage followed by shock in the Skinner box. Conditioned-freezing behavior 24-hr after shock was the dependent variable. In the first experiment, testosterone (13.5 mg/kg), or an equimolar dose of nandrolone or olive-oil vehicle were administered seven times over 17 days, prior to and concurrent with the LI procedure (N = 36). A second multi-dose experiment assessed LI 10-months after the administration of anabolic steroids during "adolescence" (N = 73). Both experiments showed a LI effect with vehicle ($p < .01$) but not the testosterone groups. Nandrolone administered 10-months earlier also disrupted LI but had a marginal effect when concurrent with LI testing. Andogenic (testosterone and nandrolone) and estrogenic (metabolism of testosterone) effects may be differentially involved in acute versus enduring influences on LI. Supported in part by USPHS grant DA011979 and MMF grant SMF68-90.

568.11

GABA LEVELS IN DIALYSATE OF RAT PERIAQUEDUCTAL GRAY DURING MORPHINE ADMINISTRATION. Y.F.Jacquet, E.Yablonsky-Alter and R.L.Napolitano*, Nathan Kline Institute, NY, 10962, CCNY Med. Sch., NY, 10031, and Bioanalytical Systems, W. Lafayette, Ind., 47906.

The role of GABA in opiate-induced analgesia remains unclear. Intraperitoneal (IP) administration of GABAergic drugs was found to potentiate morphine analgesia,while intracerebral (IC) injections of morphine into the morphine-sensitive CNS site, the periaqueductal gray (PAG), were reported to reduce morphine analgesia. The present study investigated GABA levels in dialysates obtained from the rat PAG during morphine administration. A microdialysis probe (BAS) was inserted into chloral hydrate anesthetized male rats (B.W. approx. 230 g) mounted on a Kopf stereotaxic; dialysates were collected in 30-min samples (after discarding the first 60-min dialysate) during perfusion of Ringers at 1 uL/min. Morphine was given IP (10 mg/kg) or IC (15 ug/uL) through the probe following a 2-hr control period, and collection continued for another 1.5 hrs. GABA was evaluated using the BAS HPLC electrochemical detection method. All rat brains were examined histologically to determine probe placement. Changes in GABA levels following morphine administration were found to be correlated with variations in placement site. Decreases in GABA levels were found when probes were located within the PAG, while more variable results were seen in other sites.

568.12

EFFECTS OF D-SERINE ON EEG, OVERT BEHAVIOR AND DISCRIMINITIVE STIMULUS EFFECTS OF PCP IN RATS. J. E. Moreton, L. Robles, J. M. Witkin, L. G. Sharpe and F. C. Tortella. Univ. Maryland Sch. of Pharmacy, Baltimore, MD, 21201, Neuropharmacol. Br., Walter Reed Army Inst. Res., Washington, DC, 20307 and NIDA Addiction Res. Ctr., Baltimore, MD, 21224.

The potential antagonist action of D-serine, a selective agonist for the glycine/NMDA receptor, against the EEG and behavioral effects of PCP, a noncompetitive NMDA antagonist, was investigated. In rats bearing chronic cortical EEG electrodes and iv and icv cannulae PCP (1.25 mg/kg, iv) produced a behavioral syndrome characterized by head weaving, locomotion, and ataxia. Five min pretreatment with D-serine (1 μM, icv) tended to reduce these PCP-induced behaviors, but the effect was not significant, nor dose-dependent (2 μM). Simultaneous recording of EEG frequency spectra revealed a PCP-induced dual-peak response centered about 2-3 Hz and 7-8 Hz which was also unaltered by D-serine. D-serine alone produced no significant EEG or behavioral changes. In rats trained to discriminate 1.5 mg/kg PCP ip versus saline on a FR 20 schedule of food presentation, PCP produced 95% responding on the PCP-correlated lever. This effect was mimicked by (+)-MK801 but not significantly altered by a two min pretreatment with D-serine (0.5-2 μM, icv). Collectively, these results fail to support a PCP antagonist effect by D-serine. Confirmatory icv PCP studies are in progress. (Supported in part by grant DA03173)

568.13

AN INTERACTION BETWEEN OPIOID AND NORADRENERGIC SYSTEMS IN THE MODULATION OF PULSATILE LUTEINIZING HORMONE SECRETION BY DELTA-9-TETRAHYDROCANNABINOL. L.L. Murphy , P. Keller* and M. Kohli*. Department of Physiology, Southern Illinois University, School of Medicine, Carbondale IL 62901.

We have previously demonstrated that naloxone (NAL), an opiate receptor blocker, can reverse the ability of delta-9-tetrahydrocannabinol (THC), the major psychoactive component of marijuana, to inhibit pulsatile luteinizing hormone (LH) secretion in the ovariectomized female rat. Because the effects of endogenous opioids on LH-releasing hormone (LHRH) secretion and, thus, LH release, may be mediated through hypothalamic noradrenergic systems, we attempted to block the NAL reversal of THC-induced LH inhibition by pretreatment with norepinephrine receptor blockers. Approximately 4 weeks post-ovariectomy, blood samples were drawn from adult Sprague-Dawley rats, via indwelling intra-atrial cannulae, every 10 min for 60 min pre- and 120 min post-vehicle (control) or THC (0.5 mg/kg b.w., iv) treatment at time 0 for LH determinations. Either the β-receptor antagonist, yohimbine (YOH, 0.5 mg/kg, iv), or the α-receptor antagonist, prazosin (PRZ, 0.5 mg/kg, iv), was administered 30 min prior to THC or its vehicle, and 50 min before NAL (2 mg/kg b.w., iv) treatment. Neither YOH nor PRZ altered pulsatile LH secretion in vehicle-exposed animals treated with NAL, and YOH pretreatment did not alter the ability of NAL to reverse THC action. However, pretreatment with PRZ prevented NAL reversal of THC-induced suppression of pulsatile LH release. These results suggest that THC may augment hypothalamic opioid activity which, in turn, could inhibit noradrenergic stimulatory inputs onto the LHRH secreting neurons. (Supported by DA 05042)

568.14

EFFECTS OF ANALGESIC DOSES OF MORPHINE ON REGIONAL CEREBRAL BLOOD FLOW IN THE RAT. H. Zhang*, E.A. Stein and T.C. Trusk. Departments of Psychiatry and Cell Biology and Anatomy, Medical College of Wisconsin, Milwaukee, WI 53226

The widespread distribution of opioid receptors and peptides in the central nervous system suggests an important role for this system in numerous brain functions including analgesia and regulation of numerous physiologic parameters. Included among these functions may be as mediators in an intrinsic central neurogenic control of cerebral circulation. The present study examined the effects of morphine on neuronal activity as reflected by alterations in regional cerebral blood flow (rCBF). rCBF was measured according to the method of Sakurada et al with the radioactive iodoantipyrene technique and quantitative autoradiography in awake, restrained rats. Morphine was delivered subcutaneously (2 and 4mg/kg) and rCBF measured 15 min later. Tail flick latencies increased at 2 mg/kg to 47% MPE and 100% at 4 mg/kg. The results showed that morphine caused a global dose-dependent increase in RCBF in 39/51 structures analyzed. Mean change in rCBF at 2 and 4 mg/kg were 27% and 71% respectively. At 4 mg/kg, morphine resulted in an increase in $PaCO_2$ of approximately 6 mm Hg, while not changing PaO_2, heart rate or blood pressure. Naloxone (10 mg/kg) was ineffective in changing either rCBF or physiologic parameters when delivered alone but was able to completely reverse the effects of 4 mg/kg morphine. These results suggest that opioid receptor stimulation by morphine may increase both functional neuronal activity as well as influence an intrinsic central neurogenic control of the cerebral circulation as well. (Supported by NIDA grant DA 05012)

568.15

RAPID DETERMINATION OF DRUG DISCRIMINATIVE STIMULUS EFFECTS UNDER A CTA PROCEDURE. T.M. Richardson*, E.A. Walker and A.M. Young. Psychology Dept., Wayne State University, Detroit MI 48202

Conditioned taste aversion (CTA) procedures allow efficient characterization of discriminative stimulus effects of drugs. This study developed a method for generating cumulative dose-response curves (DRCs) using multiple trial tests. Rats (n=8) were trained to discriminate 3.2 mg/kg morphine (MS) and saline (SAL) by giving either MS or SAL 15 min prior to 30-min access to a 0.1% saccharin solution, followed by post-session injections of 75 mg/kg LiCl or SAL, respectively. A control group (n=8) received identical pre-session injections, but always received SAL post-session. Five subjects from each group received single trial tests in which an acute dose of a test drug was given 15 min prior to 30-min access to saccharin. Generalization was measured by differences in the dose needed to suppress drinking in the conditioned and unconditioned groups. MS (tested at doses of 0.1-10 mg/kg) and buprenorphine (0.001-0.1 mg/kg) evoked dose-dependent generalization with MS, whereas ketamine (0.1-32 mg/kg) suppressed drinking at identical doses in both groups. Three subjects from each group received multiple trial test sessions, in which each trial consisted of a 15-min pretreatment interval and 5-min access to 5 ml of saccharin. SAL and increasing doses of a test drug were given before successive trials. Multiple trial tests generated DRCs similar to those from single trial tests. MS (0.1-10 mg/kg), buprenorphine (0.001-0.32 mg/kg) and nalbuphine (0.032-32 mg/kg) evoked generalization with MS, whereas ketamine (0.1-32 mg/kg), U50,488H (0.1-5.6 mg/kg) and *d*-amphetamine (0.1-1.6 mg/kg) did not. In conclusion, multiple trial testing in the CTA procedure allows rapid, pharmacologically-specific evaluation of discriminative stimulus effects of drugs. (APA Minority Fellowship Program and by USPHS grants DA03796 and K02-DA00132.)

569.1

LONG-TERM LITHIUM TREATMENT ENHANCES K^+-INDUCED NA,DA AND 5-HT RELEASES IN THE RAT MEDIAL PREFRONTAL CORTEX: AN IN VIVO MICRODIALYSIS STUDY. T.Koyama, Y.Odagaki*, T.Ohmori, T.Inoue* and I.Yamashita*. Dep. of Psychiatry and Neurology, Hokkaido Univ. Sch. of Med., Sapporo, Japan.

The effects of long-term lithium treatment on extracellular NA, DA and 5-HT were examined in the rat medial prefrontal cortex using the in vivo brain microdialysis method. Potassium (K^+) in the perfusion Ringer induced substantial increases in NA, DA and 5-HT outputs in a concentration-dependent manner within the range of K^+ tested(30-120mM).

Maintenance of rats on a diet containing lithium carbonate(0.23%) for 7 days resulted in significant increases in the K^+(60mM)-induced NA, DA and 5-HT releases. Previously we reported that chronic lithium treatment induced significant increases in the accumulations of DOPA and 5-HTP after NSD 1015, aromatic l-amino acid decarboxylase inhibitor, administration, and the concentrations of HVA, DOPAC, 3-MT and 5-HIAA in various brain regions.

Taken together these results are suggestive that lithium treatment enhances the activity of presynaptic monoaminergic neurons.

569.2

LITHIUM INCREASED NEURONAL EXCITABILITY WITHOUT CHANGING ENDOGENOUS LEVELS OF MONOAMINES IN RAT HIPPOCAMPAL SLICES. J.-C. Lacaille, S. Cloutier*, F. Lebel* and T.A. Reader. Centre de recherche en sciences neurologiques et département de physiologie, Université de Montréal, Montréal, Québec, Canada H3C 3J7.

Lithium is an effective mood-stabilizing agent. To study its mechanism of action, we have examined the acute effects of lithium (Li^+), choline (Ch^+), and sodium (Na^+) on field potentials and on the endogenous levels of monoamines in hippocampal slices *in vitro*. Population EPSPs (pEPSP) and population spikes (pSPIKE) were monitored in the pyramidal cell layer of the CA1 region prior to and after 20 minutes of superfusion with Li^+, Ch^+ or Na^+ (1-30 mM; n=62 slices). Li^+, Ch^+ and Na^+ produced a dose-dependent decrease in pEPSP amplitude (30.7-60.7% of control). Ch^+ and Na^+ also caused a dose-dependent decrease in pSPIKE amplitude (21.7-51.7% of control). In contrast Li^+ produced a dose-dependent increase in pSPIKE amplitude (121.4% of control). Following these electrophysiological experiments, slices were collected and immediately frozen. A second set of slices (n=162 slices) were similarly exposed to Li^+, Na^+ and Ch^+ (with field potential monitoring of slice viability) and then frozen. Slices were thawed 1-5 hours later, homogenized, centrifuged and endogenous levels of monoamines assayed in the individual slices by HPLC with electrochemical detection. Li^+ and Na^+ had no effect on endogenous levels of norepinephrine, epinephrine, dopamine and serotonin ($p>0.05$). Ch^+ had complex effects on dopamine levels ($p<0.05$) but did not affect other monoamines. These results suggest a specific acute effect of lithium on neuronal excitability but not on endogenous levels of monoamines.

[Supported by Scholarships (JCL & TAR) and a grant from FRSQ]

569.3

CHRONIC, BUT NOT ACUTE, LITHIUM ALTERS A MYRISTOYLATED ALANINE-RICH C KINASE SUBSTRATE (MARCKS) IN RAT HIPPOCAMPUS. R.H. Lenox, D. Watson*, J. Ellis, and J. Patel. Neurosci. Res. Unit, Dept Psychiatry, UVM, Burlington, VT 05405 & ICI Pharmaceuticals Group, Wilmington, DE 19897.

Previous studies in our laboratory have observed a reduction in PKC mediated phosphorylation of an 83kDa protein(s) in the hippocampus of rats chronically treated with lithium. Western blot analysis has identified this 83kDa protein as a major PKC substrate known as MARCKS. Because the therapeutic action of lithium in patients is known to exhibit a delay in the onset of therapeutic action and a persistence of efficacy beyond abrupt discontinuation, we have extended these studies to rats exposed to acute lithium and those withdrawn from chronic lithium. Four matched groups of Sprague-Dawley rats were examined: (1) control diet; (2) chronic lithium diet for three weeks; (3) acute lithium (5 meq/kg IP; 2hrs); and (4) 36 hours following withdrawal of chronic lithium. We observed a significant reduction of MARCKS and of PKC mediated phosphorylation of the 83kDa protein in the hippocampi of rats receiving chronic lithium, including those in the withdrawal paradigm. The acute lithium treatment did not produce these changes, even though equivalent brain lithium levels were attained. Our findings parallel the clinical therapeutic efficacy of lithium and suggest the possible involvement of MARCKS in its mechanism of action. (Supported by RO1-MH41571)

569.4

ACUTE ADMINISTRATION OF THE PUTATIVE ANTIDEPRESSANT NEFAZODONE DOES NOT ALTER REGIONAL RAT BRAIN CRF CONCENTRATIONS. K.C. Dole, M.J. Owens, G. Bissette, M.A. Vargas, and C.B. Nemeroff. Depts. of Psychiat. and Pharmacol., Duke Univ. Med. Ctr., Durham, NC 27710

Corticotropin-releasing factor (CRF), a CNS neurotransmitter, is believed to integrate the endocrine, autonomic and behavioral responses to stress. Moreover, there is evidence that CRF of both hypothalamic and extrahypothalamic origin is hypersecreted in major depression. Because it is plausible that antidepressants may alter CRF neuronal activity and because serotonergic agonists have been shown to increase hypothalamic-pituitary-adrenal (HPA) axis activity by direct effects on CRF neurons, we have investigated the effects of nefazodone, which interacts with both 5-HT uptake sites and 5-HT_2 receptors, on brain CRF concentrations and plasma ACTH and corticosterone levels.

Male Sprague-Dawley rats were administered increasing doses of nefazodone (1 - 100 mg/kg s.c.). CRF concentrations were measured in the hypothalamus, median eminence, and locus coeruleus. After data were obtained from the dose-response experiment, animals were treated acutely with nefazodone (33.3 mg/kg), and CRF concentrations were measured in 10 discrete brain regions including the prefrontal cortex, bed nucleus of the stria terminalis, median eminence, hypothalamus, piriform cortex, amygdala, hippocampus, raphe, cerebellum, and locus coeruleus. Nefazodone was without effect on plasma ACTH or CRF concentrations at any dose. Plasma corticosterone levels were significantly elevated only at a dose of 100 mg/kg of nefazodone. The results suggest that, at least at the dose and time point studied here, nefazodone does not cause major alterations in CRF neuronal function or HPA axis activity. (Supported by MH-42088).

569.5

DIFFERENTIAL EFFECTS OF DESMETHYLIMIPRAMINE (DMI) ON THE BRAIN NUCLEUS LOCUS COERULEUS (LC) CULTURED AT TWO DEVELOPMENTAL PERIODS. Cheryl F. Dreyfus. Dept. Neuroscience & Cell Biology, R.W.J. Med Sch / UMDNJ, Piscataway, NJ 08854

DMI is a widely used tricyclic antidepressant drug that specifically inhibits the high affinity NE uptake system, and has been reported to decrease TH activity in the adult lc in vivo. To define drug actions during development, we examined effects on the lc developing within a well-defined culture system. In previous work, lc has been explanted from two developmental stages, embryonic day 12 (E12) and day 18 (E18). E12 and E18 lc grown in culture exhibit distinct developmental capabilities. E12 lc expresses significantly less tyrosine hydroxylase (TH) /cell than E18 lc. (TH is the rate limiting enzyme in NE biosynthesis.) Moreover, E12 lc cells are less mature morphologically than E18 neurons.

In the present study, we took advantage of the distinct E12 and E18 maturational stages evident in culture. Initially, we monitored NE uptake and the ability of DMI to inhibit this process. Both E12 and E18 cultures exhibited high-affinity uptake that was blocked by DMI. To define effects of the drug on TH activity, E12 and E18 explants were grown for one week and then exposed to DMI (1uM or 10uM) for an additonal seven days. In contrast to effects on NE uptake, distinct differences were now apparent in the two culture groups. TH activity in E12 cultures was unaffected by DMI treatment. However, TH in the E18 groups was markedly inhibited by the drug. This effect appears to be selective. Drug treated E18 explants exhibit extensive neuritic outgrowth and are indistinguishable from controls. These data suggest that specific DMI responsive periods exist during brain development. Mechanisms underlying these differences in responsivity remain to be defined. (Support: NIH grant DA 05132)

569.6

EFFECTS OF CHRONIC ANTIDEPRESSANT ADMINISTRATION ON RECEPTOR REGULATION IN GUINEA PIGS. A.W. Schmidt and J. Heym. Pfizer Central Research, Groton, CT. 06340.

In rats, downregulation of β adrenergic receptors after chronic administration of antidepressants (ADs) has been well documented. Both tricyclic ADs and 5-HT reuptake blockers have been shown to produce this effect. In addition, 5-HT2 receptor downregulation has been demonstrated after chronic tricyclic AD treatment, but results are inconsistent for 5-HT reuptake blockers. To date no reports are available on the effects of chronic AD treatment on 5-HT1D receptors. Since mice and rats lack 5-HT1D receptors, the present study was conducted in the guinea pig to compare the effects of chronic AD administration on the regulation of 5-HT1D, 5-HT2 and β adrenergic receptors. The tricyclic AD, desmethyl-imipramine (DMI), a norepinephrine reuptake inhibitor, or the 5-HT reuptake inhibitor fluoxetine was given to guinea pigs at a dose of 10 mg/kg i.p. once a day for fourteen days. After a wash out period of 24 hours, animals were sacrificed and brains removed, dissected and stored frozen. Binding assays were performed according to standard literature procedures. The number of 5-HT1D receptors in guinea pig caudate were unchanged in both drug-treated groups. A significant downregulation of cortical β adrenergic receptors was found in both drug-treated groups, while only the DMI-treated guinea pigs showed a significant reduction in the number of cortical 5-HT2 receptors. The effects of chronic AD treatment on 5-HT2 and β adrenergic receptor regulation in the guinea pig appears similar to those found in the rat.

569.7

EFFECTS OF TANDOSPIRONE ON ASSOCIATIVE LEARNING IN MICE. A. Shemer, J Clemente & D Quartermain, Pfizer Inc. & NYU Medical Center. New York. Effects of tandospirone 5mg\kg s.c. buspirone 1mg\kg s.c. & diazepam 1mg\kg s.c. on acquisition and retention were studied in a CER paradigm using a drink suppression task. Drugs were administered before conditioning, before test or before both conditioning & test. All 3 drugs disrupted suppression dose dependently when given before conditioning, but d-amphetamine 2mg\kg s.c. before test reversed the impairment. State dependency was observed with buspirone only. Tandospirone & buspirone both produced a reversible disruption of retrieval but did not impair acquisition, however the state dependency suggests that the drugs produce different internal states. Diazepam alone impaired conditioned suppression when administered before test, an indication that its mechanism of action may differ from that of the 5HT1a agents. In a passive avoidance task tandospirone administered before training produced deficits in retention at test 1, 3 & 7 days later. This effect was reversed by d-amphetamine before test, supporting the hypothesis that retrieval, not learning, is effected by 5HT1a receptor activation.

569.8

IMIPRAMINE BINDING AND EFFECTS OF REPEATED TREATMENT IN ADULT FEMALE RATS. S. Maswood*, J. Williams and L. Uphouse, Department of Biology, Texas Woman's University, Denton, Texas, 76204.

Although imipramine is one of the most widely investigated antidepressants, experimental studies have been performed primarily in the male rodent. However, in the human population, females are the major consumers of antidepressants. The present research was initiated to examine the effects of imipramine in a female rodent with the ultimate objective of comparing these findings to those reported for the male population. In the first study, binding of ^{3}H-imipramine, plus or minus desipramine as a competitor, to frontal cortex of estrous, proestrous and diestrous rats was examined. In general, binding profiles resembled those reported for males. However, the K_d for ^{3}H-imipramine binding in the female frontal cortex was larger than reported for the male and, although not statistically significant, the K_d and B_{max} of estrous rats tended to be lower than that of proestrous and diestrous rats. In the second experiment, female rats were injected i.p. with 10 mg/kg imipramine or saline daily for 21 days. Such treatment regimens are often employed in male rats in order to ascertain the neurochemical changes responsible for the drug's therapeutic action, which require 2-3 weeks. Disruption of the estrous cycle occurred rapidly following imipramine treatment. During the 21 days of treatment, most of the saline-treated rats had 4 consecutive estrous cycles. In contrast, imipramine-treated rats showed only 2 episodes of vaginal estrus, indicative of disruption of estrous cyclicity. These results raise the possibility that the alteration of the reproductive cycle could occur following treatment with other antidepressants.

569.9

SEROTONIN FUNCTION AND THE MECHANISM OF ANTIDEPRESSANT ACTION **P.L. Delgado, H.L. Miller*, R.S. Salomon*, J. Licinio*, L.H. Price, G.R. Heninger, J.H. Krystal, D.S. Charney. West Haven VAMC and Dept. of Psychiatry, Yale University School of Medicine, New Haven, CT 06508**

Brain serotonin (5-HT) content is dependent on plasma levels of the essential amino acid, tryptophan (TRP). We have previously reported that rapid TRP depletion more frequently reversed antidepressant response to monoamine oxidase inhibitors and 5-HT reuptake inhibitors than to desipramine (DMI). This study further investigates the relationship of relapse during TRP depletion to antidepressant type in non-refractory depressed patients randomly assigned to treatment with either DMI or fluoxetine (FLU). METHOD: In an ongoing study, 25 drug-free depressed (DSM-III-R) patients were randomly assigned to receive antidepressant treatment with either desipramine or fluoxetine. All patients were either treatment naive (N=15) or had had previously successful antidepressant treatment (N=10). 19 of the 25 patients had therapeutic responses by predetermined criteria to antidepressant treatment (DMI 7/10; FLU 12/15) and 16 (6 DMI responders and 10 FLU responders) went on to TRP depletion testing. TRP depletion testing involved two 2-day tests, a 16-amino acid drink and a follow-up day, in a double-blind, placebo-controlled (TRP depletion and control testing), crossover fashion. On one test the drink was supplemented with L-TRP (control) and on the other test no TRP was added (TRP depletion). Behavioral ratings (Hamilton Depression Scale (HDRS)) and plasma (for TRP levels) were obtained prior to, during and after testing. Relapse was defined as a 50%increase in HDRS with total >20. RESULTS: Total and free TRP decreased 70-80% 5 hrs. after the TRP-free drink. While 5/10 FLU responders relapsed, none of the DMI responders relapsed (one DMI-treated patient experienced a partial relapse). No patient experienced significant depressive symptoms during control testing. Implications: Rapid depletion of plasma TRP transiently reverses antidepressant response to FLU but not DMI. Given the random, balanced patient assignment, depressive relapse during TRP depletion appears to be more related to antidepressant type than to patient variables. Antidepressant response to FLU appears to be more dependent on 5HT availability than that of DMI. This data suggests that antidepressants may mediate their therapeutic effects through different mechanisms.

569.10

MAZINDOL IN TREATMENT-REFRACTORY DEPRESSION **H.L. Miller*, S.M. Stine, P.L. Delgado, R.M.Salomon* and D.S. Charney. West Haven VAMC and Dept. of Psychiatry, Yale University School of Medicine, New Haven, Ct. 06508**

Mazindol is a potent dopamine and norepinephrine reuptake inhibitor which has not previously been examined for antidepressant activity in humans. This study examines the efficacy of mazindol in patients with treatment-refractory major depression. **Method:** 7 depressed patients (4 males and 3 females, ages 34-68) received placebo for the first two weeks of the study, then were treated with mazindol, at doses ranging from 1-4 mg, for 6 weeks. The study was single-blind. Severity of depression was measured weekly with the 25-item Hamilton Depression Rating Scale (HDRS). All 7 patients continued to be depressed throughout the placebo period and proceeded to active treatment. **Results:** 2 of the 7 patients (29%) treated to date had a therapeutic response to mazindol ($\geq$ 50% decrease in HDRS and total $\leq$ 10). The drug was well tolerated; common side effects included insomnia, headaches, and urinary hesitation. **Conclusions:** 30% of this treatment-refractory population improved with mazindol treatment. Mazindol may have greater antidepressant efficacy in patients less refractory to treatment.

569.11

CHRONIC FLUOXETINE TREATMENT UP-REGULATES DOPAMINE RECEPTORS IN MESOLIMBIC FOREBRAIN. J.E. Margulies, A.B. Lynn*, L.S. Brady and R.P. Hammer, Jr., Dept. Anatomy & Reprod. Biol., Univ. Hawaii Sch. Med., Honolulu, HI 96822 and Sect. Functional Neuroanat., NIMH, Bethesda, MD 20892.

Fluoxetine is a clinically effective antidepressant compound which blocks serotonin reuptake. However, the extended time course for clinical efficacy suggests that this compound may produce direct or indirect effects on other neurotransmitter systems, as well. Fluoxetine (5 mg/kg) was administered daily i.p. for 8 weeks in Sprague-Dawley rats, after which animals were decapitated, brains frozen and sectioned, and adjacent sections incubated in appropriate buffer solutions containing either 1.6 nM [^{3}H]SCH 23390 or 0.5 nM [^{3}H]spiperone, washed, dried, and exposed to X-ray film. These binding conditions reveal D_1 and D_2 receptors, respectively; the radioligand concentrations were calculated to be 1.5 x K_d in our hands. Nonspecific binding was determined in the presence of 1 μM (+)butaclamol, and an additional adjacent series of tissue sections was prepared for digital subtraction of nonspecific D_2 binding. The results were analyzed using quantitative densitometry. Receptor binding was assessed in mesolimbic and nigrostriatal terminal regions. D_1 labeling was increased selectively in rostral portions of the nucleus accumbens (NAc), but not in the olfactory tubercle nor the striatum. Specific D_2 binding also increased in NAc, but not in the striatum. The results suggest that chronic fluoxetine treatment could increase dopaminergic response selectively in the mesolimbic fields involved in motivational activity. Thus, dopaminergic activity in endogenous reward centers might be involved in depression. Supported by USPHS Awards HD01161, DA06645 and RR03061 to RPH.

569.12

G-PROTEIN α-SUBUNIT mRNA LEVELS IN RAT CORTEX ARE REGULATED BY CHRONIC LITHIUM BUT NOT CARBAMAZEPINE TREATMENT. P.P. Li, Y.K. Tam*, L.T. Young, and J.J. Warsh. Clarke Institute of Psychiatry, Toronto, Ontario, Canada, M5T 1R8.

While lithium and carbamazepine (CBZ) are effective in the treatment of bipolar affective illness, their mechanism of action is still unknown. Recent evidence suggests that lithium might exert its therapeutic effect by modulating the function of GTP-binding (G) proteins associated with CNS second messenger systems. We have therefore examined the effects of chronic lithium or CBZ treatment on rat cerebral cortical mRNA levels for Gs and Gi α-subunits. Groups of male Wistar rats were fed one of three diets for 21 days: control, 0.2% LiCl or 0.25% CBZ for 4 days followed by 0.5% CBZ. Total cellular RNA was extracted from cerebral cortex by the guanidine isothiocyanate/cesium chloride centrifugation method. Northern blots were performed using the EcoR I fragment of rat Gsα, Gi1α and Gi2α cDNAs subcloned into pGEM-2 (provided by Dr. R. Reed). The yield of total cellular RNA did not differ significantly between the control (1.02 ± 0.01 mg RNA/g tissue), lithium-treated (0.97 ± 0.03; n=5) and CBZ-treated animals (0.99 ± 0.01; n=5). Northern blot analysis revealed that chronic lithium treatment decreased Gsα mRNA abundance by 30% ($p < 0.05$) in cerebral cortex. Similarly, there was a 25-30% reduction ($p < 0.05$) in the Gi1α and Gi2α mRNA levels. In contrast, the prevalence of these mRNAs was unaffected by chronic CBZ treatment. Neither of these drug treatments altered β-actin mRNA levels. These data represent the first demonstration of a potential modulatory role of lithium on the dynamics of G protein α-subunit biosynthesis at the level of gene expression. Furthermore, our data support the notion that G protein(s) may be a molecular target for the therapeutic effects of lithium which is not shared by CBZ. Supported by the OMHF, MRC (Canada) and NARSAD.

569.13

EFFECTS OF PUTATIVE 5-HT_{1A} ANTAGONISTS ON DORSAL RAPHE UNIT ACTIVITY IN BEHAVING CATS. C.A. Fornal, F. Marrosu, K. Tada*, C.W. Metzler and B.L. Jacobs. Prog. Neurosci., Dept. of Psychol., Princeton Univ., Princeton, NJ 08544.

Somatodendritic 5-HT_{1A} autoreceptors are thought to play an important role in regulating the discharge activity of central 5-HT neurons. Recently, we have shown that blockade of the 5-HT autoreceptor with systemic spiperone produces an increase in the firing rate of 5-HT dorsal raphe nucleus (DRN) neurons in awake cats, suggesting that 5-HT autoreceptors exert a tonic inhibitory influence on the activity of 5-HT neurons under physiological conditions. The present study examines the effects of three other putative 5-HT_{1A} antagonists (BMY-7378, NAN-190, and (-)propranolol) on DRN unit activity. 5-HT neurons were recorded and identified using methods previously described (Fornal et al., Am. J. Physiol. 259:R963-R972, 1990). Drugs were administered via a jugular catheter. In contrast to the effects of spiperone (0.25-1 mg/kg), intravenous administration of BMY-7378 (0.02-0.1 mg/kg), NAN-190 (0.02-1 mg/kg), and (-)propranolol (1-3 mg/kg) suppressed the activity of DRN neurons and failed to block the inhibitory action of 8-OH-DPAT, a selective 5-HT_{1A} agonist. In addition, the suppression of DRN unit activity produced by BMY-7378, NAN-190, and (-)propranolol was antagonized by spiperone. These results indicate that these putative 5-HT_{1A} antagonists act as agonists rather than antagonists at the 5-HT_{1A} autoreceptor site.

569.14

SINGLE-UNIT RESPONSES OF N. RAPHE DORSALIS AND CENTRALIS SUPERIOR NEURONS TO 5-HT_{1A} DRUGS IN BEHAVING CATS. K. Tada*, C.A. Fornal, F. Marrosu, C.W. Metzler and B.L. Jacobs. Prog. Neurosci., Dept. of Psychol., Princeton Univ., Princeton, NJ 08544.

The somatodendritic 5-HT_{1A} autoreceptor regulating the impulse activity of central 5-HT neurons represents an important target site of action for a variety of psychotherapeutic agents. Our previous studies suggested that the sensitivity of the 5-HT_{1A} autoreceptor may vary across different raphe cell groups. The present study examines the effects of 5-HT_{1A} agonist and antagonist drugs on unit activity in the dorsal raphe nucleus (DRN) and nucleus raphe centralis superior (NCS) in behaving cats (methods described in preceding abstract). Intravenous administration of the selective 5-HT_{1A} agonists 8-OH-DPAT and ipsapirone produced a dose-dependent inhibition of both DRN and NCS unit activity. However, 8-OH-DPAT and ipsapirone were more potent in suppressing the activity of DRN neurons (ED_{50} for 8-OH-DPAT = 1.2 μg/kg; ED_{50} for ipsapirone = 4.6 μg/kg) than NCS neurons (ED_{50} for 8-OH-DPAT = 3.0 μg/kg; ED_{50} for ipsapirone = 23.8 μg/kg). The 5-HT_{1A} autoreceptor antagonist spiperone (1 mg/kg) produced a marked, and similar, increase in the firing rate of both DRN and NCS neurons. Overall, these results provide evidence for a differential sensitivity of DRN and NCS neurons to 5-HT_{1A} agonists and suggest that 5-HT neurons in both raphe nuclei are under tonic feedback inhibition.

569.15

5-HT_{1A} AUTORECEPTOR AGONISTS INDUCE HIPPOCAMPAL RHYTHMIC SLOW ACTIVITY (RSA) IN FREELY MOVING CATS. F. Marrosu, C.A. Fornal, K. Tada*, C.W. Metzler and B.L. Jacobs. Prog. Neurosci., Dept. of Psychol., Princeton Univ., Princeton, NJ 08544.

Ascending serotonergic projections from the midbrain raphe nuclei have been implicated in the regulation of hippocampal electrical activity. Previous studies have shown that serotonin derived from the midbrain raphe nuclei inhibits the activity of hippocampal pyramidal cells. Recently, we have found a subgroup of serotonergic neurons whose activity appears to be inversely correlated with the occurrence of hippocampal RSA during waking behaviors. These findings have prompted us to examine the effects of 5-HT_{1A} autoreceptor agonists (8-OH-DPAT and ipsapirone) on hippocampal EEG activity in freely moving cats. Intravenous administration of low doses of 8-OH-DPAT (10-20 μg/kg) and ipsapirone (40-100 μg/kg) induced clear RSA in the hippocampal EEG. The onset of the effect (~20 sec) coincided with a general suppression of serotonergic unit activity recorded in the midbrain raphe nuclei. These data suggest that preferential activation of somatodendritic 5-HT_{1A} autoreceptors produces RSA in cats and support the hypothesis that central serotonergic neurons inhibit the generation of hippocampal RSA.

569.16

BEHAVIORAL EFFECTS OF CAFFEINE IN RATS TREATED ACUTELY VERSUS CHRONICALLY WITH FLUOXETINE (PROZAC). A.P. Leccese, M.L. Van Etten* and M.G. Smith*. Psychology Department, Kenyon College, Gambier, OH 43022.

Anecdotal evidence from humans receiving the antidepressant fluoxetine suggested that this drug interacts with the commonly used methylxanthine, caffeine, to induce subjectively unpleasant levels of stimulation. A first experiment investigating the impact of caffeine upon rats given acute fluoxetine found that acute fluoxetine induced a reduction in most measured behaviors, while all caffeine increases in behavior were merely superimposed over acute fluoxetine-induced decreases in behavior. In the second experiment, rats were injected ip once daily for 15 days with 0.0 or 5.0 mg/kg fluoxetine. On the 15th day, fluoxetine injections were followed later by ip injections of 0.0, 5.0 or 10.0 mg/kg caffeine 30 min prior to placement within an automated activity device that measured a number of variables relating to locomotion, stereptypy and anxiety. There was no significant effect of fluoxetine nor a significant fluoxetine-caffeine interaction on any measure. There was a main effect of caffeine only upon total distance and number of stereotypy. However, despite the failure to achieve significance, rats given chronic fluoxetine followed by saline showed a trend towards elevated behavior. Increasing doses of caffeine generally caused further elevations of chronic fluoxetine-induced increases in behavior.

569.17

PHARMACOLOGICAL ACTIONS OF A-75200, A NOVEL α_2 ANTAGONIST/NOREPINEPHRINE (NE) REUPTAKE INHIBITOR AND POTENTIAL ANTIDEPRESSANT AGENT. A.A. Hancock, J.Y. Lee, R.E. Zelle, S.A. Buckner, D. Stanisic, M.E. Brune, R.B. Warner, J.F. McKelvy, and J.F. DeBernardis

Abbott Laboratories, Abbott Park, IL 60064

A-75200, (±)-(1'R*,3R*)-3-phenyl-1- [(1',2',3',4'-tetrahydro-5',6'-methylene dioxy-1-naphthalenyl) methyl]pyrrolidine-methanesulfonate, exhibits potent antagonism of α_2 adrenergic receptors (R) (K_i = 1.2 nM) plus the ability to inhibit the neuronal reuptake of NE in the CNS (IC_{50} = 0.84 μM). In vitro, A-75200 antagonized α_2 R in canine saphenous vein (pA_2 = 8.2) and rat vas deferens (pA_2 = 7.4), with much weaker effects at α_1 R (pA_2 = 6.6, rabbit aorta). A-75200 also blocked presynaptic α_2 R to release [^{3}H]NE from guinea pig hippocampal slices. In vivo, A-75200 blocked CNS α_2 R (ED_{50} = 0.35 mg/kg i.v., in antagonizing clonidine-induced mydriasis). The agent lacked anticholinergic or antihistaminergic activity. Cardiovascular studies indicated that A-75200 had minimal effects on hemodynamic parameters such as cardiac output, heart rate, stroke volume or total peripheral resistance, and did not induce orthostatic hypotension. A-75200 is a racemic mixture, and biochemical assays showed that one enantiomer antagonized α_2 R more potently while the other enantiomer had better affinity for NE uptake sites. This unique pharmacological profile suggested that A-75200 should have antidepressant activity, and behavioral tests in animals support this hypothesis.

569.18

BEHAVIORAL PHARMACOLOGY OF ABBOTT-75200, A POTENTIAL ANTIDEPRESSANT COMPOUND W. J. Giardina, D. M. Ebert*, C. T. Wismer*, J. F. DeBernardis* and J. F. McKelvy. Abbott Laboratories, Abbott Park, Il 60064

ABBOTT-75200, (±)-(1'R*,3R*)-3-phenyl-1 [(1',2',3',4'-tetrahydro-5',6'methylenedioxy-1-naphthalenyl) methyl] pyrrolidine methane sulfonate, is a potent alpha-2 antagonist and an inhibitor of norepinephrine reuptake. Because this unique profile suggests potential antidepressant activity, A-75200 was evaluated in an antidepressant screening procedure using olfactory bulbectomized (OB) rats. Sham operated (SO) and OB rats were placed in activity monitors for 11 days on a 13 hour light - 11 hour dark cycle. OB rats were markedly more active than SO rats during the dark phase. Six injections of A-75200 (10.0 mg/kg, i.p., once daily) reduced the dark phase activity of OB rats to a level equal to that of SO rats. Imipramine (10.0 mg/kg, i.p., once daily) was effective after 8 injections. A-75200 did not affect locomotor activity, rotarod, seizure threshold or nociception, and showed weak activity in the thirsty rat conflict test. The efficacy of A-75200 in the OB rat supports its potential for antidepressant activity .

570.1

GENERATIONAL GENETIC EFFECTS OF IONIZING RADIATION (I.R.)$_2$ CNS' ENERGETICS METABOLISM. E. Egaña & M.T. Ramirez. University of Chile; Faculty of Medicine; Institute of Experimental Medicine; Laboratory of Neurochemistry; Santiago 7 - Chile.

It is well known both in human & experimental that I.R. damage seriously the CNS at its successive stages (preimplantation, embryon, fetus, neonatal, puberty & adult). It describes effects acute and chronic. Our Institute has created an generational (genetic) model of I.R. We studied several CNS' parameters which have been analyzed in three successive generations. In this paper our attention was focused on the alteration of CNS' mitochondrial energetic. First generation: Wistar rats adult ♂&♀were exposed to low-dose rate 0.10-0.25 Gy three times a week to reach 10 Gy as total-dose; this crossed to get the 2nd gen. & this was treated in the same manner to get the 3rd gen. In each gen. many parameters were evaluated some of them at intrauterine life. During pregnancy the ♀ continued being exposed as also neonatals, lactants, juvenils and adults; that means exposure all life. Mitochondria crude preparation from 5 CNS' areas: cortex, hippocampus, hypothalamus, cerebellum & midbrain. Site I & II of the respiratory chain; electron transport & Ca^{2+} influx measured polarigraphically with adhoc electrodes. Results showed alterations in most mitochondrial parameters in all generations, particularly P/O & Ca^{2+} influx.

570.2

EXPRESSION OF THE NEUROFIBROMATOSIS TYPE 1 GENE PRODUCT IN THE CHICK EMBRYO L. Baizer, G. Ciment, and G. Schafer* Neurological Sci. Institute, Good Samaritan Hospital, and Department of Cell Biology and Anatomy, Oregon Health Sci. Univ., Portland, OR. 97209.

Neurofibromatosis type 1 (NF-1) is among the most common inherited disorders, and primarily affects tissues derived from the neural crest. Symptoms of this disease include café-au-lait spots, which appear to result from abnormal differentiation and/or proliferation of melanocytes, and neurofibromas, benign tumors consisting of Schwann cells, as well as other cell types. Insight into a possible mechanistic basis for this disorder was provided by the recent molecular cloning and sequence analysis of the NF-1 gene product, which appears to be similar in structure and function to the GTPase Activator Protein (GAP), which may act as a tumor suppressor.

To investigate the possible role of the NF-1 gene product in regulation of the proliferation and differentiation of neural crest cells during development we have isolated cDNAs for chicken NF-1. Chick embryo brain cDNA libraries were screened at low stringency with probes derived from the human NF-1 cDNA. Several positive clones have been isolated and amplified and their nucleotide sequences determined. Comparison of the predicted amino acid sequences for the chick and human gene products suggests that the entire protein is highly conserved, implying that plays an important role in cellular regulation. When radiolabelled and used as a probe on RNA blots the cDNAs hybridize to a low-abundance mRNA of approximately 13-14 kb, similar to the size of the human gene product. These cDNAs will be used as probes to examine the time-course and distribution of NF-1 mRNA expression in the chick embryo.

570.3

CELLULAR EXPRESSION OF p53 AND T-ANTIGEN IN HYBRID JCV-SV40 TRANSGENIC MICE. H.G.Ressetar*, O.Prakash, R.Frisque*, H.Webster and G.Stoner. NIH, Bethesda, MD 20892, Alton Ochsner Med. Fdn., New Orleans, LA 70121, and Penn State Univ., University Park, PA 16802

The p53 gene is considered to be an anti-oncogene which controls normal cell proliferation. Elevated levels of p53 have been reported in SV40 polyomavirus-transformed cells. We previously reported pathology in transgenic mice carrying hybrid polyomavirus constructs generated by exchanging the T-antigen-coding regions of SV40 and JC polyomaviruses. Using immunostaining methods to detect p53 and SV40 or JC virus (JCV) large T-antigens, we now report coexpression of p53 and T-antigen in tissues from these mice. A mouse carrying SV40 regulated JCV T-antigen-coding sequences exhibited p53 and JCV T-antigen-expressing cells within a choroid plexus papilloma, but did not express either of the proteins in a coexisting thymoma. Two mice carrying JCV regulated SV40 T-antigen-coding sequences displayed thymic depletion with p53 and SV40 T-antigen-positive cells present in the thymus of one mouse. This mouse also exhibited brain neoplasia and an abdominal lymphoma, with both tissues containing SV40 T-antigen and p53-positive cells. These results suggest that viral regulatory control over T-antigen expression influences p53 levels in pathologic tissue.

570.4

HSV-1 VECTOR MEDIATED GENE TRANSFER OF LOW AFFINITY NERVE GROWTH FACTOR RECEPTOR, $p75^{NGFR}$, AND RECEPTOR MUTANTS. M.V. Chao*, D. S. Battleman*, S. Regunathan and A. Geller. The Department of Cell Biology and Anatomy and Division of Neurobiology, Cornell University Medical College, New York, N.Y. 10021, and The Dana Farber Cancer Institute, Boston, MA 02115

The ability to study the genetic regulation and functional role of cloned nervous system genes has traditionally been limited by the inability to stably transfect post-mitotic nerve cells in culture. The recent development of defective Herpes Simplex Virus (HSV-1) vectors has provided a useful method of stable gene transfer into central and peripheral nerve cells without observable cytopathic effects (Science 241, 1667, 1988; Proc. Natl. Acad. Sci., 87, 1149,1990; Biochem. Pharm., 40, 2189, 1990).

Here we report the construction of three viral vectors containing the 2.3 kb cDNA of the low affinity nerve growth factor receptor, pHSV-NGFR, and two mutant constructs, pHSV-X1 and pHSV-5A. pHSV-X1 encodes for a intracellular truncation mutant, containing a premature termination signal shortly after the transmembrane domain coding sequence. pHSV-5A, in contrast, contains a large deletion in the ligand binding domain coding sequence. These constructs have been efficiently introduced and stably expressed in both PC12 cells and fibroblasts. Current work includes establishing stable expression in primary nerve cell culture in order to examine the functional role and molecular interactions of $p75^{NGFR}$ in neurons.

570.5

RETROVIRUS VECTOR WITH HERPES SIMPLEX VIRUS THYMIDINE KINASE GENE AND WILD-TYPE RETROVIRUS KILL GLIOMA CELLS IN CULTURE AND IN VIVO. Y. Takamiya,* M.P. Short, D. Ezzeddine,* X.O. Breakefield and R.L. Martuza. Neuroscience Ctr., Mass. General Hosp., Charlestown, MA 02129, and Neuroscience Prog., Harvard Med. Sch., Boston, MA 02115.

Retroviral vectors can selectively deliver "transgenes" to tumor cells in the nervous system. Tumor cells infected with retrovirus vectors bearing the herpes simplex virus thymidine kinase (HSV-TK) gene are killed by treatment with nucleoside analogues, such as ganciclovir (GCV) (Moolten & Wells, J. Natl. Cancer Inst., 1990; Ezzeddine et al., New Biol., 1991). To increase the efficiency of delivery of the HSV-TK gene to tumor cells, we infected C6 glioma cells, bearing the HSV-TK gene under the MoMLV LTR (C6VIK cells) with ecotropic wild-type retrovirus (MoMLV). These genetically modified tumor cells (C6VIK-WT) release both wild-type retrovirus and retrovirus bearing the HSV-TK gene. In culture, C6VIK-WT cells were 300-fold more sensitive to the toxic effects of GCV than C6VIK cells. The increased sensitivity of C6VIK-WT cells may reflect multiple integrations of the HSV-TK gene and the debilitating effects of wild-type retrovirus infection. Co-culture of C6VIK-WT cells with C6BAG cells (labelled with the lacZ gene) caused the latter to become sensitive to GCV treatment. Regression of tumors was also noted in nude mice inoculated subcutaneously with C6VIK-WT and C6BAG cells following treatment with GCV. These findings suggest that the efficiency of retrovirus-mediated gene delivery and the sensitivity to toxic agents can be increased in tumor cells using helper wild-type virus.

570.6

GENE TRANSFER INTO CULTURED SENSORY NEURONS USING HERPES VIRUS VECTOR: EXPRESSION OF BACTERIAL LAC Z USING A MAMMALIAN NEURONAL PROMOTER. J.K. Andersen, D.A.Garber*, C.A.Meaney*, and X.O.Breakefield. Neuroscience Center, Massachusetts General Hospital East, Charlestown, MA, 02129; and Neuroscience Program and Genetics Department, Harvard Medical School, Boston, MA 02115.

A recombinant herpes simplex virus type 1 (HSV) has been engineered by insertion of the rat neuronal specific enolase (NSE) promoter-bacterial lacZ cassette into the thymidine kinase gene of a wild-type KOS HSV genome. This recombinant virus, which is replication-defective in neurons, has been used to infect neuron-enriched, primary cultures of rat neonatal dorsal root ganglion cells at MOIs ranging from 10 to 0.001. After 3 and 10 days, the cells were fixed and stained for beta-galactosidase (ß-gal) activity using X-gal as a substrate. At low MOIs, some apparently healthy blue cells were observed, indicating that the NSE promotor is active in some of these cells and that gene delivery can be effected with little apparent toxicity to cells. This vector does not express lacZ in cultured Vero cells infected in a similar manner. This finding, together with the fact that the NSE-lacZ plasmid construct was active after transfection into cultured mouse neuroblastoma cells, support the conclusion that this promoter remains neuronal-specific within the herpes genome. The virus is currently being assessed by stereotactic injection into rat CNS for its ability to give efficient and stable gene transfer into the mammalian nervous system.

570.7

A POTENTIAL NEW METHOD OF DELIVERY OF AN EXPRESSED GENE TO CENTRAL NEURONS OF THE ADULT RAT

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A number of methods have been used to deliver expressible genes to cells. Viral vectors can deliver such genes with a high degree of efficiency. Retroviral vectors are believed to have an absolute requirement for DNA replication to integrate into the host genome, and thus could not mediate gene delivery to postmitotic neurons. To explore whether retroviral vectors may integrate into neurons under certain conditions, we engrafted the right striata of male Sprague-Dawley rats with a mouse fibroblast cell line that constitutively produces retroviral vector containing the reporter gene *E. coli* lacZ (vector provided by Dr. C. Cepko). Rats were perfused 10 days postoperatively with paraformaldehyde. Sections of brains (40 μm) were reacted with 5-bromo-4-chloro-3-indolyl-ß-D-galactoside (X-gal), yielding an insoluble blue reaction product when metabolized by ß-galactosidase. Analysis revealed marked ß-galactosidase activity in clusters of large (approximately 20 μm diameter) cells with neuronal morphology. Prominent gene expression was found bilaterally in the ventral forebrain (ventral pallidum), certain thalamic subnuclei and the substantia nigra pars compacta. We hypothesize that the high efficiency of integration may have been caused by the production of helper virus by the grafted cell line, combined with ongoing DNA repair/replication in neurons. We have thus demonstrated the potential utility of an implanted retroviral vector packaging cell line for the delivery of a gene to adult neurons of the CNS.

570.8

Alterations in Levels of Choline Acetyltransferase in Somatostatin Transgenic Mice. M.L. Oster-Granite, C.F. Hohmann, S.L. Kinsman, and J.T. Coyle, Departments of Psychiatry, Physiology, and Neuroscience, The Johns Hopkins University School of Medicine, and The Kennedy Institute, Baltimore, Maryland.

Various studies of normal and mutant (e.g., *dwarf*) mice during development have indicated a dynamic interaction between the cholinergic and somatostatinergic systems in the forebrain. Furthermore, somatostatinergic regulation of cholinergic activity has been reported in cortical structures. We investigated the effect of increased somatostatin levels in the brains of transgenic mice on the expression of cholinergic markers.

These mice express a transgene consisting of the mouse preprosomatostatin gene, 9 kb of 5' sequences, and 1.5 kb of 3' sequences, in addition to their own endogenous preprosomatostatin gene. Preprosomatostatin mRNA levels in heterozygous mice from this line are elevated in hypothalamus (3 fold), neocortex (1.7 fold), and brainstem (1.8 fold), but are not elevated appreciably in hippocampus or striatum (1.2 fold), when compared to nontransgenic controls.

We assayed levels of choline acetyltransferase in various brain regions of homozygous transgenic mice. Levels of choline acetyltransferase were elevated an average of 1.5 fold in neocortex, striatum, and hippocampus, but were not elevated in the remaining brainstem-ventral forebrain sample.

These data lead us to suggest that a complex interaction exists between somatostatin gene expression and cholinergic innervation in the forebrain. Current studies are analyzing cholinergic markers in other lines of somatostatin transgenic mice with different patterns of expression of preprosomatostatin.

570.9

FETAL GROWTH RETARDATION IN TRANSGENIC MICE OVEREXPRESSING SOMATOSTATIN, S.L. Kinsman* and M.L. Oster-Granite, Kennedy Research Institute and Depts. of Neurology and Physiology, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Because of its early expression during ontogeny, the neuropeptide somatostatin (SS) is considered to play an important role in fetal growth and development To study the developmental roles of somatostatin we produced mice that overexpress SS. Using transgenesis to introduce extra copies of a 15kb mouse genomic fragment, which included the preprosomatostatin (PPSS) coding region and 9kb of 5' sequences, we produced mice with extra functional copies of this neuropeptide gene. Three lines of adult SS transgenic mice have up to three-fold elevations in PPSS message levels from hypothalamus, striatum, hippocampus, and brain stem. Brain regions that did not express PPSS signal in control tissue, such as cerebellum, were also negative in transgenic tissue. These mice were used to generate lines of transgenic mice that were homozygous for the PPSS transgene. Their homozygous state was confirmed by demonstrating 100% transmission of the transgene when these mice were mated to non-transgenic mice. Litters of homozygous transgenic mice from one of these lines were used to study the effects of the transgene on fetal growth. The homozygous SS transgenic mice showed a 10% reduction in size compared to non-transgenic controls on embryonic day 15 (body weight 384.0 ± 7.5 mg (n=64) vs. 427.1 ± 7.6 mg (n=16) ; $p < 0.05$). Preliminary Northern blot analysis of total RNA from embryonic day 15 embryos heads showed at least two-fold overexpression of PPSS message. These studies show that an unaltered 15kb mouse genomic construct containing the PPSS coding region could be used to produce transgenic mice that overexpress SS during prenatal stages. Homozygous transgenic mice exhibit a reduction in body size evident in the fetal period. This indicates a novel role for SS expression in the regulation of fetal growth and development. Future experiments will study the relationship between the degree of growth retardation and the level and distribution of SS overexpression. The mechanism by which SS overexpression exerts this growth reduction remains to be elucidated.

570.10

Immunochemical localization of somatostatin in transgenic mouse. S.B. Plano, S.L. Kinsman, M.L. Oster-Granite, J.T. Coyle, and C.F. Hohmann. Depts. of Psychiatry and Neuroscience, The Johns Hopkins University School of Medicine, and The Kennedy Research Institute, Baltimore, Maryland.

Previous studies on transplants of trisomy 16 [Ts16] and euploid tissue into mouse neonatal cortex showed differences in the numbers of Somatostatin (Smst)-expressing neurons in cortex. Since the gene encoding preprosomatostatin (Smst) is located on mouse chromosome 16, the increase in Smst expressing cells could be the result of the increased gene dosage. Alternatively, such increases may reflect altered developmental regulation. To determine whether the increase in somatostatinergic cells results from an increase in the gene dosage of Smst alone, we studied transgenic mice which have extra copies of a genomic fragment including the entire Smst gene.

We examined paired transgenic (n=8) and nontransgenic control (n=8) adult mice histologically using immunocytochemistry according to the ABC method. The survey of the somatostatin-like immunoreactivity (SOMLI) was conducted blindly. Transgenic and control animals revealed similar patterns of SOMLI, with similar numbers of cells being specifically stained. Previous neurochemical and molecular biology studies showed an approximately 60% elevation of Smst peptide and mRNA levels in cortex of these transgenic animals. Subtle differences in the number of Smst cells between transgenic and control animals may become apparent only upon completion of the quantitative cell counts of these tissue sections. The findings here suggest that the observed increases in Smst expression in these transgenic mice cannot be completely accounted for by increases in the number of somatostatinergic cells.

EPILEPSY: BASIC MECHANISMS III

571.1

NMDA-RECEPTOR MEDIATED ELECTRICAL EPILEPTOGENESIS IN THE ORGANOTYPIC CULTURE OF RAT HIPPOCAMPUS. C. Shin, Y. Tamaki, L. Butler* and J. Wilson*, Epilepsy Research Laboratory, Duke and VA Medical Centers, Durham, N.C. 27705.

Post-traumatic epilepsy develops after brain injury through the process of epileptogenesis. Mechanisms underlying the long-term plasticity in epileptogenesis can best be studied in a chronic *in vitro* preparation. Organotypic slice culture combines the long survivability with preserved neuronal cytoarchitecture and intrinsic connectivity. We sought to establish epileptogenesis in this preparation and study its mechanism.

Hippocampal slice cultures (7-15 DIV) were prepared according to the methods of Gähwiler (*TINS*, 1988) and studied in standard aCSF (95/5, O_2/CO_2) at 36°C. Extracellular fields were recorded in CA1 pyramidal layer. Schaffer collaterals were stimulated with 1-second trains of rectangular pulses (0.1 msec, 60 Hz, 500 μA) every 10 minutes.

Initially seizures were not always evoked, but, with subsequent stimulations, seizures could be reliably elicited with initial tonic discharges followed by phasic bursts. Progressive lengthening was seen over 1-2 hours (N=10), resembling the evolving seizure pattern of kindling model. D-APV (50 μM), an NMDA antagonist, blocked the development of seizures (N=7). The inactive stereoisomer, L-APV (50 μM), was without effect.

We have demonstrated that the electrical stimulation induces seizures reliably and gradually. This process of *in vitro* epileptogenesis appears to be mediated in large part through NMDA receptors. It offers a unique opportunity to study the long-term consequences of seizures and NMDA receptor activation in this chronic *in vitro* preparation.

571.2

HIGH POTASSIUM INDUCED SEIZURES IN ORGANOTYPIC CULTURES OF RAT HIPPOCAMPUS. Y. Tamaki, L. Butler*, J. Wilson*, and C. Shin, Epilepsy Research Laboratory, Duke and VA Med. Ctr., Durham, N.C. 27705

In adult rat hippocampal (HPC) slices, modest elevation of extracellular K^+ (up to 8.5 mM) results in spontaneous seizures (SZs) in a small proportion of these slices (Traynelis and Dingledine, 1989). Many reports document more epileptiform activity in organotypic HPC cultures than in acute adult HPC slices. We therefore used the organotypic HPC cultures to study the mechanism underlying SZs induced by high K^+ medium.

Organotypic cultures were prepared from 6-day-old rat HPC according to the methods of Gähwiler. Extracellular field recordings were made from the CA1 and CA3 pyramidal layers in standard aCSF (95/5, O_2/CO_2) at 36°C.

In 30 out of 35 cultures (10-15 DIV), spontaneous SZs were induced in both CA1 and CA3 after 2-19 minutes of 8.3 mM K^+. During the first 15-25 min of high K^+ application, 3.6 ± 0.8 (mean ± SEM) SZs per 10 min were recorded with an average duration of 39.1 ± 6.1 sec. The SZs recurred as long as high K^+ was maintained (1.5-2.5 hrs; n=7; 15.8 ± 2.4 SZs/hr, 37.6 ± 6.7 sec/seizure). In 11 cultures, D-APV (50 μM) was added for 20 min after 20 min of high K^+. The number of high K^+ induced SZs decreased significantly during D-APV application: before, 4.3 ± 0.7 vs. during, 1.8 ± 0.5/10 min ($p < 0.004$, Wilcoxon). The duration of SZs decreased slightly: before, 30.6 ± 5.2 vs. during, 24.6 ± 3.0 sec ($p > 0.1$, Wilcoxon). L-APV (50 μM) did not suppress the SZs and was not significantly different from control high K^+ bath.

Application of high K^+ medium reliably induced spontaneous SZs in the organotypic HPC cultures. The NMDA antagonist, D-APV, stereoselectively suppressed the number of SZs by 60% but not the duration of each SZs. This suggests that NMDA-receptor mechanisms may mediate events leading up to the initiation of SZs induced by high K^+.

571.3

PAROXYSMAL INHIBITORY POTENTIALS MEDIATED BY $GABA_B$ RECEPTORS IN DISINHIBITED HIPPOCAMPAL SLICE CULTURES. Massimo Scanziani, Beat H. Gähwiler, Margaret Schlumpf, and Scott M. Thompson. *Brain Research Institute, Univ. of Zurich, 8029 Zurich Switzerland*

What is the role of $GABA_B$ receptors in the regulation of hippocampal excitability? The $GABA_B$ antagonist CGP 35 348 blocked the late IPSP in CA3 cells, without causing epilepsy, unlike $GABA_A$ antagonists. Epileptic activity induced by 40 uM bicuculline was changed by low concentrations of the glutamate antagonist CNQX (2uM) into a cycle of epileptic bursts alternating with paroxysmal inhibitory potentials (PIPs), consisting of an EPSP followed by a long-lasting (1-2 s) hyperpolarization. PIPs were as large as 30 mV, were prolonged by the GABA-uptake blocker nipecotic acid, were reduced by the opioid-agonist FK 33-824, and reversed near E_{K^+}. CGP 35 348 abolished the PIPs, indicating that they are mediated by $GABA_B$ receptors. Unlike control $GABA_B$ IPSPs, the amplitude of the PIP was reduced by the NMDA-receptor antagonist D-APV. Regular stimulation elicited a cyclical pattern of responses consisting of gradually increasing PIPs followed by an epileptic burst, which then reset the cycle. Similarly, low concentrations of bicuculline (2uM) alone increased the amplitude of control $GABA_B$-mediated synaptic potentials 2-3 fold. Like the PIP, these responses were D-APV-sensitive and could be cyclical. Unlike control, however, application of CGP 35 348 under these conditions lead to intense epileptic discharge. Amplification of $GABA_B$-mediated inhibition may be a self-protective mechanism, preventing epileptogenesis after partial disinhibition.

571.4

A REVERSIBLE LOSS OF DENDRITIC SPINES ACCOMPANIED BY A DECREASE IN EXCITABILITY IN A MODEL OF CHRONIC EPILEPSY IN VITRO. Michael Müller*, Beat H. Gähwiler, Esther Peterhans and Scott M. Thompson. *Brain Research Institute, University of Zürich, 8029 Zürich, Switzerland.*

Chronic epilepsy was induced in mature rat hippocampal slice cultures by adding bicuculline methochloride (BMC) or picrotoxin to the medium for 3 days. After 3 days, there was vacuolation of the soma, the number of spines in HRP injected cells was considerably reduced, and dendritic swellings appeared. The damage affected CA1, CA3 and granule cells. Intracellular recordings showed a significant reduction in spontaneous synaptic activity. However, IPSPs could still be evoked and were similar to those in untreated cultures. GABA immunohistochemistry suggested that interneurons were intact in treated cultures. Acute addition of BMC to control cultures resulted in spontaneous, long-lasting (>5 s) ictal bursts followed by spontaneous and evoked interictal bursting (<1 s). In treated cultures, however, ictal bursts were never observed, although interictal bursts were still evoked. Moreover, Timm's staining of treated cultures suggested either a loss of mossy fiber terminals, or a depletion of zinc. BMC-treated cultures returned to normal medium for 1 week appeared to have recovered: high levels of spontaneous activity were observed, HRP injected cells possessed normal spines, and acute application of BMC resulted in spontaneous ictal bursts. The data suggest that chronic epileptiform activity causes a transient decrease in excitability, perhaps as a result of reversible morphological changes.

571.5

THE EFFECTS OF EXTRACELLULAR POTASSIUM ON EPILEPTIFORM BURST DYNAMICS IN CULTURED MONOLAYER NEURONAL NETWORKS. B.K. Rhoades and G.W. Gross. Dept. of Biological Sciences, Univ. of North Texas, Denton, TX 76203.

Neuronal cultures from embryonic mouse CNS tissue form networks whose native, spontaneous activity is characterized by consolidation of spikes into coordinated, patterned bursting. This activity may be regarded as epileptiform with regards to its coarse-grained synchrony at multiple sites across the networks, its pharmacological responses to convulsant and anticonvulsant drugs, and its responses to the external ionic milieu. Dissociated cell cultures of 100-200 neurons and underlying glial cells were established from the whole spinal cord, ventral spinal cord, and olfactory bulb, taken at embryonic day 14. For explant cultures, transverse spinal cord slices (400μm) were transferred to culture at the same age. The culture substrate was a multimicroelectrode plate, with 64 photoetched electrodes forming a $1mm^2$ extracellular recording matrix. Extracellular K^+ levels were varied by KCl additions to the bathing medium. Each culture was tested in the native activity state and in a disinhibited state produced by 30μM bicuculline. In the native state, increasing extracellular potassium promoted burst consolidation and burst periodicity. Disinhibition produced a more periodic pattern of bursting and more uniform burst amplitudes and durations. In the disinhibited state, increasing extracellular potassium promoted burst periodicity, increased burst rate, decreased burst duration, and decreased burst amplitude. In both states increasing extracellular potassium promoted synchronization of bursts across the network. These results held for both dissociated cell and explant tissue slice cultures. The results also parallel those reported for induced epileptiform bursting in the adult hippocampal slice, and support the cultured monolayer neuronal network as an *in vitro* model of epileptic activity. (Supported by grants from the Texas Advanced Research Program and the Hillcrest Foundation of Dallas, founded by Mrs W.W. Caruth, Sr.)

571.6

COOPERATIVE PHENOMENA IN NEURONAL NETWORKS: DYNAMICAL MODELS OF EPILEPTIFORM ACTIVITY IN SMALL NEURONAL ENSEMBLES. J.M. Kowalski*, G.L. Albert*, B.K. Rhoades, and G.W. Gross. Depts. of Physics* and Biological Sciences, Univ. of North Texas, Denton, TX 76203.

A new class of neuronal network models is being developed (Kowalski & Gross, 1990) where the basic structural units are neurons of Hodgkin-Huxley type and synaptic interactions are represented by a simple model of the neurotransmitter release and subsequent generation of postsynaptic microcurrents. Each neuron is governed by a scalar equation for membrane potential, a subsystem of kinetic equations for ionic variables, and equations describing neurotransmitter kinetics at synaptic junctions. The model can accommodate a wide range of network interconnection schemes and types of interactions, and allows parametric changes in ionic gradients and conductances. Networks composed of non-endogenous bursters bifurcate into approximately synchronous and phase-locked bursting states. Local network disinhibition results in the emergence of activity patches which may grow, decay, or remain stationary. The model qualitatively mimics some observed effects of extracellular $[K^+]$ on epileptiform bursting in neuronal cultures from embryonic mouse CNS tissue (Rhoades & Gross, this conf.). Simulations with excitatorally interconnected networks match the experimental results from pharmacologically disinhibited cultures; in that increasing extracellular $[K^+]$ decreases both burst and interburst durations. The inclusion of inhibitory interactions may reveal the more complex $[K^+]_o$ effects observed in non-disinhibited cultures. With the further elaboration of network substructure, we expect the model to also show rapid state transitions to synchronized bursting and the local emergence and network spread of coordinated activity patterns. (Funded by grants from the Texas Advanced Research Program and the Hillcrest Foundation of Dallas, founded by Mrs W.W. Caruth, Sr.)

571.7

SPONTANEOUS SYNAPTIC POTENTIALS IN CULTURED SPINAL CORD NEURONS DURING DEVELOPMENT OF EARLY CONVULSANT ACTIVITY PRODUCED BY TETANUS TOXIN. G.K. Bergey and P.J. Franaszczuk*. Departments of Neurology and Physiology, University of Maryland School of Medicine, Baltimore, Maryland 21201

Tetanus toxin is distinct from other convulsants in acting at a presynaptic locus to produce a gradual onset of paroxysmal depolarizing events (PDE) following a dose dependent period (Bergey et al., 1983, 1987). To investigate the changes in synaptic activity occurring during the evolution of convulsant activity, an algorithm was developed for continuous detection of spontaneous postsynaptic potentials (Franaszczuk and Bergey, submitted). Intracellular recordings from dissociated mouse spinal cord neurons in culture (4-10 wk of age) following the addition of tetanus toxin (100-1000 ng/ml final con). Typically 200-600 PSPs/min were detected during the period prior to the onset of established convulsant activity. During this time period the absolute numbers of spontaneously occurring EPSPs increased while the amplitudes of the observed spontaneously occurring EPSPs variably remained constant or diminished. The numbers of spontaneously occurring IPSPs decreased and the amplitudes of the observed IPSPs invariably decreased. Spontaneously occurring large IPSPs disappeared during the period preceding organized convulsant activity. Following toxin addition, spontaneous EPSPs became clustered in advance of the onset of organized convulsant activity (PDE).

Tetanus toxin provides a model for evolution of convulsant action resulting from increased network excitation produced by reduced synaptic inhibition and increased numbers of excitatory synaptic events. No evidence exists to suggest augmentation of monosynaptic excitation in this model.

571.8

IMMATURE RAT CA1 SHOWS AN INCREASED PROPENSITY FOR NON-SYNAPTIC EPILEPTIFORM ACTIVITY. S.N. Roper*†, A. Obenaus and E.E. Dudek. Mental Retardation Research Center, †Division of Neurosurgery, UCLA School of Medicine, Los Angeles, CA 90024.

Immature hippocampal slices show an increased propensity for epileptiform activity in several in vitro preparations. In the absence of synaptic activity, the low-$[Ca^{2+}]$ model produces synchronous bursts of population spikes that are enhanced by osmotically reducing the size of the extracellular space (Dudek et al., Neurosci. Lett. 120:267-270, 1990). Hippocampal slices (N=10/group) were obtained from rats aged 7-10 days (1 week), 11-14 days (2 weeks), 20-23 days (3 weeks), and >60 days (adult). Extracellular recordings were made from the CA1 area of the hippocampus in a low-$[Ca^{2+}]$ solution, low-$[Ca^{2+}]$ diluted 20% with H_2O, and low-$[Ca^{2+}]$ with 20 mM mannitol. Slices from all age groups, except 1 week, generated bursts in the control low-$[Ca^{2+}]$ solution (2 weeks 60%, 3 weeks 80%, adults 40%). In low-$[Ca^{2+}]$ diluted 20% with H_2O, 90%, 80%, and 40% of slices showed bursting in the 2-week, 3-week, and adult groups, respectively. In both of the low-$[Ca^{2+}]$ solutions, burst duration was two-fold greater in the 2- and 3-week old groups compared to adults. This study suggests that CA1 hippocampal neurons from 2- and 3-week-old rats are more susceptible than adults to low-$[Ca^{2+}]$-induced epileptiform activity; this implies that the increased propensity of immature hippocampus for seizure activity is not dependent on synaptic mechanisms. Supported by NIH grant HD05958.

571.9

GIANT IPSC'S RECORDED IN HIPPOCAMPAL PYRAMIDAL CELLS IN THE PRESENCE OF EXCITATORY AMINO ACID BLOCKERS.

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Whole-cell voltage clamp recording from pyramidal cells in transverse slices of guinea pig hippocampus in the presence of 4-aminopyridine(75 uM) revealed large synaptic events which were in part composed of a giant (500-2000 pA, varying among slices) outward current. These giant outward currents(ipsc's) continued to occur spontaneously 1 to 4 times per minute in the presence of CNQX (10uM) and CPP (10 uM). The intracellular solution consisted of (mM) potassium gluconate 120, Hepes 10, EGTA 10, and NaCl 10. The giant ipsc's had at least two components. One component reversed at the same potential as the spontaneous unitary ipsc's; therefore it probably is a GABA-mediated chloride current. The other component reversed at a more negative potential.

The giant ipsc's presumably reflect the synchronous firing of inhibitory interneurons. The amplitude of the giant ipsc's was 25-85 times the amplitude of the unitary ipsc's. This number can be used to estimate the number of inhibitory interneurons innervating a single pyramidal cell in a hippocampal slice.

(Supported by NIH and by a NSF Graduate Fellowship to KLP.)

571.10

MEMBRANE CURRENT IN HIPPOCAMPAL NEURONS DURING ELECTRICALLY PROVOKED ELECTROGRAPHIC SEIZURES.

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Interictal epileptiform discharges have in the past been studied in much greater detail than the neuron discharges occurring during actual seizures. We have previously suggested (Somjen et al., J. Neurophysiol., 53: 1079, 1985) that in hippocampal neurons, ictal events are generated by a sustained inward current in the neuron soma. The present experiments used conventional sharp elecrode recording in current clamp mode, and whole cell patch in voltage clamp mode, to further investigate ictal events in CA1 pyramidal cells. Rat hippocampal slices were maintained in an interface chamber with slightly elevated bath K^+ (5.0 mM). Population responses were monitored by extracellular electrodes. Orthodromic stimulation (2-10 sec trains, 0.1 msec, 80-150 μA, 10-50 Hz) provoked electrographic seizures consisting of tonic-sustained as well as clonic-intermittent discharges. The current required to hold membrane potential at hyperpolarized levels reversibly increased during both tonic and clonic phases of a seizure. Voltage-current (V-I) plots shifted, indicating depolarization and decreased input impedance. The findings are consistent with sustained inward current caused by increased ion conductance in membrane regions near the patch electrode during seizure discharge. (Supported by grants NS 17771, NS 18670, NS 06233).

571.11

HYPOXIA MODULATES THE EPILEPTIFORM ACTIVITIES INDUCED BY MAGNESIUM-FREE MEDIUM IN SLICES OF RAT HIPPOCAMPUS - ENTORHINAL CORTEX.

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Combined slices of rat hippocampus (HC) and entorhinal cortex (EC) exhibit electrographic seizures when exposed to medium containing no added magnesium (0-Mg medium). These seizures are eventually replaced by interictal bursts (IIBs). It has been previously reported that the IIBs, arising in CA3, disturb the seizure generation in EC. We have hypothesized that the suppression of IIBs in CA3 promotes seizures in EC. Here we describe that mild hypoxia, suppressing IIBs in CA3, promotes seizure generation in EC.

Extracellular recordings were made in area CA3 and EC of HC/EC slices of male Sprague-Dawley rats using a submersion chamber. The bath PO_2 was monitored with a Clark-type oxygen electrode in some cases (n=13). In the 0-Mg medium, seizures appeared first but eventually they gave way to IIBs in both regions. After the seizures were completely replaced by IIBs, mild hypoxia was introduced by switching the oxygenation gas from $95\%O_2+5\%CO_2$ (bath PO_2=551mmHg) to $30\%O_2+65\%N_2+5\%CO_2$ (bath PO_2=211mmHg). In all the slices tested (n=19), the IIBs were suppressed in CA3 and the seizures reappeared in EC during the hypoxic period. In regular artificial cerebrospinal fluid, orthodromic population spikes in CA3 were almost completely suppressed by the mild hypoxia whereas those in EC were less affected (n=10).

In conclusion, hippocampal area CA3 is more susceptible to hypoxia than EC and the suppression of IIBs in CA3 by mild hypoxia promotes seizures in EC.

571.12

BICUCULLINE-INDUCED INTERICTAL BURSTING DECREASES THE DURATION OF STIMULUS-INDUCED ICTAL BURSTING IN THE RAT HIPPOCAMPAL SLICE.

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We demonstrated previously that in a 0-Mg^{+2} model of electrographic seizures (EGS's) in the rat hippocampal slice, interictal (II) activity suppressed ictal activity; application of the $GABA_B$ agonist baclofen (2-4 uM) inhibited II bursting and reversed this effect. We now show similar results using stimulus-induced EGS's in the in vitro hippocampal slice.

EGS's were induced in the slice preparation by repeated stimulus trains (Science, vol 245:648-651, 1989); then the $GABA_A$ antagonist bicuculline (10 uM) was added to the bath to initiate II bursting. EGS duration was reduced markedly in 11 of 12 experiments. Baclofen (1 uM) was then applied to the bath in the presence of bicuculline to suppress all II activity. Application of baclofen allowed partial to near complete recovery of EGS duration in 9 of 10 slices. In 8 of these experiments, interictals were simulated electrically in the presence of bicuculline and baclofen, and EGS duration was once again significantly diminished.

II bursting is viewed clinically as a symptom of epileptiform activity. These studies suggest that II bursting may actually exert negative feedback control of ictal burst duration.

571.13

GAD mRNA INCREASES IN NEURONS FROM CHRONIC EPILEPTIC FOCI INDUCED IN RATS BY INTRAHIPPOCAMPAL TETANUS TOXIN.

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Intrahippocampal tetanus toxin induces chronic epileptic foci in both injected and contralateral hippocampi[1]. Epileptic activity recurs intermittently for at least 6 weeks, usually remitting by 8 weeks[2]; it is associated with impaired GABAergic inhibition. The low dose of toxin used (ca. 6-12 mouse LD_{50}) causes no gross histopathology.

Here we used in situ hybridization (ISHH) of mRNA for GAD to determine the continued presence and condition of inhibitory interneurons[3]. Slices were prepared for recording in vitro from 2 rats at each survival time (1, 2, 4, 8 weeks), and epileptic responses were recorded from slices from both hippocampi in every case. ISHH revealed no loss of inhibitory neurons in any hippocampal subregion in the toxin injected rats. In the vehicle-injected rats the amount of GAD mRNA per neuron fell in the hilus and CA3 up to 2 weeks after injection, recovering thereafter. In contrast, inhibitory neurons in the toxin-injected group did not show this dip in GAD mRNA, and at 4 weeks, showed a significant increase, which reached double the control value in hilar neurons.

The increase in GAD mRNA in these epileptic rats indicates plastic, possibly adaptive responses to recurring epileptic discharges.

1. Jefferys JGR & Empson RM. 1990. *Exp.Physiol.* 75:733-736.
2. Jefferys JGR. 1989. *J.Neurophysiol.* 62:458-468.
3. Najlerahim A, Harrison PJ, et al. 1990. *Mol.Brain Res.* 7:317-333.

571.14

INHIBITION IN PRIMARY AND SECONDARY CHRONIC EPILEPTIC FOCI INDUCED BY INTRAHIPPOCAMPAL TETANUS TOXIN.

Ruth M. Empson* and John G.R. Jefferys. Dept. Physiology & Biophysics, St. Mary's Hosp. Med. Sch., Imperial Coll., London W2 1PG, England.

Injecting tetanus toxin into one hippocampus of a rat induces chronic epileptic foci in both hippocampi[1], which remain epileptogenic for at least 6 weeks. We know that GABA release is depressed 2 weeks after bilateral injections of the toxin[2]. Here we compare inhibitory function in the primary and independent, secondary, "mirror" foci.

Hippocampal slices were prepared 10-16 days after injection of 12 mouse LD_{50} into one hippocampus and used for two studies in vitro:-

1. The Ca^{++}-dependent, K^+-evoked release of ^3H GABA was depressed for slices from the injected hippocampus, in accord with previous acute and chronic studies[2]. In contrast, it remained normal in the contralateral slices, although they generated similar epileptic activity.

2. In control slices, both fast and slow IPSPs are reliably recorded from CA3 pyramidal cells following afferent stimulation. Both were consistently abolished within the primary focus in slices from injected hippocampi. In contrast, fast and slow IPSPs were present in a minority of CA3 cells from the focus in the contralateral hippocampi (respectively 4 and 8 of 17 cells).

The secondary focus thus differs from the primary in retaining some inhibitory function, in spite of producing similar epileptic discharges.

Supported by the S.E.R.C. and Wellcome Trust

1. Jefferys JGR & Empson RM. 1990. *Exp.Physiol.* 75:733-736.
2. Jefferys JGR, Mitchell P, et al. 1991. *Neurochem.Int.* 18:373-379.

571.15

RHYTHMIC SLOW ACTIVITY AND OSMOTIC CHANGE IN CA3 PYRAMIDALE OF RAT HIPPOCAMPUS. E.P. Osehobo* and R.D. Andrew. Anatomy Department, Queen's University, Kingston, Ontario K7L 3N6

Osmolality inversely affects epileptiform activity both clinically and in brain slices (Andrew, R.D., *J. Neurol. Sci. 101*, 7-18). Rhythmic slow activity (RSA) encodes memory in lower mammals and represents a non-pathological oscillation of cortical neurons. We examined the effect of osmolality on the duration and frequency of RSA which consists of a 4-10 Hz oscillation (theta rhythm) overriding a periodic slower depolarization (SD). RSA was induced by 40 μM carbachol and intracellularly recorded in CA3 neurons. Artificial CSF (ACSF) elevated to +40 mOsm with mannitol slowed SD frequency (16 of 16 cells). Conversely -40 mOsm ACSF increased SD frequency (9 of 13). Glycerol however (+40 mOsm) had no effect (4 of 4), indicating that the frequency effects were osmotic in origin. There was no consistent change in theta frequency (n=9) but overriding action potentials were often reduced or arrested in hyperosmotic ACSF (8 of 12). The lack of osmotic effect on theta, which is intrinsically generated by CA cells (and synaptically synchronized) fits with previous data showing no endogenous or synaptic CA3 changes in ± 40 mOsm ACSF. On the other hand, changes in SD frequency and action potential firing may reflect elevated accumulation and slower clearance of K^+ from the reduced extracellular space induced by hyposmotic ACSF. Conversely, hyperosmolality may reduce $[K^+]_{out}$, lowering SD frequency. This hypothesis will be investigated further.

571.16

OSMOTIC EFFECTS UPON FIELD POTENTIALS: EXCITABILITY CHANGE OR SIMPLY RESISTIVE CHANGE? S.J. Quackenbush*, J. Goh and R.D. Andrew. Depts. of Anatomy & Pharmacology, Queen's University, Kingston, Ontario K7L 3N6

Lowered osmolality leads to increased seizure susceptibility both clinically and in brain slice preparations (Andrew, R.D. *J. Neurol. Sci. 101*, 7-18). The field EPSP (fEPSP) and population spike (PS) amplitude recorded in CA1 stratum pyramidale or radiatum are inversely proportional to artificial CSF (ACSF) osmolality. In -40 mOsm ACSF, the orthodromic PS evoked from CA3 radiatum increased by 46 ± 11% at maximal stimulation but increased by 107 ± 39% at sub-maximal stimulation (n=6). The antidromic PS increased by 43 ± 12% and 67 ± 16% (n=9) at max and sub-max stimulation respectively. A +50 mOsm increase using D-glucose *decreased* control ortho PS amplitude by 45 ± 21% at max stim and 69 ± 24% at sub-max stim (n=6). The antidromic PS also decreased by 28 ± 2% and 41 ± 4% (n=9), max and sub-max respectively. The consistently greater sub-max changes suggest that the increased field potentials in hyposmotic media result from neuronal recruitment and not simply from increased extracellular resistance presented by cell expansion. Furthermore unlike the CA1 fEPSP, the preceding CA3 afferent volley was not significantly altered in -40 and +50 mOsm ACSF nor at max and sub-max stim (n=5). With a purely resistive change, both fields should be equally affected. We propose that axons and their glia are less susceptible to volume changes than somatic and dendritic regions.

571.17

EPILEPTIFORM DISCHARGES INDUCED BY TETRAETHYL-AMMONIUM (TEA) IN THE RAT HIPPOCAMPAL SLICE. Y. Fueta and M Avoli. MNI and McGill Univ. Montreal, Quebec, H3A 2B4.

TEA is a K' channel blocker and a convulsant drug in the CNS. We have shown that 4-aminopyridine (4-AP), which blocks a different K' channel, induces epileptiform discharges and synchronous GABA-mediated depolarizing potentials in the CA3 subfield of the rat hippocampal slice (J Neurophysiol, 65:771,1991). Therefore intra- and extracellular recordings were performed to study whether TEA could disclose similar patterns in the CA3 subfield. Like 4-AP, TEA induced interictal-like epileptiform field potentials, which were associated with an intracellular burst of action potentials, were blocked by the non-NMDA antagonist CNQX in a dose-dependent manner, and were resistant to CPP, which is an NMDA receptor antagonist. However TEA did not disclose any GABA-mediated depolarization, although inhibitory mechanisms appeared to be operant. Our data show that TEA induces epileptiform discharges mediated through non-NMDA receptors, but it is not capable of modifying GABA-mediated transmission in a way similar to that reported for 4-AP.

571.18

4-AMINOPYRIDINE-INDUCED SPREADING DEPRESSION EPISODES IN CA3 AREA OF IMMATURE HIPPOCAMPAL SLICES AND THEIR RESPONSE TO EXCITATORY AMINO ACID ANTAGONISTS. C. Psarropoulou*, V. Tancredi and M. Avoli. MNI, McGill University, Montreal, QC, Canada H3A 2B4

Perfusion with 4-aminopyridine (4-AP, 50μM) induced the appearance of spontaneous epileptiform activity and spreading depression (SD) episodes in CA3 hippocampal subfield of slices obtained from 2-30 day-old rats. SD was observed in 34% of all slices tested, a percentage increasing to 50% when only 11-20 day-old slices were considered and declining to 23% for 21-30 day-old slices. SD appeared in all CA3 strata concurrently, having the largest amplitude and duration in the pyramidal layer. Under these conditions, SD was also recorded in CA1, preceding or following that in CA3 area. The negative field potential accompanying SD had an amplitude of 18.1±1.1mV (mean±SEM, n=31), duration ranging from 20-250 s and it occurred every 4.5-33 min. SD was blocked by the application of the NMDA receptor antagonist CPP (2-10μM, n=10), but not by CNQX (5μM, n=6) which is a non-NMDA receptor antagonist. These results demonstrate that: 1) SD episodes can be generated in immature tissue under conditions that do not provoke them in adult tissue and 2) excitatory amino acid receptor subtypes are differentially involved in their generation.

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571.19

IRON INDUCES "EPILEPTIFORM" ACTIVITY IN RAT HIPPOCAMPAL SLICES. **RA Palovcik, BM Uthman, C Koch, and BJ Wilder. Neurol. Dept. VAMC and Univ. of Florida, Gainesville, FL 32608.**

Intracerebral iron deposits have been suspect as a cause of posttraumatic epilepsy.

"Epileptiform" activity induced by iron occurred after bath application of $FeCl_3$ to achieve a 5 x 10^{-5} M solution. Lower doses (2.5 x 10^{-5} M $FeCl_3$) only increased single unit firing rates. Alterations of $[K^+]_o$ from 3.0 mM to 6.25 mM had no effect on iron-induced "seizure-like" activity but enhanced spontaneous background activity. Bursts of "seizure-like" activity were often followed by periods of depressed activity, similar to an epileptic post-ictal stage.

Most slices had an apparent CA3 to CA1 direction of spike propagation although phase reversal and uncoupling of large "epileptiform spikes" at the two sites often occurred during periods of maximum firing.

Iron application often doubled CA1 recorded population spike amplitude elicited by stimulation of the Schaffer collateral-commissural pathways in stratum radiatum. The acute excitatory and convulsive effects of iron may therefore contribute to its long-term epileptogenicity.

571.20

GENETIC CONTROL OF OSCILLATING BRAIN EXCITABILITY IN INBRED GERBIL STRAINS. J.W. Collins and W.B. Iturrian, Pharmacology, University of Georgia, Athens, GA 30602.

The spontaneous seizures of the gerbil model of epilepsy do not occur randomly, but rather show a periodicity across days. Each individual has its own rhythm suggesting that seizure diathesis involves an oscillator of brain excitability. We show a genetic basis for such long term control mechanism by selective breeding. The Black Inbred UGA gerbil was derived from animals that seizure with an intertest interval (iti) of 7 days. The commercial inbred Mon/Tum do not kindle at this iti. F_1 hybrids and parental lines were sensory kindled by handling at itis of 1,3,4,7 or 14 days. The most effective regimen was highly strain dependent with Mon's preferring a 3 or 4 day while BI show 7 days and F_1 are intermediate. A 14 day iti markedly increased severity in all previously kindled gerbils. This kindling is a sensory dependent phenomenon independent of previous seizure and number of tests but varies as a function of iti. Once kindled, seizure susceptibility persists for months. A postictal phase lasts 3-4 days but habituation is the more important refractory state. An overlap for kindling and refractory regimens causes difficulty in sensory kindling Mon and non-inbred gerbils. Although the neurological mechanism(s) for kindling, habituation and postictal protection are unknown, all are controlled by genetically regulated oscillators that can either increase or decrease brain excitability.

572.1

EFFECTS OF BETA AMYLOID (B1-28 & B25-35) ON HIPPOCAMPAL SLICE CULTURES. M.R. Harrigan*, D.D. Kunkel and A.T. Malouf. Dept. of Neurological Surgery, University of Washington, Seattle, WA 98195

Beta amyloid alters neuronal viability in dissociated hippocampal cultures, and may play a direct role in the etiology of Alzheimer's Disease (Whitson et al., *Science* 243, 1488-1490). We are analyzing the effects of B1-28 and B25-35 on rat hippocampal slice cultures; a culture system that maintains the organotypic structure and synaptic connectivity of the intact hippocampus. Slice cultures were exposed to 30uM B1-28 for 2-14 days, and individual CA3 pyramidal neurons were filled with Lucifer yellow or neurobiotin. No gross differences in the overall health of the cultures or morphology of individual cells were observed between B1-28-treated and control cultures. Cumulative length and number of branch points of apical dendrites were quantified using the Eutectics image analyzer. A small increase in number of branch points was observed. Slice cultures were exposed to 50ng/ml NGF + B1-28 (30uM or 100uM), since NGF was reported to enhance the potency of beta amyloid (Yankner et al., *PNAS* 87, 9020-9023). Small increases in length and number of branches of the apical dendrites were observed after exposure to 30uM B1-28 + NGF. We are presently examining the effects of B25-35 (100uM) on hippocampal slice cultures. B25-35 has been reported to be more potent than B1-28 (Yankner et al., *Science* 250, 279-282). Cultures exposed to B25-35 for 24 hr appear less healthy than untreated controls. Cumulative dendritic length and number of branch points are being quantified. Beta amyloid appears to have small effects on the viability of neurons in hippocampal slice cultures.

Supported by Alzheimer's Assoc. Grant IIRg-90-126.

572.2

AMYLOID β-PROTEIN IMMUNOREACTIVITY IN SPLEEN, LYMPH NODE AND OTHER NON-NEURAL TISSUES USING A NEW AβP ANTISERUM.

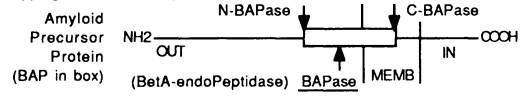
C. Lemere*, H. Yamaguchi*, C. Joachim-Morris, D. Selkoe, Harvard Medical School, Boston, MA 02115.

AβP-immunoreactive deposits have previously been observed in certain non-neural tissues (e.g., skin, intestine) in numerous patients with Alzheimer's disease (AD) and about 1/4 of aged controls. Here, we report the presence of occasional vascular AβP-reactive deposits in lymph node, spleen, omentum and mesentery from 4 autopsied AD patients, using a new antiserum to synthetic A P_{1-40} (R1280). Other than occasional vascular staining in lymph nodes, no R1280 reactivity was found in matching non-neural tissues of 2 young and 3 aged controls. Staining was markedly reduced by absorption with synthetic AβP1-40. Affinity purified R1280 (AP-R1280) was compared to the whole antiserum, the flow-through (FT) fraction and the preimmune serum on AD brain, intestine and lymph node. Preimmune serum did not label any AβP deposits. R1280 and AP-R1280 both labeled plaques and blood vessels in brain, but only R1280 labeled the vascular deposits in non-neural tissues. The FT R1280 fraction stained blood vessels in AD brain and also in non-neural tissues.The staining was markedly reduced with AβP absorption. Our results extend the detection of small amounts of AβP-reactive deposits to additional non-neural tissues and further support the hypothesis that AβP deposition can occur independently of neuronal alteration and may be of vascular origin.

572.3

THE APPREP SYSTEM, PROTEIN PROCESSING AND BAP PRODUCTION: CHARACTERIZATION OF APP-REPORTER EXPRESSION M.P.Vitek, J. Jacobsen, J. Sonnenberg, S. Sahasrabudhe, H. Muenkel, M. Spruyt and A. J. Blume. Molecular Neurobiology, Lederle Laboratories, American Cyanamid, Pearl River, NY 10965.

We wish to understand how and why BAP levels increase in AD brain. Using an S1 nuclease protection assay, total APP RNA levels increase 2 to 3 fold ($p<0.01$) in 16 AD vs. 10 normal brains (Jacobsen,etal. Neurobiol. Aging 12(1991)in press) which partially explains the increased BAP levels in AD. The remainder may be due to protein processing of the Amyloid Peptide Precursor (APP) into BAP. To test this, we have reconstituted a marked APP substrate with BetA-endoPeptidase (BAPase) enzymes. APP must be cut at the ends of its internal BAP domain by amino-BAPase (N-BAPase) and carboxy-BAPase (C-BAPase) activities which may change in AD. Alternatively, the Dutch and London mutations found in the BAP region of APP may alter these BAPase activities. To measure BAPase activities and resolve the contribution of substrate mutations, we have engineered analogs of **APP** to contain **REP**orter epitopes flanking the BAP domain. Proteolytic cleavage of APPREP is followed by immunological detection of reporters associated with protein fragments whose lengths reflect cleavage location. We have expressed and specifically detected APPREP with antibodies to the Kunitz Protease Inhibitor domain of APP and to Substance-P. Further characterization of APPREP with additional antibodies, aminoacid sequencing, peptide mapping and HPLC analysis are in progress.



572.4

MOLECULAR CLONING OF A NOVEL AMYLOID PROTEIN IN ALZHEIMER'S DISEASE.

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Although β/A4-protein was found to be a major constituent of amyloid in Alzheimer's disease (AD) brain, other components might also be found in AD amyloid. Recently, we have discovered a new component of amyloid from AD brain and cloned the corresponding cDNA. Amyloid was purified in the presence of SDS, solubilized in formic acid, digested with cyanogen bromide, then with endopeptidase and analyzed by HPLC. Analysis of the amino acid sequence showed, in addition to the β/A4-protein fragment, a peptide with unknown sequence. We named this peptide NAC signifying New Amyloid Component. This sequence was used to raise a rabbit antiserum, which stained the amyloid core of neuritic plaques on brain sections and a 40kD protein in Western blots. Oligonucleotides were generated from this sequence to amplify the cDNA from human brain cDNA library using PCR. A 247-nucleotide DNA was amplified. DNA sequencing showed this PCR product to contain a nucleotide stretch which encodes the exact peptide sequence of NAC. The amino acid sequence deduced from the DNA sequence demonstrated a striking feature of NAC, i.e., five K#K##V sequences in this 82-amino acid stretch. Furthermore, a 10-amino acid homologous sequence is found to be repeated in it. The characterization of NAC should shed light on the amyloidogenesis in AD and may help in the search for the etiology of this disease.

572.5

BIOLOGICAL ACTIVITY OF THE SECRETED FORM OF AMYLOID β/A4 PROTEIN PRECURSOR II: FUNCTIONAL ANALYSIS OF APP FRAGMENTS.

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The secreted form of APP-695 affects the growth regulation of fibroblasts (Cell 58:615-622, 1989). Using proteins made in E. coli or purified from human brain, we are testing N-terminal fragments of APP for growth regulating activity. Several plasmids which drive the synthesis of various fragments of the extracellular domain of APP-695 were constructed using a prokaryotic expression vector. These fragments were purified from bacterial lysates and were tested for the ability to support the growth of A1 fibroblasts, a cell line which produces very low levels of APP and depends on the presence of exogenous APP in the medium for normal growth. Lysates of bacteria carrying only the expression vector were used as negative controls. Whereas some fragments showed no growth stimulating activity, others were active. Our results suggest that this biological activity of the secreted form of APP-695 is contained within a region 40 amino acids in length. Experiments using peptides corresponding to the region of interest are now under way to further define the site of activity. In addition, a fragment of the secreted form of APP from human brain is being studied. This 35 kD fragment, recognized by antibodies to N-terminal epitopes, is increased in Alzheimer (AD) cortex (Neurosci. Lett. 100:340-346, 1989). Investigation of this fragment may yield information on the processing and the biological activity of APP in AD. The purification of this peptide is in progress.

572.6

EXPRESSION OF ALZHEIMER'S AMYLOID PROTEIN BY HUMAN NEUROGLIOMA CELLS: SYNTHESIS, DISTRIBUTION, AND SECRETION.

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Using antisera against full length AAP695 expressed by baculovirus, we followed synthesis and secretion of AAP in human brain neuroglioma cells (ATCC HTB 148) to determine the site of proteolysis preceeding secretion. These cells were found to express mostly AAP695, some AAP770 and no AAP751 by PCR. Pulse-chase with [35S]met followed by immunoprecipitation, SDS-PAGE and autoradiography reveals a prominent 120-kDa species that is accumulated at 15°C and is Endo H sensitive. After warming to 20°C, a family of proteins appears at 145-165 kDa that are not Endo H sensitive and are sulfated at 20°C suggesting that immature AAP has moved from ER to late Golgi. Shift to 37°C results in rapid and constitutive secretion of a 135-kDa species into the medium. Chase from the immature to the secreted form, which is not detected intracellularly at 20°C, 37°C or in the presence of weak bases, takes 2 h. Immunofluorescence microscopy of permeabilized cells shows that AAP is located mostly in a perinuclear compartment that also labels with wheat germ agglutinin. AAP is not colocalized with cathepsin B nor is it detected at the plasma membrane. These results indicate that AAP is a late Golgi membrane glycoprotein that is cleaved in a neutral, post-trans Golgi compartment enroute to the cell surface.

572.7

Participation of the Amyloid A4 Precursor Protein in Lymphocyte Activation

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Amyloid deposition in senile plaques and the cerebral vasculator is a marker of Alzheimer disease. It is encoded as part of a larger precursor(Pre A4) that maps to chromosome 21. Three mRNA-bands have now been accounted for by the demonstration of three alternative splicing products.

The precursor proteins(Pre $A4_{695}$, Pre $A4_{751}$, and Pre A_{770}) of Alzheimer disease A4 amyloid protein are integral, N-glycosylated membrane proteins of unknown function.

Here we report that normal human lymphocytes express pre A4 mRNA and protein. Pre A4 is detectable on the surface of lymphocytes. Lymphocyte Pre A4 surface abundance is increased by cell activation. We conclude that Pre A4 is a lymphocyte surface molecule that may participate in cell activation.

572.8

Phagocytosis and Vascular Deposition of Alzheimer Amyloid in Rat. S.A. Frautschy, G.M. Cole and A. Baird, Dept.of Cellular and Molecular Biology, Whittier Institute, 9894 Genesee, La Jolla, Ca.92037 and Dept. of Neurosciences, UCSD, La Jolla, Ca. 92093

Deposits of extracellular β amyloid occur in plaques and vessels in Alzheimer's disease(AD), but they are not found in normal rodent aging, nor have they been induced. In order to develop an animal model for β amyloidosis, we injected amyloid cores from AD brains and, contralaterally, similarly isolated control lipofuscin fractions into cortex and hippocampus of adult rats. Rats were sacrificed 2 d (n=2), 7 d (n=2) and 30d (n=10) and 60d (n=2) after injection and sections were silver or Congo red stained or immunostained for β amyloid. Amyloid and lipofuscin moved medially and laterally from the injection site and persisted for at least 2 months. The astroglial and microglial responses indexed by glial fibrillary acidic protein and OX-42 immunostaining were similar on the amyloid and control sides. By 7d much of the amyloid was in or near phagocytes which appeared to migrate to the vessels and the ventricles. At 30d, there was clear vascular β protein immunostaining. These results show that in the rat, AD amyloid deposits in the neuropil can be aggressively phagocytosed and deposited in vessels.

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572.9

MULTIPLE CLEAVAGE SITES OF ALZHEIMER AMYLOID PRECURSOR IN NEURONALLY DIFFERENTIATED PC-12 CELLS J. Anderson, F. Esch, P. Keim, K. Sambamurti, I. Lieberburg, and N.K. Robakis
Dept. of Psychiatry, Mt. Sinai Med Ctr, New York, NY 10029 and Athena Neurosciences, S. San Francisco, CA 94080

Studies in transfected cells have shown that the secreted form of Alzheimer amyloid precursor protein (APP) is generated by cleavage of full-length, membrane-bound APP on the N-terminal or C-terminal side of lys^{16} of the β amyloid peptide (βAP). Since βAP is deposited in amyloid fibers, cleavage within βAP cannot lead to amyloid formation. We examined secreted APP purified from medium conditioned by untransfected PC-12 cells which had been differentiated to a neuronal phenotype by NGF. Analysis of the C-terminal peptides obtained after CNBr digestion indicated that cleavage occurs between βAP lys^{16} and leu^{17}. In addition, a portion of the original secreted APP reacted with a monoclonal antibody directed against βAP leu^{17}-asp^{23}, suggesting that a portion of secreted APP may be capable of forming amyloid. Investigation of the site of this alternative cleavage is in progress.

572.10

DISTRIBUTION AND ULTRASTRUCTURE OF CEREBROVASCULAR AMYLOID IN AGED SQUIRREL MONKEYS. L.C. Walker and D.L. Price. Neuropathology Laboratory, The Johns Hopkins University School of Medicine, Balto., MD 21205.

Cerebrovascular amyloidosis occurs in the context of several neurological disorders, including Alzheimer's disease. Aged squirrel monkeys (*Saimiri sciureus*) often develop cerebrovascular amyloidosis that is similar in distribution to that of humans. In immunocytochemical studies of animals 18-23 years of age, we have found that: vascular amyloid deposition can be pronounced in the late teens; the incidence and distribution of vascular (and nonvascular) amyloid vary markedly among animals of similar age; and the focal clustering of lesions, which is particularly apparent in mildly affected brain areas, suggests selective involvement of the branches of a common blood vessel. Ultrastructurally, various vascular abnormalities are evident, and foamy, debris- and lipid-laden macrophages often abut amyloidotic vessels. These findings indicate that cerebral amyloid angiopathy in aged squirrel monkeys can selectively affect certain vessels, that the pattern of lesions varies among individuals, and that macrophages may play a role in the processing of mural amyloid in cerebral blood vessels.

572.11

STRESS-INDUCED ALTERATION OF APP mRNAs. B. Border, S. Pardue*, E.K. Miller*, and M. Morrison-Bogorad. Dept. of Neurology, Southwestern Medical School, Dallas TX 75235

Although amyloid deposition may be related to changes in absolute or relative amounts of different amyloid precursor proteins (APPs), factors regulating their levels in vivo are unknown. We have shown that two in vivo stressors result in rapid changes in specific APP mRNA levels in rat hippocampus. Rats were made hyperthermic by exposure to heat or injection of amphetamine and sacrificed 2 hours after attaining a temperature of 42°C. Hybridization to an 18s rRNA oligonucleotide probe revealed similar levels of hybridization to neurons in the dentate gyrus and CA1-4 regions in all rats, indicating that signal intensity with mRNA-specific probes could be directly compared. Heat shock alone did not change overall levels of the APP mRNAs, whereas they were increased after amphetamine-induced hyperthermia. There was a reduction in levels of the APP_{695} mRNA in heat shocked rats but a strong induction in the amphetamine-treated rats. Our results suggest that there is a several fold change in the relative amounts of different APP mRNAs after heat shock, while amphetamine induction of both total APP and APP_{695} mRNAs show a separate drug effect over and above that produced by heat shock. Supported by NIH AG08013

572.12

APP IMMUNOPOSITIVE GRANULES ARE PRESENT IN A SUBSET OF SENILE PLAQUES WITHIN ALZHEIMER'S DISEASE

B.J. Cummings, J.W. Geddes°, W. Van Nostrand*, S. Wagner*, D.D. Cunningham*, and C.W. Cotman. Depts of Psychobiol., Microbiol.* and Neurosurgery° *University of California: Irvine, CA 92717 USA*

Protease nexin-II has been identified as homologous to the end terminal region of the amyloid precursor protein (APP) containing the Kunitz inhibitor domain. We have examined the distribution and co-localization of APP immunostaining using a monoclonal APP antibody and a polyclonal antibody against β/A4 (1-28) protein. We found that this APP antibody is sensitive to fixation and yields the best results in embalmed tissue. No APP positive staining was observed in controls. Within AD brains, the distribution of APP positive plaques varied greatly, e.g. some AD cases showed the strongest staining within the hilus while other cases had no hilar staining. Total plaque number varied across regions examined, but anti-APP, anti-β/A4 or Bielschowsky staining detected an equal number of plaques within each region except parahippocampal cortex, where the number of β/A4 positive plaques was more than three times greater than APP positive plaques. APP positive plaques were composed of aggregates of punctate, granular staining and were co-localized with thioflavin S. APP positive staining was not limited to plaque-like structures: pyramidal neurons of fields CA3 through CA1, curly fibers surrounding and entering plaques and some blood vessels were also stained. Using confocal microscopy and double labeling, we found that all APP positive plaques were also positive for β/A4, but many β/A4 positive plaques did not contain APP. These data are consistent with the possibility that APP is present in immature plaques and is degraded to form β/A4 plaques.

572.13

β-AMYLOID PROTEIN POTENTIATES INJURY BY GLUCOSE DEPRIVATION IN NEURONAL CORTICAL CULTURES. A.G. Copani, J. Koh and C. W. Cotman. Department of Psychobiology, *University of California, Irvine, CA 92717 U.S.A.*

Alzheimer's disease (AD) is associated with a generalized decline in cerebral glucose metabolism, which occurs most severely in the temporal and parietal cortex. In the present study, we used an *in vitro* paradigm to examine the possibility that β-amyloid protein deposited in AD plaques may increase neuronal vulnerability to glucose deprivation. Mature murine cortical cultures deprived of glucose for 3-6 hours developed neuronal degeneration estimated both by examination with phase-contrast microscopy and by lactate dehydrogenase assay. This toxicity was increased when cultures were simultaneously exposed to 50 μg/ml synthetic β-amyloid (β 1-42). Cultures treated with β 1-42 in the presence of a physiological concentration (5.5mM) of glucose did not exhibit toxicity in comparison to controls. In a previous study, β 1-42 was reported to enhance glutamate neurotoxicity (J. Koh et al.,1990); thus the increase in hypoglycemic neurotoxicity by β 1-42 may be due to the potentiation of endogenous excitatory amino acid neurotransmitters which have been reported to mediate glucose deprivation injury. Consistent with this idea, the antagonist of NMDA-receptor MK 801 (5μM) largely attenuated the toxicity-increasing effect of β 1-42. The present results suggest that β-amyloid protein could exacerbate the neuronal cell loss in the presence of a defective cerebral energy metabolism occurring in Alzheimer's disease.

572.14

SYNTHETIC β-AMYLOID IS NEUROTOXIC *IN VITRO* FOLLOWING AGING OF PEPTIDE. C.J. Pike, A.J. Walencewicz, C.G. Glabe† and C.W. Cotman. Departments of Psychobiology and Molecular Biology and Biochemistry†, *University of California, Irvine, CA 92717 USA.*

Beta-amyloid is an approximately 42 amino acid residue protein and is the major component of the senile plaques found in Alzheimer's disease. We have found that short-term incubation of synthetic β1-42 solution can result in the formation of peptide aggregations. In order to characterize the biological activity of these β1-42 aggregates, we have conducted experiments comparing the effects of freshly solubilized and pre-incubated β1-42 on neuronal survival. Amyloid peptide was added to immature hippocampal cultures (16,000 cells/cm^2) from embryonic (E-18) rats at concentrations of 0, 0.1, 1.0, 10, 50, and 100 μg/ml. After twenty-four hours under these conditions, cultures treated with freshly solubilized β1-42 showed increased neurite-outgrowth at both the 50 μg/ml and 100 μg/ml concentrations but did not show a significant reduction in neuronal viability when compared to controls. Cultures treated with pre-incubated β1-42 displayed significant cell death that approached 100% at the 100 μg/ml concentration. Both the neurite-enhancement and neurotoxicity effects were dose-dependent, showing differences from controls only above the 10 μg/ml concentration. Analysis with SDS-PAGE revealed that freshly solubilized β1-42 migrated primarily as a ~4 kD monomer whereas pre-incubated β1-42 had additional high molecular weight bands. These data suggest that pre-incubation of β1-42 results in both a change in biological activity from neurite-promoting to neurotoxic and the formation of SDS-resistant peptide aggregations. These findings are consistent with the hypothesis that neurodegeneration in Alzheimer's disease is related to insoluble β-amyloid found in senile plaques.

572.15

SITE-SPECIFIC NICKING OF ALZHEIMER ß/A4 AMYLOID ALTERS THE AGGREGATION STATE. T. Honda and C.A. Marotta. MGH, McLean Hospital and Harvard Medical School, Boston, MA 02114.

ß/A4 Amyloid peptide (1-28) aggregated to 15kDa on SDS/urea PAGE. While a variety of proteases readily degraded the complex, endopeptidase Arg-C produced a new aggregate of higher molecular weight (16kDa) on denaturing gels without degraded smaller products. The new aggregate was comprised of three peptides: ß/A4(1-28), ß/A4(1-5) and ß/A4(6-28). The same results were obtained by treatment of ß/A4 with other arginine-specific proteases: the gamma subunit of Nerve Growth Factor and clostripain. The results indicate that arginine-specific proteases, including a growth factor processing enzyme, can nick aggregated ß/A4 amyloid and alter the configuration to produce a more complex form. Should partial cleavages, or nicking, of this type occur in the AD brain then proteolytic processes themselves may promote aggregation of amyloid. This mechanism may contribute to formation of the relatively insoluble amyloid core of senile plaques. Supported by grants from Mitsubishi Kasei Corporation, the National Institute on Aging (AG02126), and a Metropolitan Life Foundation Award to C.A.M..

572.16

KAINIC ACID LESIONING SELECTIVELY INCREASES THE AMYLOID PRECURSOR PROTEIN mRNA ENCODING PROTEASE NEXIN II IN GRANULE NEURONS OF THE DENTATE GYRUS. D. A. Willoughby, S. A. Johnson, G. M. Pasinetti, G. Tocco*, I. Najm*, M. Baudry*, and C. E. Finch. Andrus Gerontology Ctr. and *Program in Neurosciences, Dept. of Biol. Sci. Univ. So. Cal., Los Angeles, CA 90089

The ability of the amyloid precursor protein (APP) to inhibit serine proteases depends upon the inclusion of the 168 nt exon 7 sequence in the fully processed mRNA. Alternative splicing of the APP pre-mRNA generates 5 distinct mRNA transcripts. Three of these transcripts contain exon 7 and encode either 751, 770 or 568 amino acid proteins which are functionally and chemically identical to protease nexin II. The APP mRNA which is the predominant form in rodent brain, lacks exon 7 and encodes a 695 amino acid protein. In Alzheimers disease, hippocampal pyramidal neurons show an increased 751/695 mRNA ratio (Johnson et al., 1990, Science 248; 854-857). It is therefore of interest to develop rodent models which show similar changes in APP mRNA in response to experimental lesion. 12 hours after i.p. administration of kainic acid we observed an 80% increase in APP mRNA molecules containing exon 7 in the rat dentate gyrus. In situ hybridization was used to localize the response to the granule cell layer. No change in the prevalence of APP-695 mRNA was detected. No significant change in the APP mRNA content of the CA1, or CA3 pyramidal cell layers was observed. Because granule neurons are resistant to kainate induced cell death, the elevation of exon 7-containing-APP mRNA can occur in neurons independent of cell degeneration. Supported by NIH grant AG7907 to C. E. F. and NINCDS N18427 to M. B.

572.17

HUMAN MICROGLIA EXPRESS AMYLOID PRECURSOR PROTEIN. D.W. Dickson, S.C. Lee*, L.A. Mattiace, W. Liu*, A. Fishman* & S.H. Yen*. Department of Pathology, Albert Einstein College of Medicine, Bronx, New York 10461.

Microglia are associated with virtually every amyloid deposit in Alzheimer brains. Amyloid is composed of a 4kd molecule (beta/A4) derived from a larger precursor protein (APP) with several alternative transcripts, including one that is identical to protease nexin 2. APP is widely distributed in a number of tissues, and it has trophic effects on some cultured cells. In order to study amyloidogenesis, we raised antibodies to amino, carboxy and beta/A4 domains of APP. In adult brains, the antibody to the carboxy terminus (APPc) immunostained dystrophic neurites in some senile plaques, blood vessels with amyloid angiopathy and microglia in tissue that had been briefly fixed in paraformaldehyde. Microglia stained by APPc were associated with amyloid deposits, but also more widespread in gray and white matter. In fetal brains, APPc stained immature cells and ameboid microglia in the subventricular zone, but few ramified cells. To better characterize these results, microglia were isolated from mixed glial cultures from second trimester fetal brains using shaking and a differential adhesion method. Isolated microglia were immunoreactive with macrophage antibodies, internalized acetylated low density lipoprotein and produced interleukin-1 in response to lipopolysaccharide stimulation. Cultured microglia were immunoreactive with antibodies to all three domains of APP. Double-labeling immunocytochemistry of mixed astrocyte-microglia cultures showed a largely non-overlapping pattern of GFAP and APP staining. Future studies will explore molecular biology of APP synthesis in cultured microglia; however, the initial results suggest that microglia express APP and that microglia may be a source of amyloid in Alzheimer's disease. In fetal brains, APP may play a role in cell growth and differentiation.

573.1

TRANSIENT GLOBAL ISCHEMIA INDUCES EXPRESSION OF SULFATED GLYCOPROTEIN-2 (SGP-2) IN RODENT BRAIN. P.C. May, J. Clemens, K.S. Fuson, E.B. Smalstig* and P. Robison*. Lilly Research Laboratories, CNS Division, Eli Lilly and Company, Indianapolis, IN 46285.

Sulfated glycoprotein-2 (SGP-2), the rodent homologue to a human hippocampal cDNA clone (pADHC-9) elevated in Alzheimer's disease, increases in rat hippocampus (HC) following intrinsic HC lesions or extrinsic deafferentation (May et. al, 1990, Neuron 5:831). To further explore its *in vivo* regulation, SGP-2 expression was examined in rat HC and caudate nucleus (CN) 3 days after 30 min of transient global ischemia induced by 4 vessel occlusion (4VO). Northern blots of total RNA isolated from HC showed significant increases in SGP-2 RNA and GFAP RNA prevalence following ischemia (1.8 fold and 5 fold, respectively; $p<0.05$, ISC vs CTL); slightly greater increases in SGP-2 and GFAP RNA levels (2.2 and 6 fold, respectively; $p<0.05$, ISC vs CTL) were observed in RNA isolated from CN. Protein immunoblots showed marked increases in SGP-2 and GFAP protein in homogenates from 4VO-lesioned HC and CN. As observed above, greater increases in SGP-2 protein were observed in CN samples than HC samples, consistent with the earlier onset of neurodegeneration in CN vs. HC following 4VO. This well characterized animal lesion paradigm should prove useful in defining the role of pADHC-9/SGP-2 in brain and may point to novel mechanisms of neurodegeneration ongoing in AD.

573.2

APP DECREASE IS AN EARLY RESPONSE TO ISCHEMIA IN RODENT BRAIN. P. Robison*, J. Clemens, E.B. Smalstig* and P.C. May. Lilly Research Laboratories, CNS Division, Eli Lilly and Company, Indianapolis, IN 46285.

The function of Amyloid Precursor Protein (APP) in the brain is unknown. We studied APP regulation in caudate nucleus (CN) and hippocampus (HC) after 30 minutes of transient global ischemia induced by four vessel occlusion (4-VO). Previous histological examination of these two tissues at 24 and 72 hours shows that the CN loses neurons before the HC. To document neurodegeneration we used microtubule associated protein (MAP-2), a neuronal somatodendritic marker, glial fibrillary acidic protein (GFAP), a marker for reactive astrocytes and beta-tubulin as a control. Steady state levels of APP were evaluated by western blot analysis of tissue homogenates using an antibody to aa 595-695. In the CN 24 hours after 4-VO, APP and MAP-2 levels were decreased, while GFAP levels were only slightly elevated, consistent with neurodegeneration. Beta-tubulin was unchanged. In the HC at 24 hours post 4-VO, APP was more decreased than in the CN, while MAP-2 was not not affected. GFAP was elevated to the same level as CN. This confirms that neuronal degeneration was not taking place in the HC and that decreased APP precedes neuronal degeneration. Since neuronal degeneration does not appear until 48 hours after a decrease in APP is seen, it will be important to determine whether full length APP is cleaved to a neurotoxic fragment which promotes degeneration.

573.3

AMYLOID PRECURSOR PROTEIN (APP) IN ALZHEIMER'S DISEASE (AD): A COMPARATIVE IMMUNOHISTOCHEMICAL STUDY. M. Gearing, E.R. Unger*, E.R. Shelton*, H.W. Chan* and S.S. Mirra. VA Medical Center and Emory University School of Medicine, Atlanta, GA 30322 and Syntex Research, Palo Alto, CA 94303.

The distribution and modifications of APP in AD are being widely investigated with antibodies to the beta A4 peptide and other APP epitopes. Yet, differences in antibodies and histochemical techniques militate against ready comparison of data from center to center. We tested one approach to possible standardization using an automated slide stainer allowing concomitant staining of up to 60 slides with minimum quantities of immunoreagents under varying conditions (Brigati et al. J Histotechnol 1988;11:165). Multiple tissue sections were simultaneously treated using three immunohistochemical techniques with different enzyme and chromagen combinations: The more commonly used avidin-biotin peroxidase method with chromagens 3,3 diaminobenzidine tetrahydrochloride (DAB) and 3-amino-9-ethylcarbazole (AEC) was compared with the avidin-alkaline phosphatase method using 5-bromo-4-chloro-3-indoyl phosphate-nitro-blue-tetrazolium (McGadey chromagen). A battery of affinity-purified antibodies directed against domain-specific sites of APP as well as antibody to beta A4 (kindly supplied by Dr. Colin Masters) were used to label amyloid in cerebral cortex and striatum from AD and control patients. We found that the alkaline phosphatase method gave the most striking signal. This difference was most clearly seen in the labelling of diffuse plaques in the AD cases, where antibodies to beta-A4 and its components produced intense and frequent label of diffuse plaques in striatum and cortex. The flexibility of this automated system enabled ready control of variables including concentration of antisera and pretreatment of tissues with reagents such as formic acid, proteases, and phosphatases. The automated approach also permitted processing of multiple tissue sections under identical conditions and could potentially facilitate standardization of diagnostic and research methods among laboratories.

573.4

IN VIVO EFFECTS OF ß-AMYLOID PROTEIN IN PRIMATES: IMPLANTATION STUDY. D.T. Stephenson[1], M. Podlisny*[2], D. Games*[3], S. Little*[1], J. Lieberburg*[3], D. Selkoe[2] and J.A. Clemens[1]. [1]Eli Lilly & Company, Indianapolis, IN, 46285; [2]Center for Neurologic Diseases, Brigham and Women's Hospital, Boston, MA 02115; [3]Athena Neurosciences, South San Francisco, CA 94080.

Extracellular deposition of ß-amyloid protein (ßAP) is a hallmark of the neuropathology of Alzheimer's disease. Direct, toxic effects of ßAP on neurons have become a recent focus of investigation. Since most of these studies have been confined to in vitro model systems, we chose to study its effects in vivo in a species that exhibits age-related ßAP deposits. The present study was designed to evaluate the effects of ßAP when it is implanted into the brain in a lipophilic, non-toxic vehicle. Adult rhesus monkeys were anesthetized and their heads placed into a sterotaxic instrument. Sterile implants of synthetic ßAP 1-43 prepared in cocoa butter were made stereotaxically through a stainless steel canula into the frontal cortex. The contralateral hemisphere received implants of a control peptide (N-acetyl scrambled ßAP 25-35) or vehicle alone. After 30 days, monkeys were perfused transcardially with periodate-lysine-paraformaldehyde. Cortical blocks were excised, postfixed for 5 hours, processed as cryosections and stained by conventional histological and immunostaining techniques. Evaluation of three monkeys to date has not revealed characteristic AD-like pathology at any of the implant sites. However, the following differences between control and experimental implant sites were observed in these initial experiments: 1) ßAP implants produced larger necrotic lesions than those produced by control implants. 2) axonal spheroids, morphologically abnormal processes and swollen neurites, as revealed by Bielschowsky silver staining, appeared to be more numerous in the ßAP than the control implants. 3) ßAP implants demonstrated more extensive astrogliosis, as observed by immunolocalization of glial fibrillary acidic protein and S-100 protein. Studies in progress are aimed at implanting more animals with varying amounts of the peptide, testing other control peptides and evaluating the lesion sites with multiple antigenic markers to further define the effects of ßAP in the primate brain.

573.5

IN VIVO EFFECTS OF ß-AMYLOID PROTEIN IN PRIMATES: MICRO-INJECTION STUDY. [1]M.Podlisny*, [2]D.Stephenson, [1]M.Frosch, [3]D.Tolan*, [4]J.Lieberburg, [2]J.Clemens and [1]D.Selkoe. [1]Brigham & Women's Hosp., Boston, MA 02115; [2]Eli Lilly & Co., Indianapolis, IN 46285; [3]Boston University, Boston, MA 02215; [4]Athena Neurosciences, San Francisco, CA 94080.

The progressive deposition of ß-amyloid protein (ßAP) is an early pathogenetic event in Alzheimer's disease (AD). Recent studies suggest both neurotoxic and neurotrophic effects of ßAP in vitro. Since ßAP deposition and surrounding neuritic dystrophy occur spontaneously in aging primates, we sought to evaluate the effects of ßAP in vivo by microinjecting ßAP into monkey cortex. Samples of ßAP were injected into multiple neocortical sites of adult rhesus monkeys in a vehicle of either artificial CSF or 35% acetonitrile/0.1% TFA (AN); control samples were identically injected into homologous contralateral sites. The experimental samples were synthetic ßAP 1-40; ßAP 25-35; or sonicated SDS-extracted amyloid cores purified from AD cortex. Control samples were scrambled synthetic ßAP 1-40; scrambled ßAP 25-35; or SDS-insoluble particles purified from control cortex. All injections were at least duplicate, and the animals were allowed to survive for 2-3 weeks, 8-10 weeks or 3-4 months. Monkeys were perfused with periodate-lysine-paraformaldehyde and cortical blocks were excised and post-fixed for 4 hours. Tissue was paraffin-embedded and injection sites were screened by H&E, modified Bielschowsky silver and immunocytochemistry. In our initial analysis of 5 monkeys, we found experimental injection sites with a range of morphologies. The largest lesions showed liquefactive necrosis with cavitation, indicative of general cellular toxicity. To date, we have found a greater number of such cavitary necrotic lesions in peptide injections made in the AN vehicle than in CSF. Other lesions showed focal neuronal loss accompanied by astrocytic gliosis and microgliosis. The cavitary necrotic lesions occured more commonly with experimental peptide injection; however histologically similar changes were found with control peptide. Experiments are currently underway to define the role of the AN vehicle in causing cavitary necrosis and to determine which cellular effects can be attributed to the experimental peptide itself. Cellular changes that are closely reminescent of AD pathology have not yet been seen.

573.6

POSSIBLE CONTRIBUTION OF THE C-TERMINUS OF β AMYLOID PROTEIN PRECURSOR (APP) TO ALZHEIMER PAIRED HELICAL FILAMENTS (PHFs). C.B.Caputo, C.Wischik*, I.R.E.Sobel*, D.A.Kirschner*, P.E.Fraser*, W.F.Brunner*. ICI Pharms. Gr., Wilm. DE 19897, Lab. Mol. Biol., MRC, Cambridge, UK, Children's Hosp. & Harvard Med. School, Boston, MA 02115.

Polyclonal antibody BR188, which recognizes a peptide corresponding to the C-terminal 20 amino acids of APP, was found to react with pronase-treated PHFs isolated from five different Alzheimer brains. This reactivity was blocked by pretreating the antibody with the peptide antigen but not with peptides with unrelated sequences. The antibody immunogold-labeled these PHFs producing a periodic pattern of labeling. Upon extensive extraction of PHFs and electrophoresis of the soluble fraction, a band was obtained which, when subjected to micro-sequencing, yielded a sequence that corresponded to the last 17 amino acids of APP. Further, the 20 aa peptide used as antigen was found to display amyloid-like properties, including a fibrillar morphology by electron microscopy, birefringence with Congo red staining, fluorescence with thioflavin S, proteolytic resistance, and a characteristic X-ray diffraction pattern for cross-beta pleated sheets. Thus, a fragment from the C-terminus of APP may bind to PHFs and provide them with some amyloid-like properties that are not produced by τ protein, the only other confirmed component of PHFs.

573.7

APP GENE EXPRESSION IN SPECIFIC CELL TYPES OF THE RAT CNS. AC LeBlanc, HY Chen *, L. Autilio-Gambetti and P. Gambetti.Inst. Pathol. Case Western Reserve U. Cleveland, OH 44106

Differential amyloid protein precursor (APP) gene expression was investigated in neonatal rat brain cortex, meninges, and primary astroglial, neuronal and microglial cultures. Relative to brain cortex levels, total APP mRNA was higher in meninges (4.5x) and neuronal cultures (1.4x) and lower in astroglial and microglial cell cultures (0.25x). Ribonuclease protection assays revealed that APP_{695} mRNA was predominant in cortex and cortical neuron (~95% of total APP mRNA) while KPI-containing forms were more abundant in meninges, astrocytes and microglia (~70% of total APP mRNA). The ratio of APP mRNA species changed in neurons and astrocytes with time in culture. Northern blot analysis revealed that cortical astrocytes have an additional APP mRNA of 2.7kb. APP assessed on immunoblots closely paralleled APP mRNA expression except in microglial cells where APP was barely detectable. In addition, lower MW C-terminal fragments were detected in intact tissues but not in the cell cultures. These results indicate that (1) APP gene expression in the CNS is subject to cell specific transcriptional and post-transcriptional regulation (2) high turnover of APP or negative translational regulation is unique to microglia (3) astrocytes express an additional 2.7kb APP mRNA (4) abnormal processing or high turnover of APP C-terminal fragments might occur in primary cell cultures (5) neurons and astrocytes maintained in culture undergo changes in alternative splicing of APP mRNA. Supported by NIA MA AGO8155

573.8

NEUROTROPHIC REGULATION OF AMYLOID PRECURSOR PROTEIN (APP) mRNA SPLICING IN RAT BASAL FOREBRAIN. C.A. Sherman[1], K.S. Chen[2], F.H. Gage[2] and G.A. Higgins[1] [1]Mol. Neurobiol., NIA/NIH, Baltimore, MD 21224; [2]Dept. of Neurosci., U.C.S.D., La Jolla, CA, 92093.

The amyloid precursor protein (APP) gene produces several transcripts. These include APP-695, that is largely 'neuron-specific', as well as a family of mRNAs with a Kunitz serine protease inhibitor (KPI-APP) motif which are expressed in many cell types. We have previously shown that KPI-APP transcripts are elevated relative to APP-695 in the CNS of aged rats with cognitive impairments. In the present study, the developing rat CNS was shown to contain the highest amounts of APP-695 mRNA relative to KPI-containing APP transcripts at postnatal day 15, paralleling the temporal expression of the low affinity NGF-R in the basal forebrain. Chronic infusion of human recombinant NGF into the striatum of adult rats resulted in an induction of APP-695 mRNA, reiterating the pattern of APP expression observed in the developing basal forebrain. These results demonstrate that trophic factors such as NGF can regulate APP mRNA splicing *in vivo*.

573.9

EXPRESSION OF THE C-TERMINAL 100 RESIDUES OF THE AMYLOID PRECURSOR PROTEIN IN CULTURED 293 CELLS. S.P. Little*[1], E. M. Johnstone*[1], K. Bales*[1], R. F. Santerre*[1], T. Oltersdorf*[2], N. W. Fox*[1] and H. U. Bryant[1]. [1]Eli Lilly and Company, Indianapolis, Indiana 46285; [2]Athena Neurosciences, Inc., San Francisco, CA 94080.

The expression of the C-terminal 100 (APP-C100) residues of the amyloid precursor protein (APP) may provide a mechanism for studying processing of APP into the 42-43 residue beta amyloid peptide (ßA4) implicated in Alzheimer's disease. Expression of human APP-C100 in mammalian cells reportedly causes "toxicity" and amyloid-like fibrils. We have expressed the APP-C100 in human embryonic kidney cells (293 cells) in a transient assay and compared it to the expression of transfected full length APP. Products were characterized by Western blot analysis using antibody to the carboxy-terminal region of APP-C100 and the amino-terminal region of ßA4. The APP-C100 produced in 293 cells is the same size as the fragment left in cells after protease nexin II cleavage from full length APP (Esch et al., Science 248:1122, 1990), and is only recognized by anti-carboxy terminal antibody and therefore may not contain the amino terminal amino acids of ßA4. Moreover, APP-C100 appears to be a substrate for "APP secretase" whether it is expressed with the natural APP signal peptide or as APP-C100.

573.10

Beta-AMYLOID PROTEIN POTENTIATES H_2O_2-INDUCED NEURON DEGENERATION IN VITRO. R.D. Saunders, C.A. Luttman*, P.T. Keith* and S.P. Little*. Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285.

In Alzheimer's disease, the accumulation of neuritic plaques and tangles has been associated with selective neuronal degeneration. The fact the beta-amyloid protein accumulates in these plaques suggests that it may be involved in neuronal degeneration, either by exerting a direct neurotoxicity on neurons or by making neurons more vulnerable to endogenous toxins. Free radicals and oxidants are believed to contribute to neuronal damage in various disease states. We tested the ability of beta-amyloid protein (beta1-43) to increase the vulnerability of cultured hippocampal neurons to H_2O_2-induced degeneration. Neurons were incubated with beta1-43 for 1 to 3 days before addition of H_2O_2. Neurons were incubated with 1, 10, 50, and 100 uM H_2O_2 for 15 min and then examined the next day for evidence of degeneration. In cultures that had not been preincubated with beta1-43, only 50 and 100 uM H_2O_2 induced significant neuronal degeneration. Preincubation with beta1-43 increased the susceptibility of the neurons to damage at 1 and 10 uM H_2O_2. The results suggest that beta-amyloid potentiates the toxicity that oxidants exert on neurons and therefore may increase the vulnerability of neurons to damage mediated by various endogenous toxins.

573.11

MECHANISMS OF NEUROTOXICITY OF CARBOXYTERMINAL FRAGMENTS OF THE ALZHEIMER AMYLOID PRECURSOR PROTEIN. A. Kammesheidt, C.F. Hohmann, P. Brandt, K.J. Ivins, A.F. Spanoyannis and R.L. Neve. Dept. of Psychobiology, U. of California, Irvine, CA 92717; Dept. of Psychiatry, Johns Hopkins Univ. Sch. of Medicine, Baltimore, MD 21205.

We have shown that the C-terminal 104 amino acids of the Alzheimer amyloid precursor protein (APP-C104) is toxic to neurons in vitro. To facilitate our studies of the neurotoxicity of this and other fragments of APP, we have attached a sequence termed FLAG (MDYKDDDDK) to the N-termini of the fragments. Fusion of FLAG to APP-C104 and to the beta/A4 fragment allows us to track the molecules as they exert their neurotoxic activity in vitro, so that we may study the mechanism by which they cause the degeneration of neuronal cells. Immunocytochemical analysis of SV-T2 fibroblasts stably transfected with FLAG-APP-C104 reveals localization of the recombinant protein predominantly on the surface of the cells. Conditioned medium from the FLAG-APP-C104 transfectants causes NGF-differentiated PC12 cells and primary cortical neuronal cultures to degenerate more rapidly than those exposed to APP-C104 conditioned medium. Purification of FLAG fusion proteins following their expression in bacteria will enable us to study their activity. We are also examining the neurotoxic effects of these APP fragments in vivo. Preliminary results indicate that transplantation of APP-C104 transfectants into mouse brain results in progressive cortical atrophy and neurodegeneration.

573.12

THE NEUROTOXIC CARBOXYTERMINAL FRAGMENT OF THE ALZHEIMER AMYLOID PRECURSOR BINDS SPECIFICALLY TO A NEURONAL CELL SURFACE MOLECULE. M.R. Kozlowski, A.F. Spanoyannis, S.P. Manly*, E. Hall, and R.L. Neve. Dept. of Screening and Biochemical Research, Bristol-Myers Squibb Company, Wallingford, CT 06492 and Dept. of Psychobiology, U. of California, Irvine, CA 92717.

Recent studies have implicated carboxyterminal fragments of the Alzheimer precursor protein (APP) in the processes of amyloidogenesis and neurodegeneration. In particular, the carboxyterminal 104 amino acids of APP (termed APP-C104) exhibits neurotoxic activity in vitro and in vivo. We hypothesized that APP-C104 may cause the degeneration of neurons by interacting with a cell surface receptor. In support of this hypothesis, we have shown that APP-C104 synthesized in vitro binds specifically and with high affinity to the surface of NGF-treated PC12 cells. Both the cell surface binding and the neurotoxicity of APP-C104 are pH dependent with peak activity at pH 7.0-7.2. Neither the binding nor the neurotoxicity is inhibited by tachykinins. Mutational analysis suggests that both the binding and the neurotoxicity are dependent at least in part on the presence of a tyrosine residue that is a potential site of phosphorylation at the carboxyterminus of the fragment.

573.13

PROPERTIES OF MEMBRANE BOUND AMYLOID β/A4 PEPTIDE HYDROLASE(APP-SECRETASE) ACTIVITIES. [1]T. Yamamoto, [2]F. Kametani* [1]T. Kato* and [3]D. Allsop*. [1]Lab. of Molecular Recognition, Yokohama City Univ. Yokohama 236,Japan, [2]Dept. of Molecular Biology, [3]Psychiatric Res. Inst. of Tokyo, Tokyo 156, Japan, and [3]Division of Biochemistry, The Queen's Univ. of Belfast, Northern Ireland, UK.

We have recently reported the binding of Alzheimer amyloid β/A4 peptide fragments to rat cortical membranes and the degradation of β/A4 peptide(Brain Res., in press). In this study, we further examined proteolytic cleavage of synthetic β/A4 peptide, 8-15, 8-17 and 8-28 using cerebral membranes from rats. The major degraded products of β/A4 8-17 identified to 8-14(13) and 8-15. We could find no peak corresponding to 8-16, suggesting that β/A4 8-17 was probably cleaved internally at Gln^{15}-Lys^{16}, not Lys^{16}-Leu^{17} by APP-secretase-like protease. 8-14(13) was also formed by other degrading enzyme which newly identified in this study. A wide range of protease inhibitors had little or no effect on these enzymes. EDTA completely inhibited these degradation and reversed by the addition of Ca^{2+} or Mn^{2+}. No degradation were observed of β/A4 8-15 and 8-28 as a substrate. These observations indicate that membrane-bound metaloendopeptidase(s) may act as APP-secretase to the membrane-spanning region of APP including the β/A4 protein, and speculate that the change of C-terminal side of β/A4 may affect on the conformation of protein toward inactive form to normal degrading protease(s).

573.14

EXPRESSION OF β-AMYLOID PEPTIDE mRNA IN TRANSGENIC MICE. K. R. Bales*, E. M. Johnstone*, S. P. Little*, P. E. Setler[1] and R. F. Santerre*. Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285 and [1]Athena Neurosciences, Inc., San Francisco, CA 94080.

Extracellular plaques and cerebrovascular deposits containing a 4-kilodalton beta-amyloid peptide (β/A4) are pathologic manifestations of Alzheimer's disease (AD). How full length amyloid precursor protein (APP) is processed yielding the shorter β/A4 peptide is unclear. Cleavage of APP within the β/A4 region occurs constitutively, suggesting that deficiencies within this pathway may contribute to β/A4 accumulation. To develop a rodent model of β/A4 deposition and to identify the role of β/A4 accumulation in AD, transgenic mice containing the plasmid pAMYminiAMY were produced. This minigene construction contains 2.9 kilobases (kB) of the human APP promoter, the genomic sequence of β/A4, including the 5.9 kB intron and a SV40 3' polyadenylation sequence. Six germline founder mice were identified and their hemizygous progeny were analyzed. Northern blot analysis of total RNA isolated from the fore- and hindbrain regions and testes showed significant expression of the transgene message. The level of endogenous APP transcript from testes of transgenic animals appeared to be suppressed. No β/A4 protein has been detected by immunoblotting techniques, suggesting that mice are capable of processing the human β/A4 peptide, perhaps constitutively.

573.15

LACTOFERRIN IMMUNOCYTOCHEMISTRY IN ALZHEIMER BRAIN TISSUE. T. Kawamata*, S.C. Sung and P.L. McGeer. Kinsmen Laboratory for Neurological Research, Univ. of British Columbia, Vancouver, B.C., V6T 1W5, Canada.

Lactoferrin (LF) is a protein of unknown function which appears in many secretory fluids of the body. It also occurs in Alzheimer Disease (AD) plaques and tangles (Osmand and Switzer, Neurobiol Aging 11:284, 1990). We studied its distribution in brain immunohistochemically, and confirm the report of Osmand and Switzer. However, we also found it to be present in many neurons of elderly brain, as well as in some astrocytes and microglia, but not in baby brain. At the EM level, LF immunoreactivity (IR) occurred primarily in lysosome-like particles, and was frequently associated with lipofuscin. It was also occasionally distributed diffusely in the cytoplasm. In AD, LF-IR was compared with that of Alz-50, ubiquitin, and Tau-2 to assess its association with neurofibrillary tangles and neuropil threads; with C3d, C4d and β-amyloid protein to assess its association with extracellular amyloid; with GFAP to assess its association with astrocytes; and with LCA and HLA-DR to assess its association with microglia. LF-IR was associated with almost all amyloid deposits, with reactive microglia in consolidated plaques, with a majority of extracellular neurofibrillary tangles, but with only a minority of intracellular neurofibrillary tangles and neuropil threads. These data suggest that lactoferrin may be closely associated with the development of amyloidosis in AD.

573.16

MICROGLIA PROCESS AMYLOID PRECURSOR PROTEIN SEGMENTS IN ALZHEIMER'S DISEASE. P.L. McGeer[1], T. Yamada*[1], T. Kawamata*[1], H. Akiyama*[2] and T. Ishii*[2]. [1]Kinsmen Lab, University of British Columbia, Vancouver, B.C., Canada and [2]Psychiatric Res Inst of Tokyo, Tokyo, Japan.

The immunohistochemical distribution of APP was studied in normal and Alzheimer Disease (AD) brain with antibodies to residues 527-540 (R36), 597-620 (R17, 1-24 of BAP) and 681-695 (R37) (Ishii et al., Neuropath Appl Neurobiol 15:135-147, 1989) at light and em levels. Antibodies to segments flanking BAP (R36 and R37) prominently stained dense membrane-bound granules in normal and AD pyramidal neurons, suggesting that APP is concentrated in an intraneuronal organelle and is not a cell surface protein. In Alzheimer disease (AD), R36 and R37 stained the cytoplasm of some dystrophic neurites but did not stain their surface membranes and only sparsely stained extracellular deposits. By contrast, the BAP antibody (R17) did not stain intraneuronal material, indicating inaccessability of the epitope, but did strongly stain extracellular deposits. All 3 antibodies prominently stained intramicroglial material. BAP deposits in microglial cells were seen communicating with extracellular BAP deposits. Since APP mRNa has not been found in microglia, these data suggest that microglia ingest a large carboxy terminal APP segment containing R36, R17 and R37 sequences. BAP may be primarily produced by breakdown of this segment and then be extruded into amyloid deposits where it accumulates.

573.17

WITHDRAWN

573.18

SHORT BIOTINYLATED OLIGONUCLEOTIDES BIND NON-SPECIFICALLY TO SENILE PLAQUE OF ALZHEIMER'S DISEASE. S.Syrjänen*, O.Heinonen*, H.Soininen, R.Miettinen*, K.Beyreuther[1], L.Paljärvi*, K.Syrjänen* and P. Riekkinen. Depts. of Neurology and Pathology*, Univ. of Kuopio, P.O.B.1627, SF-70211 Kuopio, Finland. Univ. of Heidelberg, Center for Molecular Biology[1], D-6900 Heidelberg, Germany.

We have used in situ hybridization with biotinylated oligonucleotide (antisense) probes and sreptavidin-biotinylated alkaline phosphatase method to detect the amount of amyloid β-protein mRNA in the paraffin embedded and formalin-fixed brain samples of patients affected by Alzheimer's disese and those of non-affected controls. Instead of the expected specific binding to neuronal cytoplasm, the probes did constantly bind to neuritic senile plaques, the strongest binding being in the hippocampus and the cerebral cortex. We have been able to constantly repeat this peculiar phenomenon with other oligonucleotide probes, e.g., the sense probes for β-amyloid protein and those specific for Human papillomavirus (HPV) 18- and c-erb B-2 mRNAs. An identical nonspecific binding of the oliconucleotides was seen also in one skin biopsy derived from a patient with Alzheimer's disease. Intrestingly, this particular skin biopsy presented with a basal cell carcinoma, a tumor frequently encountered in patients with Down's syndrome. Further experiments to correlate the staining pattern of oligonucleotide probes to accumulation of β-amyloid detected by immunohistochemistry are in progress.

573.19

POSITIVE UBIQUITIN IMMUNOREACTIVITY IN CEREBRAL AMYLOID ANGIOPATHY. F.F. Cruz-Sanchez*, C. Marin, I. Ferrer* and E. Tolosa. Neurological Tissue Bank, Service of Neurology, HCP and Dept. of Pathology, HPE, Universitat de Barcelona, SPAIN.

Vascular amyloid deposit, senile plaques (SP) and neurofibrillary tangles (NFT), are encountered in both cerebral amyloid angiopathy (CAA) and Alzheimer's disease (AD). Ubiquitin (UBQ) is a polypeptide related to ATP-dependent abnormal protein metabolism. Immunohistological studies using antibodies for UBQ have demonstrated similar antigenic determinants between (SP) in CAA and AD. In the present study, we describe immunohistochemical findings in the cerebral blood vessels of CAA compared to the AD ones. Formalin fixed paraffin embedded brain tissue from 4 AD and 4 cerebrovascular patients and 2 from elderly patients without neurological disorders were studied. Sections were stained for the polyclonal antibody for UBQ. Wall vessels in CAA cases showed strong UBQ-positivity in subendothelia and/or in the media layers. SP showed strong positivity with a negative central core immunoreactivity. AD brains showed immunoreactivity for UBQ in SP and NFT, but, vessels were negative. Control brains showed few positive SP and NFT and vessels were negative. These results may indicate that vascular amyloid deposit in CAA and AD might have different physiopathogenic mechanisms.

DEGENERATIVE DISEASE

574.1

6-HYDROXYDOPA SELECTIVELY DISPLACES [^{3}H]AMPA BINDING: A POTENTIAL MECHANISM FOR HUNTINGTON'S DISEASE. J. J. Cha, L. S. Dure IV, S. Y. Sakurai, J. B. Penney, and A. B. Young. Neuroscience Program and Department of Neurology, University of Michigan, Ann Arbor, MI 48104-1687.

Severe atrophy of the neostriatum is a main characteristic of Huntington's disease (HD). Intrastriatal lesions with excitatory amino acids (EAA) mimic certain features of HD leading to the hypothesis that EAA's play an etiologic role in HD. However, the EAA hypothesis of HD does not readily account for the striking involvement of the striatum with relative sparing of other structures which also participate in EAA circuitry.

6-HydroxyDOPA (6OH-DOPA), a derivative of the dopamine precursor L-DOPA, has been reported to have neurotoxic properties (Olney et al., **Exp. Neurol. 108**:269, 1990). 6OH-DOPA displaced rat brain [^{3}H]AMPA binding in an autoradiographic assay. Ineffective displacers of [^{3}H]AMPA binding included: dopamine, L-DOPA, D-DOPA, *threo*-DOPS, carbiDOPA, and DOPAC. 6OH-DOPA also displaced 20% of [^{3}H]kainate binding, but did not displace any NMDA-sensitive [^{3}H]glutamate, [^{3}H]MK-801, [^{3}H]SCH-23390 (D1 receptor), or [^{3}H]spiroperidol (D2 receptor) binding. The IC50 of 6OH-DOPA in displacing [^{3}H]AMPA binding was 31.7 µM, as potent as kainate in displacing [^{3}H]AMPA binding.

These data raise the possibility that 6OH-DOPA, or another abnormal L-DOPA metabolite, could act as an excitotoxic agent via action at AMPA receptors. If the genetic defect in HD resulted in production of 6OH-DOPA, neuronal death would be expected to occur in those structures with a coincidence of L-DOPA (as toxin precursor) and postsynaptic AMPA receptors (mediators of 6OH-DOPA-induced cell death), such as the striatum.

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574.2

NEUROPSYCHOLOGICAL CHANGES ASSOCIATED WITH CT SCAN MEASURES OF NEUROANATOMICAL ATROPHY IN HUNTINGTON'S DISEASE. J.K.A. Roberts, E.A. Loh, S.L. Sally* & E. Mohr*. Dept. of Neuropsychiatry, Royal Ottawa Hospital, Ottawa, Canada, K1Z 7K4. CT scan measures of basal ganglia regions of 15 patients at various stages in the progression of Huntington's disease (HD) were compared with patient's scores on a neuropsychological test battery. Indices of caudate atrophy: Frontal horn of ventricle width and Bicaudate distance were determined from measures taken from digitized CT scans. Lentiform region atrophy was assessed by measures of width of the third ventricle and distance between the third ventricle and insular regions. Cognitive tests were often as well correlated with atrophy of the lentiform regions as more traditionally used measures of caudate atrophy. Of particular interest, measures of attention and perseveration were highly correlated ($P<.05$) with the lentiform measures even after the effects of the choreiform movements were factored out. These data suggest that measurements of the lentiform regions provide additional information on the progress of HD.

574.3

A QUANTITATIVE LAMINAR ANALYSIS OF CEREBRAL CORTICAL DEGENERATION IN HUNTINGTON'S DISEASE. SM Hersch, V Rosenfeld*, C-A Gutekunst, HD Rees, RC Green. Department of Neurology, Emory University School of Medicine, Atlanta, Georgia 30322

The extent and significance of cerebral cortical atrophy occurring in Huntington's disease (HD) and its implications for understanding the symptoms and pathophysiology of the disease are of great interest. One-micron thick plastic sections, stained with toluidine blue, from defined regions of cerebral cortex from the brains of patients with Huntington's disease (HD) are being examined using a Macintosh computer based image analysis system for data acquisition and analysis. Right hemispheres from 10 patients, aged 45-69, with grade 3 or 4 HD were obtained from the Brain Tissue Resource Center of McLean Hospital. Age-matched control tissue was obtained from routine autopsies. Measurements are based on 250µm wide strips of cortex spanning its full depth and include the total area of each strip, the area contained in each layer, the total numbers of neurons, astrocytes, and oligodendrocytes in each layer, and the size of each neuron. These measurements are adjusted for shrinkage and then neuronal densities, glial densities, and volume densities are calculated. This study will survey primary, association, and limbic cortices. Detailed information about the relative involvement of different areas of cortex, different cortical layers, different neuronal types, and gliosis is being collected. Data from the analysis of frontal lobe regions will be presented. (Supported by the Huntington's Disease Society of America).

574.4

EVIDENCE FOR NEURONAL DEGENERATION IN THE SUBTHALAMIC NUCLEUS IN HUNTINGTON'S DISEASE. Karen M. Harrington, Robert J. Ferrante and Neil W. Kowall. Dept of Neuropathology and Experimental Neuropathology Laboratory, Massachusetts General Hospital, Boston MA 02114.

Recent anatomical, physiological, pharmacological and pathological evidence suggests that decreased excitation of the internal segment of the globus pallidus (GPi) by the subthalamic nucleus (STN) produces hyperkinetic movement disorders in man and primates. It has been further theorized that the hyperkinetic movement disorder in Huntington's disease (HD) results from selective depletion of inhibitory enkephalinergic striatal projections to the external segment of the globus pallidus (GPe) which in turn leads to pathological disinhibition of inhibitory projections to the STN and thus the physiological equivalent of a STN lesion. Because we did not find evidence of disproportionate depletion of enkephalinergic fibers in the GPe in HD, we theorized that the basis for the hyperkinetic movement disorder may lie in the STN itself. We therefore examined the STN for evidence of neuronal degeneration in HD utilizing a polyclonal antibody against ubiquitin, a sensitive marker for neuronal injury and stress response. In a series of HD cases (n=9) of various grades the maximal number of ubiquitin positive cells in the STN was 147±33 per mm^2. In normal age-matched STN (n=5) the mean maximal density of ubiquitin positive cells was 57±29 per mm^2 ($p<0.05$). Our findings suggest that the STN is affected in HD. The basis for the hyperkinetic movement in HD may, therefore, lie in abnormalities of the STN itself rather than in the striatum or pallidum.

574.5

UBIQUITIN IMMUNOREACTIVITY IS INCREASED IN HUNTINGTON'S DISEASE CEREBRAL CORTEX.

Alida J. Evans and Neil W. Kowall. Neurology Service, Massachusetts General Hospital, Boston MA 02114.

Neurons respond to injury by increasing the synthesis of endogenous stress proteins including ubiquitin which is conjugated to abnormal proteins destined for non-lysosomal proteolysis. In several neurodegenerative diseases including Alzheimer's disease, Parkinson's disease, and Amyotrophic Lateral Sclerosis, neurons contain ubiquitin immunoreactive inclusions. In Huntington's disease (HD), specific subsets of neurons degenerate in the cerebral cortex and striatum without evidence of specific cytopathological alterations by standard histological techniques. We examined the cerebral cortex of 12 HD patients using a polyclonal ubiquitin antibody to determine if abnormal ubiquitin-conjugated proteins are produced in response to neuronal stress or injury in HD. In all HD cases, variable amounts of dot-like immunoreactivity were found in the superficial cortical layers and white matter identical to that seen in controls. In both low and high grade HD, scattered, diffusely ubiquitin positive neurons were found within the cortex and infracortical white matter. In one high grade HD case with severe cortical changes, ubiquitin immunoreactivity was especially prominent within diffusely stained neurons, intracytoplasmic inclusions and axonal spheroids. The presence of ubiquitin immunoreactivity within a subset of neurons in HD cortex suggests that abnormal proteins may accumulate during the degenerative process. The nature of these proteins and their contribution to neuronal degeneration is unknown but their identification may clarify the pathogenesis of HD.

574.6

NEUROPATHOLOGY OF FRONTAL CEREBRAL CORTEX IN HUNTINGTON'S DISEASE. C.E. Peyser, M.V. Wagster, J.C. Hedreen, T.M. Dawson, M. Fotuhi, D. Bredt, S.H. Snyder, S.E. Folstein,* C.A. Ross. Psychiatry, Neuropathology, and Neuroscience Departments, Johns Hopkins, Baltimore, Md. 21205.

Huntington's disease (HD) results in massive degeneration of neurons in the basal ganglia, but neuronal loss in HD cerebral cortex has not yet been clearly established. We therefore counted neurons in individual laminae of the dorsal frontal cortex in 5 HD (Vonsattel grade IV) and 5 age matched control autopsy brains. Significant neuronal loss was found in layer VI (HD 117±29 vs control 207±32, or greater than 40% loss, $p<0.005$), and layer V (30%, $p<0.05$), and possibly layer III (13%, ns), but no change in layer II and IV. Neurons in layer VI project to thalamus, claustrum, and other regions of cerebral cortex, but not to the basal ganglia; thus their loss is unlikely to be the result of retrograde degeneration secondary to striatal pathology. We are currently performing autoradiography for glutamate receptor subtypes and immunocytochemistry for nitric oxide synthetase in order to explore possible neurochemical mechanisms underlying the neuronal loss.

574.7

EFFECTS OF CHRONIC STRIATAL LESIONS ON CORTICAL NEUROPEPTIDES. S. Garside, JCS Furtado and MF Mazurek. Department of Biomedical Sciences, McMaster University Medical Centre, Hamilton, Ontario, Canada.

We have previously reported widespread increases in CCK-LI as well as more localized changes in VIP-LI and NPY-LI in Huntington's disese (HD) postmortem cerebral cortex. It is not clear whether these differences are the result of abnormal feedback from the striatum or the result of independant cortical pathology. To investigate this issue we examined the effect of chronic striatal lesions on cortical peptide levels in the rat. Animals were given unilateral intrastriatal injections of QUIN (240nmols/µl) or saline (1µl) and sacrificed by decapitation 9-12 months later. Two 2mm coronal sections incorporating the lesion were sliced from each brain and photographed. The striatum was removed from the posterior slice and processed for neurochemistry. Striatal SP-LI in QUIN animals was decreased by at least 50% as compared with controls. The lesion size as assessed with Bioquant II encompassed at least 60% of the striatum. CCK, SS, NPY and VIP levels were studied in 8 regions of cerebral cortex. No significant changes were found between QUIN and saline animals.

These results suggest that the widespread neuropeptide changes in HD cerebral cortex are independant of the striatal pathology seen in HD.

574.8

RELATIVE SURVIVAL OF STRIATAL PROJECTION NEURONS AND INTERNEURONS AFTER INTRASTRIATAL INJECTION OF QUINOLINIC ACID. K.D. Anderson and A. Reiner. Dept. Anat. and Neurobiol., Univ. TN, Memphis.

Huntington's disease (HD) is characterized by extensive loss of striatal projection neurons with no evident loss of somatostatin/neuropeptide Y (SS/NPY)-containing or cholinergic interneurons. Several studies have reported that intrastriatal injection of the NMDA receptor-specific excitotoxin quinolinic acid (QA) induces a pattern of neuronal death in rats that mimics HD, favoring the hypothesis that such excitotoxicity is involved in the neurodegenerative process in HD. Other investigators, however, have reported that striatal SS/NPY-containing neurons are vulnerable to QA. We conducted immunohistochemical studies in rats 2 months after injection of 50 nmol QA to determine the relative survival of 4 types of neurons as a function of distance from the injection site: 1) striatopallidal neurons labeled for enkephalin (ENK); 2) striatonigral neurons labeled retrogradely by fluorogold (FG); 3) SS/NPY-containing neurons; and 4) cholinergic neurons. By quantifying the number of cells in 0.2-mm wide concentric zones at increasing distances from the injection site, we were able to assess the relative vulnerability of each type to QA. Within the injection site, cholinergic neurons were normal in abundance, but ENK+, FG+, and SS/NPY+ neurons were sparse. Moving away from the injection site, the abundance of the latter three returned to normal in the sequence: ENK+, FG+, SS/NPY+. Thus, SS/NPY+ interneurons are more vulnerable to QA than are striatal projection neurons. These results indicate that intrastriatal injection of QA does not induce a pattern of neuronal loss in rats that mimics HD, but the results do not necessarily refute the excitotoxin model of HD. NS-19620, NS-28721 (AR)

574.9

MULTINUCLEATED, TAU POSITIVE ASTROCYTES IN PROGRESSIVE SUPRANUCLEAR (PSP) BRAIN TISSUE. T. Yamada*, P.L. McGeer and E.G. McGeer. Kinsmen Lab., Dept of Psychiatry, Univ. of British Columbia, Vancouver, B.C., Canada, V6T 1W5.

We reported earlier on Tau-2 positive oligodendroglial microtubular masses (OMMs) (Yamada and McGeer, Neurosci Letts 120:163, 1990) in some neurodegenerative diseases. We now report the occurrence of significant numbers of Tau-2 positive astrocytes in PSP but not other neurological diseases. Three PSP, 10 Parkinson's disease, 2 striatonigral degeneration, 3 Huntington's chorea, 8 Alzheimer's disease and 5 non-neurological brains were examined immunohistochemically with the following antibodies: Alz-50, Tau-2, anti-PHF and anti-GFAP. Tuft fibers were identified within large glial cells, which surrounded a single large or smaller paired nuclei. They were positive for Tau-2, Alz-50 and anti-PHF. Double immunostaining with Alz-50 and anti-GFAP revealed that they were Alzheimer type I astrocytes. These astrocytes also stained positive with the Bielschowsky silver stain. They were predominantly localized in areas where a mild gliosis and occasional neurofibrillary tangles were seen such as the striatum, thalamus and frontal cortex. By EM paired nuclei without an obvious separating membrane could be seen. Alz-50 was localized to fiber bundles with a diameter of 25nm. These data suggest that there is active differentiation of astrocytes in PSP.

574.10

A SIMPLE METHOD FOR ASSESSING NEURONAL NUMBER IN THE HUMAN SUBSTANTIA NIGRA. C. Zarow and H.C.Chui. University of Southern California, Rancho Los Amigos Medical Center, Los Angeles, CA 90089-0191.

We report a simple method of assessing numbers of neurons in the substantia nigra pars compacta (SNpc). Formalin fixed, paraffin embedded blocks of the midbrain were obtained from 9 Parkinson disease (PD), 4 progressive supranuclear palsy (PSP), 17 Alzheimer disease (AD) and 13 age-matched control cases. Blocks were sectioned at 15 µm and stained with cresyl-violet. The number of pigmented cells per section was counted in every 25th section throughout the rostral-caudal length of the SNpc using a Quantimet 970 image analyzer (Cambridge Instruments). The mean number of neurons/section (N_{total}) was calculated for each case. N_{total} was significantly lower in PD (mean =89.2, sd =84.4) and PSP (mean =46.7, sd =17.8) compared to AD (mean =262.8, sd =151.9) and controls (mean =371.3, sd =152.7) (ANOVA, F=10.7). The mean number of neurons/section was also calculated from a subset of sections which contained the third cranial nerve (N_{CN3}), where it exits from the mesencephalon. There were no significant differences between N_{total} and N_{CN3} for any of the four diagnostic groups. Thus, the number of neurons/section in the region of the third nerve provides a reliable estimate of the mean number of neurons/section through the entire SNpc. This method is reliable even when the degree of neurodegeneration is severe. The exit of cranial nerve III (oculomotor nerve) from the brainstem can be identified easily by observation of the ventral surface of the brainstem. This affords an readily identifiable, externally visible, anatomic landmark. (Supported by NIA 1P50AG05142 and the Veterans Administration).

574.11

MOLECULAR GENETIC ANALYSIS OF THE X-LINKED DYSTONIA-PARKINSONISM SYNDROME (XDP). M.B. GRAEBER[1], K.G. KUPKE[1,2*], A.P. MONACO[3*], U. MÜLLER[1*]. Divisions of Genetics[1] and Newborn Medicine[2], Children's Hospital, Harvard Medical School, Boston, MA 02115; Institute of Molecular Medicine[3], John Radcliffe Hospital, Oxford University, Oxford OX3 9DU.

We have performed linkage analysis in 14 Filipino families with XDP, a severe, X-linked neurological disorder of high penetrance which is characterized by adult-onset dystonia and parkinsonism. Previously, the XDP-Locus (DYT3) has been assigned to Xq21 (Neurology 40:1438). Focusing on chromosomal region Xp11-Xq21, we studied 10 marker loci in DNA samples from a total of 94 family members, including 34 affected males. Highest LOD scores were 5.45, 4.52, 7.7, 4.4 for Loci DXS106, DXS159, DXS72, DXS367 at θ_{max}=0.00 and 4.28 for PGK1 at θ_{max}=0.01. Analysis of linkage and linkage disequilibrium suggests that DYT3 lies in proximity to DXS72 with DXS159 and DXS95 being flanking markers (Kupke et al., in preparation). Presently, we are isolating from yeast artificical chromosomes highly polymorphic, $(CA)_n$-based human DNA sequences close to DYT3. Updated lod scores and data obtained with these probes will be presented.

574.12

EFFECTS OF IBOTENIC ACID LESIONS OF THE STRIATUM ON DOPAMINE CELL NUMBER IN THE ADULT RAT SUBSTANTIA NIGRA. R.M.E. Chalmers[1] and A. Fine[2]. [1]Dept. of Anatomy and [2]Dept. of Physiology & Biophysics, Dalhousie Univ., Halifax, Nova Scotia, Canada. B3H 4H7.

Striatal derived factors may influence outgrowth and survival of tyrosine hydroxylase-immunoreactive (TH-IR) cells of the substantia nigra (SN) in vitro (Del Toso, et al. J. Neuroscience, 1988). To examine whether striatal trophic factors may influence continued survival of dopaminergic SN neurons in the adult, we lesioned the striatum in 250g rats by stereotaxic unilateral injection of ibotenic acid, and after six weeks determined the number of TH-IR cells in the SN. TH-IR cells were counted bilaterally in lesion as well as in control animals.

The SN ipsilateral to the lesioned striatum had a consistent 10 to 15% decrease in the number of TH-IR cells when compared to the contralateral SN while intact animals showed a side to side difference of less than 4%. The number of TH-IR cells in the contralateral SN of lesioned animals was comparable to the unlesioned animals (16-18 x 10^3 cells per SN).

The results suggest that the absence of a trophic factor normally supplied by the striatum, but decreased in our lesioned striatum, is necessary for the continued survival or maintenance of TH expression in a population of dopamine cells in the adult SN. (Sponsored by the MRC of Canada)

574.13

GENE MAPPING STUDIES IN FAMILIAL ALS. D.H.Donaldson, C. Nusbaum,* P.Sapp,* T.S.Barnes,* R.H.Brown,Jr,* and D. Patterson.* Dept. of Neurology, Univ. of Colorado Health Sciences Center, Denver, CO 80262; Eleanor Roosevelt Institute, Denver, CO 80206; Dept. of Neurology, Mass. General Hospital, Boston, MA 02129.

Multipoint linkage analysis of 23 pedigrees with familial ALS suggests that a gene important in the disease might lie in the region q22.1-22.2 of chromosome 21. Within this region lie the genes for superoxide dismutase, the trifunctional purine biosynthetic enzyme GART, the interferon α/β receptor, and the interferon γ response element. We report the production of new probes for this region prepared from $(CA)_n$-containing cosmids isolated from somatic cell hybrids that contain only this region of chromosome 21. We are constructing contig maps with these cosmids and YACs homologous to chromosome 21-specific DNA probes mapped to this region of 21, using somatic cell hybrids which allow dissection of this region of the chromosome into more than 10 parts.

574.14

ALTERATIONS IN DIHYDROPYRIDINE RECEPTOR BINDING KINETICS IN AMYOTROPHIC LATERAL SCLEROSIS (ALS) SKELETAL MUSCLE. R.G. Smith*, F. Kimura*, K. McKinley*, Y. Harati*, and S.H. Appel. Dept. of Neurology, Baylor Col. of Med., Houston, TX 77030.

Immunoglobulin from ALS patients alters the electrophysiological parameters of rat skeletal muscle dihydropyridine-sensitive Ca^{++} channels in vitro. To determine whether Ca^{++} channels in ALS muscle are altered, subsarcolemmal membrane fractions enriched for transverse tubules were prepared from muscle biopsy specimens taken from ALS, disease control, and normal control patients. These fractions were then assayed for the presence of dihydropyridine-sensitive voltage-dependent Ca^{++} channels (DHPR) with ^{3}H-PN-200/100. Results indicate an increased PN-200 binding affinity in ALS muscle (high affinity Kd $\pm$ S.D. = 0.17 $\pm$ 0.06 nM:n=26) compared to normal and myopathic controls (high affinity Kd $\pm$ S.D. = 0.92 $\pm$ 0.17 nM:n=10). In some ALS muscle specimens, there was also up to a 5-fold increase in the number of DHPR binding sites relative to controls, correlating crudely to the degree of denervation noted histologically. However, five non-ALS denervated muscle specimens (from one familial ALS, two spinal muscular atrophy, and two traumatically denervated control patients) yielded elevated Bmax values, but with DHPR Kd values in the control range. Conversely, specimens from four patients with autoimmune motor neuropathies had control range Bmax values, but Kd values indistinguishable from those of ALS muscle. This difference in ALS muscle DHPR Kd is not solely explainable by denervation state, and may reflect changes attributable to immunological alterations of Ca^{++} channels in vivo.

Supported by the Muscular Dystrophy Association.

574.15

GANGLIOSIDES MODIFY DIABETES-INDUCED CHANGES IN AXONAL ELEMENTAL COMPOSITION. J. Eichberg, C.M. Castiglia*, A.J. Saubermann and R.M. LoPachin. Dept. Biochem. and Biophys. Sci., Univ. Houston, Houston, TX 77204 and Dept Anesthesiology, Med. Sch., Stony Brook, NY 11794..

Ganglioside (GA) treatment corrects certain functional and biochemical deficits caused by experimental diabetes (DB). Previously, we demonstrated that intraaxonal concentrations of K and Cl were increased significantly in tibial nerve of streptozocin-DB rats. We have now assessed the ability of GA (mixed bovine brain) to modify DB-induced alterations of axonal elemental composition. X-ray microanalysis was used to measure concentrations of Na, P, S, Cl, K, Mg and Ca in tibial nerve axons. As reported earlier, DB rats had elevated axoplasmic K and Cl. In DB rats treated with GA (10 mg/kg/day x 30 days) axoplasmic contents (mmol/kg dry weight±SE) of K and Cl were increased relative to both DB and control (CON) levels (e.g. large axon K: CON=1668±51; DB=2131±80; DB-GA=2393±86), whereas axoplasmic Na was decreased (e.g. large axon Na: CON=237±12; DB=240±19; DB-GA=115±12). GA-control rats displayed changes in Na, K and Cl which were in the same direction as those of the DB-GA group, but smaller in magnitude (e.g. large axon K: CON-GA=1891±98; Na; CON-GA=164±18). DB-induced changes in axonal elemental composition might reflect compensatory mechanisms which are enhanced, either directly or indirectly, by GA treatment. Supported by NIH grants ES03830, NS21445 and DK30577.

574.16

ACETYL-L-CARNITINE COUNTERACTS EXPERIMENTALLY INDUCED DIABETIC NEUROPATHY **L. Pacifici*[1], A. Bellucci*[1], P. Piovesan*[1], F. Maccari*[1], A. Gorio[2], A.M. Di Giulio[2], M.T. Alderdice[3], L. Ferraris*[1]. [1]Sigma-Tau Institute for Research on Senescence, Pomezia, Rome, Italy; [2]Dept. Pharmacology, School of Medicine, Univ. of Milano, Italy; and [3]Sigma Tau Pharmaceuticals, Gaithersburg, MD 20878, USA.**

Acetyl-L-Carnitine (ALCAR) exerts trophic effects on neurons. Here we report that similar effects were also observed in Sprague-Dawley rats, 3 months of age, in which a chronic diabetic peripheral neuropathy was induced by a single injection of streptozotocin 50 mg/kg i.v. or alloxan 100 mg/kg s.c. By means of a radioimmunoassay, a significant reduction (30-50%) of Met-enkephalin and substance P in the gut of diabetic rats was found. Such a reduction was successfully prevented by ALCAR administration (for 12 consecutive weeks) at the dose of 50 mg/kg/day i.p. or 300 mg/kg/day in drinking water. Treatment with ALCAR also fully prevented both the reduction (20%) in neuromuscular conduction velocity, as recorded on the sciatic nerve soleus muscle preparation, and the decrease (30%) in muscle contraction force. ALCAR also improved the concomitantly reduced motor performance on Rota-Rod test. These data suggest for a potential therapeutic use of ALCAR in the treatment of diabetic peripheral neuropathy and further confirm ALCAR capability to ameliorate behavioral and morphological aspects of degenerative and age-dependent disorders.

574.17

THE EFFECT OF DNA METHYLATION ON THE EXPRESSION OF BC-200, A PUTATIVE GENE-REGULATORY ELEMENT IN NEURONS. J.H. Uhm*, N.P. Dooley, J. Nalbantoglu*, H.D. Durham, N.R. Cashman. Montréal Neurological Institute, Montréal, Québec, CANADA, H3A 2B4.

We have hypothesized that selected neurodegenerative diseases are due to altered DNA methylation in neurons with aging (Schwob *et al*, Ann Neurol 1990). Neuron-specific gene expression in various species may be regulated in part by repetitive DNA elements known as ID (identifier) sequences, which are transcribed by Pol III predominantly in brain; the primate ID element is BC-200 (Watson & Sutcliffe, Mol Cell Biol 1987). BC-200 is also expressed in cultured cells representing a variety of lineages. We have investigated the effect of agents that influence DNA methylation on BC-200 expression in four human cell types: two neural cell lines (H4, SK-N-SH), and two non-neural cells (HeLa, primary skin fibroblasts). Cells were grown in medium containing 5-azacytidine (AZA, 3 μM), which inhibits CpG maintenance methylase, or in methylazoxymethanol (MAM, 50 μM), a carcinogenic methylating agent found in the neurotoxic Cycad nut of Guam. Total RNA extracted by guanidinium-thiocyanate-phenol-chloroform was Northern-blotted with ^{32}P-labeled probes for BC-200, 18S RNA, and actin; the latter two RNAs control for lane loading. Autoradiographs were quantitated by densitometry. BC-200 expression was detected in all four cell types, but was not apparently affected by treatment with AZA; this may reflect hypomethylation of this element prior to AZA exposure. In contrast, MAM decreased expression of BC-200 by 33-43% in neural cell lines (H4, SK-N-SH), but not in non-neural cultures. Our data suggest that abnormal DNA methylation can alter BC-200 expression preferentially in neural tissue.

574.18

EXPRESSION OF WILD TYPE AND MUTANT HUMAN PRION PROTEINS BY RECOMBINANT BACULOVIRUS INFECTED INSECT CELLS. D. Goldgaber, R. Bhasin, E.A. Barnes* and J. Safar*. Dept. of Psychiatry, SUNY, Stony Brook, NY 11794; Laboratory of Central Nervous System Studies, National Institutes of Neurological Disorders and Stroke, National Institutes of Health.

A host protein encoded by the gene specifying the human scrapie amyloid precursor protein (Prion Protein, PrP^{CJD}, PRIP gene) is linked with the development, and apparently affects the pathogenesis of the transmissible spongiform encephalopathies: Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker's syndrome (GSS) and Kuru. At least four point mutations of the human PRIP gene are known to be associated with the occurrence of CJD and GSS. The wild type human PRIP gene and two point mutants, one at codon 102 (Pro to Leu) and one at codon 200 (Glu to Lys) have been expressed in the baculovirus expression vector system. The recombinant proteins were detected in recombinant baculovirus infected insect cells two days post-infection. No PrP proteins were detected in the culture medium of infected cells indicating that the proteins were not secreted. The cell associated recombinant proteins migrated as a triplet of bands with apparent Mr 29-30, 27-28 and 25-26 kDa. The apparent Mr of the highest Mr band was 3-5 kDa lower than the human PrP^{CJD} protein isolated from brains of patients with CJD. Studies on the subcellular distribution and hydrophobicity of the recombinant proteins indicated similar behavior to proteins isolated from human brains. The recombinant proteins produced by the baculovirus expression system provide an abundant source of prion proteins which can be used for comparative study and determination of the role of point mutations in the infectivity of prion proteins.

574.19

A CHICKEN PRION-LIKE PROTEIN UNDERGOES TWO POST-TRANSLATIONAL CLEAVAGES. D.A. Harris. Dept. of Cell Biology & Physiology, Washington Univ. School of Medicine, St. Louis, Missouri 63110.

We have previously identified a chicken prion-like protein (ch-PLP) as the major sequenceable protein in purified preparations of an acetylcholine receptor-inducing activity (ARIA) from brain (D.A. Harris, et al., Soc. Neurosci. Abstr., 15:164, 1989). We have prepared stably transfected lines of mouse neuroblastoma (N2a) cells that express recombinant ch-PLP detectable by immunoblotting and immunoprecipitation. A 45kD form of ch-PLP can be released from intact cells by incubation with bacterial phosphoinositol-specific phospholipase C (PIPLC), suggesting that the protein is anchored to the cell surface by a glycosyl-phosphatidylinositol (GPI) linkage. Two forms of ch-PLP having molecular weights of 33-43kD and 10kD are also released into the extracellular medium in the absence of added enzyme. The 10kD fragment is derived from the N-terminal half of the molecule since it reacts with an antibody raised against a synthetic peptide corresponding to pro^{47}-pro^{65} of the ch-PLP sequence. This fragment can be metabolically labeled with ^{3}H-proline but not with ^{35}S-methionine, suggesting that it probably does not extend beyond met^{137}. Ch-PLP is also detectable in a 100,000xg membrane fraction of chicken brain, from which a 45kD form can be released by PIPLC. 33-43kD and 10kD forms are also detectable in a 100,000xg supernatant fraction of brain. We propose that the the ch-PLP molecule undergoes at least two posttranslational cleavages: one within or adjacent to the GPI anchor that releases the 33-43kD form, and a second near the middle of the protein that generates the 10kD fragment. The role of these cleavages in the deposition of amyloid in prion diseases is under investigation.

574.20

Ca^{2+}-INDUCED SPONGIFORM VACUOLATION IN SQUID GIANT NERVE FIBERS: A MODEL OF NEURODEGENERATIVE PROCESSES. H.M. Fishman, J. Metuzals* and K.P. Tewari. Univ of Texas Medical Branch, Galveston, *Univ of Ottawa, Ottawa, Canada.

Extensive vacuolation as observed by light and electron microscopy in stressed, isolated giant axons and surrounding Schwann cells of squid resembles spongiform changes seen in tissues of patients with neurodegenerative diseases (ND). Internal dialysis of unstressed axons with dialysates containing Ca^{2+} (100 μM to 10 mM) induced vacuolation similar to that observed in stressed axon preparations with Ca^{2+} externally. These results suggest that neurodegeneration is mediated via a common mode of action involving intracellular Ca^{2+} regulative processes. Membranes involved in Ca^{2+}-induced spongiform changes form a layer as a peripheral portion in fibers and originate from axolemma and endoplasmic reticulum (ER) as well as from adjoining plasmalemma and ER of Schwann cells. The membranes of vacuoles demonstrate regions of membrane damage, especially axolemma. Modified cytoskeletal filaments (10 to 20 nm diameter) are associated with these damaged regions. The large size of the squid giant nerve fibers and the relative ease with which morphological, biochemical and physiological methods are applied and integrated make giant axons of squid a unique preparation in which to study processes associated with degeneration. Support: Office of Naval Research, Medical Research Council of Canada and American Health Assistance Foundation.

574.21

REDUCTION OF KAINATE-INDUCED SPECTRIN BREAKDOWN BY CALPAIN INHIBITORS. [1]D. Eveleth, [1]J. Estrada*, [2]S.J. Mennerick, [1]D. Lutz, [1]R. Dean, [3]J. Powers and [1]R. T. Bartus. [1]Cortex Pharmaceuticals, Irvine, CA 92718, [2]Washington Univ., St. Louis, MO 63110, [3]Georgia Institute of Technology, Atlanta, GA 30332.

Excitotoxicity is associated with many neuropathologies, including ischemia, stroke, and epilepsy. The mechanism of excitotoxicity is thought to involve elevations of intracellular calcium, which in turn activates a variety of potentially damaging enzymatic events. One such event is the activation of the calcium-dependent protease calpain, which degrades intracellular proteins including the cytoskeletal component spectrin.

Systemic administration of the excitotoxic glutamate analog kainate to rats produces an acute phase of convulsions and seizures followed by extensive cell death in the hippocampus and other brain regions. Cell death is dependent upon the induction of seizures, manifested externally by convulsions, in individual animals. The breakdown of spectrin correlates well with the extent of cell death observed histologically. Administration of Calpain Inhibitor 1 (Ac-Leu-Leu-Nle-H), a potent inhibitor of calpain, reduced the breakdown of spectrin measured 4 days after administration of kainate. The inhibitor also reduced the extent of convulsions associated with kainate toxicity.

These data argue that Calpain Inhibitor 1 interferes with the cascade of events leading to cell death and neurodegeneration following kainate toxicity. Calpain activation, which is reduced by the inhibitor, may be necessary for the initiation or maintenance of the cascade of events leading to cell death. Calpain inhibitors may thus represent a useful therapeutic approach to stroke and other forms of glutamate-related neurodegeneration.

574.22

OVERCOMING CONDUCTION BLOCK IN A CENTRAL DEMYELINATING LESION BY COOLING, BUT NOT BY 4-AMINOPYRIDINE (4-AP). P.A. Felts[1], K.J. Smith[2] and F.J. Liuzzi[1], Dept. of Anat. and Neurobiol., Eastern Va. Med. Sch., Norfolk, Virginia[1] and Depts. of Neurol. and Anat., UMDS, Guy's Campus, London[2].

The symptoms of multiple sclerosis (MS) can sometimes be relieved by a reduction in body temperature, or by the administration of 4-AP, a potassium channel blocking agent. The improvement in symptoms has been attributed to a restoration of conduction to central axons in which conduction has been blocked by demyelination. To examine this possibility in more detail, focal, unilateral demyelinating lesions were produced in the dorsal columns of fifteen adult rats by the intraspinal injection of ethidium bromide under general anesthesia. Two chronically prepared animals were then examined at multiple time points, and the remaining animals were examined acutely. During the period of demyelination (2-6 weeks post-injection) a reduction of lesion temperature from 38°C to 33°C increased the number of axons conducting through the lesion by up to approximately 280%. The newly conducting axons had the long latency expected of demyelinated axons. However, in the same preparations the administration of 4-AP, in a dose sufficient to have caused limb tremors in preliminary experiments with lightly anesthetized animals (1.7-3.4mg/kg, i.v.), had little or no effect on the number of conducting fibers.

In view of these findings, we suggest that the restoration of conduction to blocked axons contributes to the improvement in symptoms observed with body cooling. However, it seems that the improvement caused by 4-AP may result primarily from other effects, perhaps by the facilitation of transmission at central and neuromuscular synapses, thereby augmenting the action of pathways which are already functional. *Supported in part by grants from the NINDS and the National Multiple Sclerosis Society.*

574.23

ATTENUATION OF EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS (EAE) BY THE 21-AMINOSTEROID TIRILAZAD MESYLATE (U-74006F). S.J. Karlik*, E. Grant*, C. Norley* and E.D. Hall. University of Western Ontario, London, Ontario, and The Upjohn Company, Kalamazoo, MI 49001.

In EAE, a model of multiple sclerosis, there is an autoimmune attack on the CNS causing demyelination, blood-brain-barrier (BBB) disruption, edema, and neurological dysfunction. Macrophage-derived free radicals and lipid peroxidation (LP) have been postulated to play a role in EAE. Thus, we tested the protective efficacy of the LP inhibitor U-74006F in a guinea pig EAE model. Vehicle or U-74006F (1, 3, or 10 mg/kg) was administered i.p once daily from days 0-12 after innoculation with whole CNS tissue in Freund's adjuvant. Disease was evaluated by a clinical grading scale and by magnetic resonance imaging (MRI). Gadolinium MRI enhancement was used to determine BBB compromise. U-74006F reduced the incidence of clinical symptoms from 81% in the untreated animals to 44 and 56% with 3 and 10 mg/kg, respectively. The maximum MRI scores were significantly decreased, and the incidence of gadolinium-enhanced scans (BBB opening) was attenuated from 63% to 36-44%. These results support a role of LP in the pathogenesis of EAE.

574.24

EFFECT OF GM_1 ON EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS IN FEMALE LEWIS RATS E.L. Orr and N.C. Stanley Dept. of Anatomy & Cell Biology. Texas College of Osteopathic Medicine. Ft. Worth, TX 76107.

Experimental autoimmune encephalomyelitis (EAE) is a model for CNS associated autoimmune disease (eg., multiple sclerosis). Fluctuations in clinical signs of EAE parallel changes in histamine in certain regions of the CNS. Agents known to alter histamine levels or release from neurons or CNS-associated mast cells attenuate the course and severity of EAE. Since the ganglioside GM1 has been reported to inhibit histamine release from basophils and mast cells, we investigated the effect of GM1 on the clinical course and severity of EAE. In all experiments adult female Lewis rats were inoculated to induce EAE and assessed for clinical symptoms beginning on day 7 postinoculation (pi). Parameters assessed were: day of onset of clinically significant disease (score of 4+), peak clinical score, day the peak score occurred, and duration of clinically significant disease (number of days at a score of 4+). In our initial experiments, the effect of intraperitoneal injections of GM1 (30mg/kg/day) or equal volumes of saline from days 6 pi through day 10 pi on the clinical course of acute (experiment 1) and recurrent (experiment 2) EAE were investigated. Compared to Controls, only a slightly shorter duration of acute EAE was observed in GM1 treated rats and no effect was observed in the clinical course or severity of recurrent EAE. The last experiment assessed the effect of prolonged, continuous administration of GM1 or saline (delivered subcutaneously using Alzet minipumps) on acute EAE. Pumps were implanted on day 6 pi and delivered an average daily dose of 30 mg GM1/kg for 25 days (i.e., through day 31 pi). Again, no significant differences between experimental or control rats were observed for any of the clinical parameters assessed. We conclude that GM1 has no effect on the clinical course or severity of EAE in female Lewis rats.

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SCHIZOPHRENIA

575.1

DELAYED PHENCYCLIDINE-INDUCED ALTERATIONS IN LOCAL CEREBRAL GLUCOSE UTILIZATION IN RAT BRAIN. X.M. Gao, O. Shirakawa, C.A. Tamminga. Maryland Psychiatric Research Center, University of Maryland at Baltimore, Baltimore, MD. 21228

Phencyclidine (PCP) is a psychotomimetic drug which produces psychotic symptoms in humans, closely resembling clinical manifestation in schizophrenia. PCP has been observed to produce delayed, reversible intracellular vacuolizations after a single dose (Olney Science 244:1990). Because this action might be related to the long lasting psychotic manifestations of PCP, we studied the delayed regional metabolic alterations in rat brains following the administration of PCP, using glucose autoradiography. PCP (8.6 mg/kg) was administered 3,6,and 12 hours prior to the 14C-2DG experiment. At 6 hours intracellular vacuolizations were observed with PCP on thin light microscopic sections in the posterior cingulate cortex, hippocampus, and subiculum. The rCMRglu was decreased selectively in parallel limbic regions with vacuolizations (e.g. posterior cingulate, retrosplenial cortex, amygdala, ventral tegmental area)($p<0.05$), and, in addition, in auditory regions ($p<0.001$) and in visual cortex ($p<0.05$). However, no metabolic changes remained either in the neocortex or in the extrapyramidal areas, and vacuolizations were not present. Glucose metabolism was decreased in the lateral habenula, an action opposite to that produced by antipsychotic drugs. The overall distribution of altered glucose utilization observed in this study, is distinct from the acute actions of PCP (Tamminga et.al. Synapse 1:1987) in its restricted limbic effects. This may be a more precise animal model for psychosis because of the extended time course of the psychotomimetic action of PCP. Moreover, we have previously observed an altered distribution of regional metabolism in schizophrenia precisely in limbic areas, similar to the effects of delayed PCP.

575.2

CEREBRAL METABOLIC ACTIVITY AND ABNORMAL EYE MOVEMENTS IN SCHIZOPHRENIC PATIENTS. D.E. Ross*, G.K. Thaker, H.H. Holcomb, N.G. Cascella, P.Morris*, S. Cassady, C.A. Tamminga. Maryland Psychiatric Research Center, University of Maryland at Baltimore, Baltimore, MD 21228.

Schizophrenic patients have abnormal smooth pursuit and saccadic eye movements, and it is likely that these defects are associated with abnormalities in supra-brain stem oculomotor areas. We hypothesize that these abnormal eye movements are associated with abnormal metabolism in the frontal eye fields (FEFs) and/or supplementary motor area (SMA).

To test this hypothesis, we examined regional cerebral metabolic rates of glucose utilization (rCMRglu) with PET/FDG in these areas and correlated them with measures of eye movements in schizophrenic patients (N=13). For the analysis of PET images, regions of interest for the FEFs and SMA were defined by stereotactic atlas coordinates reported by Fox et al. (J. Neurophysiol. 54: 348-369, 1985). 12 of the 13 subjects had an MRI scan of the brain which was used to determine the PET slices of interest. Eye movements were tested with infrared oculography, and tasks included smooth pursuit and an antisaccade paradigm. One measure of the latter is distractibility, which is defined as the percentage of trials in which the patient makes a reflexive saccade inappropriately toward the target. The results show that saccadic eye movement distractibility correlates with decreased metabolism in the frontal eye fields ($r=0.58$, $p=0.05$) and supplementary motor area ($r=0.64$, $p=0.02$). Distractibility did not correlate significantly with metabolism in non-oculomotor areas. Other oculomotor measures did not correlate significantly with metabolic values. These preliminary results may indicate that increased saccadic eye movement distractibility in schizophrenic patients results from a failure of FEFs and SMA to inhibit lower centers of oculomotor control.

575.3

HIPPOCAMPAL EFFERENTS TO THE NUCLEUS ACCUMBENS MODULATE SENSORIMOTOR GATING OF STARTLE IN THE RAT. S.B. Caine, M.A. Geyer and N.R. Swerdlow*. Departments of Psychiatry and Neuroscience, UCSD School of Medicine, La Jolla, CA 92093-0804.

"Prepulse inhibition" (PPI) occurs when the startle response to a startling stimulus is reduced by the prior presentation of a weak lead stimulus. PPI is an operational measure of sensorimotor gating that can be studied in both humans and rats. Schizophrenic patients exhibit deficits in PPI. In the rat, infusion of the cholinergic agonist carbachol into the dentate gyrus (DG), but not into the neocortex, disrupts PPI. Because the nucleus accumbens (NAC) is an important neural substrate of PPI, the present study examined the modulation of PPI by hippocampal efferents to the NAC. Male Sprague-Dawley rats were surgically equipped with bilateral steel cannulae aimed above the DG, ventral subiculum (VS) or CA1 of the hippocampus. Starting a week later, carbachol was infused into each site and startle amplitude was measured following 50 presentations of a startle pulse (118 db[A]) either alone or 60, 120 or 500 msec after a weak prepulse (80 dB[A]). The normal inhibition of startle by the prepulse was reversed by carbachol dose dependently (0.1,0.2,0.4 ug/side) in the DG, VS and CA1. The magnitude of the disruption of PPI was CA1>DG>VS, ventral hippocampal infusions having a longer duration of action than dorsal hippocampal infusions. Coinfusion of atropine (0.4 ug/side) restored PPI, indicating that carbachol disrupted PPI via a receptor-mediated mechanism. In a separate experiment, infusion of atropine alone (0.2,0.4,0.8 ug/side) failed to alter PPI.

Other studies in our laboratory indicate that the ventral pallidum and pedunculopontine nucleus are also important neural substrates of PPI. The results presented here suggest that the hippocampus may modulate sensorimotor gating through efferents to the NAC- ventral pallidal- pedunculopontine circuitry, and that pathology within the hippocampus may play a role in the deficits in sensorimotor gating observed in schizophrenic patients.

575.4

CHRONIC HALOPERIDOL AND CLOZAPINE TREATMENT AT THE CENTRAL AMYGDALA NUCLEUS. M.C. Schiess. Dept. Pharmacology, Univ. Texas Med. Branch, Galveston, TX 77550

The dopaminergic cell bodies of the ventral tegmental area (A_{10}) project to the central nucleus of the amygdala. Hyperactivity of the dopaminergic system at this level may be responsible for the dysfunctional emotional symptomatology of schizophrenia. *In vitro* intracellular recording from the amygdala slice was used to assess the effects of chronic neuroleptic treatment. At 25 weeks of 1 mg/kg/ day s.c. haloperidol, all rats displayed vacuous chewing movements, catalepsy and stereotypy. After six weeks chronic treatment with clozapine at 25 mg/kg/day i.p. all rats displayed passivity and apathy. Haloperidol and clozapine did not produce significant changes from control in passive membrane properties; depolarization blockade was not induced. Haloperidol produced a heightened response to superfusion with muscarine and quisqualate. Clozapine dramatically altered the central nucleus response to dopamine (1 μM) superfusion; producing membrane depolarization, an increase in spontaneous firing, a blockade of the s-AHP and a broadening of the action potential. These data suggest that clozapine's antipsychotic efficacy may be mediated by a modulation of dopaminergic transmission at the amygdala level and that haloperidol's antipsychotic efficacy may be mediated by a modulation in excitatory transmission. (Supported by Scottish Rite Schizophrenia Research Program)

575.5

DOPAMINERGIC DYSFUNCTION IN SCHIZOTYPAL PERSONALITY DISORDER. Larry J. Siever MD, Farooq Amin MD*, Oren Kalus MD*, Robert Trestman MD*, Emil Coccaro MD*, Ren-Kui Yang PhD*, Peter Knott PhD, Kenneth L. Davis MD. Dept. of Psychiatry, Mt. Sinai School of Medicine, NYC, NY 10029

In order to evaluate dopaminergic activity, suggested to be associated with psychosis in schizophrenia, in patients with schizotypal personality disorder (SPD), who have attenuated symptoms related to schizophrenia, plasma and cerebrospinal fluid (CSF) concentrations of HVA were determined by HPLC in SPD patients (n=21) and controls (19 other personality disorder [OPD] patients and 14 normal controls) in the AM after an overnight fast and 3 days of low monoamine diet. An overlapping group of patients were evaluated by CT-scan and neuropsychologic testing utilizing the Wisconsin Card Sort (WCST) and Trails B in order to evaluate pre-frontal function. Both plasma and CSF HVA concentrations were significantly increased in the SPD patients compared to OPD patients and normal controls ($p<0.05$), and both were correlated with the number of psychotic-like symptoms ($p<0.01$). Both frontal horn and lateral ventricular-brain-ratio were increased in the SPD patients compared to the two comparison groups ($p<0.05$) and lateral VBR was negatively correlated with psychotic-like symptoms ($p<0.05$). Plasma HVA was correlated negatively with frontal horn size and both WCST perseverative errors (increased in SPD) and Trails B performance ($p<0.05$). These results suggest a bidirectional relationship between dopamine activity and pathophysiology of SPD with increased dopaminergic function associated with the psychotic-like symptoms of SPD.

575.6

THE USE OF MAGNETIC RESONANCE IMAGING (MRI) TO QUANTIFY CORTICAL CEREBROSPINAL FLUID: A COMPARISON OF THREE METHODOLOGIES. M.L. Kesler, D.S. O'Leary, G.A. Cohen*, O. Stibolt*, and N.C. Andreasen. NIMH-CRC, Psychiatry Department, University of Iowa, Iowa City, IOWA 52242.

In a preliminary study by our group (see O'Leary *et al.*, this conference), 54 male patients with schizophrenia were found to have significantly more cortical CSF than 33 male controls in frontal and temporal regions. A visual rating method was used to rank MRI scans by the amount of cortical CSF seen within the subarachnoid space.

To validate the visual rating method and to begin to understand the etiology of increased cortical CSF, two computerized methods were used on coronal MRI slices (forward of the anterior horns of the lateral ventricles). Using image analysis software developed in our laboratory, inner cranial and cerebral surfaces were semi-automatically traced, and the surfaces were used to define three-dimensional volumes. Ratios of peripheral CSF volume to total cranial volume were calculated and were found to be significantly correlated with the visual ratings for the same subjects ($n=29$, $r=.70$, $p<.001$). Additional tracing data suggests that the increased cortical CSF results, in part, from sulcal widening.

A segmentation algorithm was used to classify MRI voxel intensities as gray matter, white matter, or CSF for each subject's series of frontal lobe slices. Ratios of the number of voxels classified as CSF to the total number of voxels classified were calculated. These ratios were found to be significantly correlated with the visual ratings ($n=15$, $r=.57$, $p<.05$) and with the tracing ratios ($n=16$, $r=.67$, $p<.01$). Gray matter and white matter volume changes which correlate with increased cortical CSF are being explored.

The comparison of three different methodologies used to evaluate MRI scans serves to validate each, and each may prove useful in generating hypotheses concerning brain changes in schizophrenia and schizophrenic subtypes.

575.7

DECREASED CRANIAL AND CEREBRAL VOLUME IN SCHIZOPHRENIA: A MAGNETIC RESONANCE IMAGING STUDY. M.A. Flaum*, D.S. O'Leary, V.W. Swayze II*, R. Alliger*, O. Stibolt* and N.C. Andreasen. Mental Health Clinical Research Center, University of Iowa, Iowa City, IA 52242.

Objectives: To assess volumes of various brain structures using MRI in a group of schizophrenics as compared with healthy control subjects.

Methods: The groups were selected so as to be highly comparable for the following variables: age, gender (all male), race and parental socioeconomic status. All patients (N=54) met DSM-III-R criteria for schizophrenia, and had no concurrent medical illness or history of substance abuse. Controls (N=33) were recruited from the community and were in good medical and psychiatric health. All subjects were scanned on a 1.5 Tesla GE Signa MR scanner, with 5 mm coronal slices and a 2.5mm gap. All tracings were performed by a single rater, blind to diagnosis, using a semi-automated method. Intra-rater reliability was consistently high, and inter-rater reliability was acceptable (kappa >0.70) for all structures except for the amygdala and thalamus. Subarachnoid CSF volume was estimated based on subjective ratings using a 0-5 scale.

Results: Using two tailed t-tests, schizophrenics were found to have significantly smaller cranial volume, right and left cerebral volume, and right and left thalamic volume than controls. Ratings of subarachnoid CSF were higher in the schizophrenic sample. Third ventricle volume was significantly larger in the schizophrenic group. All of these differences remained significant ($p<0.05$) after cranial volume and age were covaried. There were no significant differences in the volumes of the following structures: lateral ventricles, caudate, putamen, globus pallidus, hippocampus, amygdala or cerebellum.

Conclusions: The finding of smaller cranial and cerebral volume in schizophrenia was replicated, even after carefully controlling for socioeconomic factors, suggesting an early developmental defect. The findings of smaller cerebral volumes and increased subarachnoid CSF, even after cranial volume is covaried, suggest that an atrophic process may also be considered.

575.8

COGNITIVE FUNCTION AND CEREBRAL SPINAL FLUID MEASURES IN SCHIZOPHRENICS AND NORMALS: A MAGNETIC RESONANCE IMAGERY STUDY. D.S. O'Leary, M.L. Kesler, M.A.Flaum*,O. Stibolt*, & N.C. Andreasen. Mental Health Clinical Research Center, University of Iowa, Iowa City, IA 52242.

The relationships of cognitive functions and putative measures of cerebral atrophy were studied in schizophrenic and normal males 50 years of age or younger. Visual ratings of cerebral spinal fluid (CSF) surrounding Frontal, Parietal/Occipital, and Temporal lobes were made by two raters blind to diagnosis on coronal MRI films. Volumes of ventricles were obtained using semi-automated tracings (see Flaum et al., this conference). Neuropsychological tests were administered to obtain summary scores for IQ and other cognitive domains. Schizophrenics (n=54) met DSM-III-R criteria and had no concurrent medical illness or history of substance abuse. Controls (n=33) were recruited from the community.

Schizophrenics had significantly higher ratings of CSF surrounding the Frontal and Temporal lobes and had significantly larger Third Ventricular volumes than normals. These findings remained when age was covaried. IQ showed no relationship to any CSF measure. In both normals and patients, significant correlations were found between CSF measures and long- and short-term verbal memory, visual memory, and attentional measures. The effects of age differed in schizophrenics and normals but significant correlations remained in both groups when age effects were controlled. Future studies will investigate whether increased CSF reflects changes in gray or white matter using semiautomated segmentation techniques (see Kesler *et al.* this conference for discussion of computer methods of MRI analysis).

575.9

ALPHA$_2$ ADRENERGIC BLOCKADE WITH YOHIMBINE DISRUPTS AUDITORY SENSORY GATING IN RATS. K.E. Stevens, J. Meltzer, S.L. Stryker* and G.M. Rose. Department of Pharmacology, UCHSC and Medical Research, VAMC, Denver, CO 80262

In unmedicated rats and normal humans, midlatency auditory evoked potentials recorded in response to the second of a closely-spaced pair of clicks are reduced compared to the first. Amphetamine treatment of rats or humans disrupts this "gating" in a manner similar to that seen in schizophrenics. Animals studies using selective antagonists have implicated alterations in both noradrenergic and dopaminergic neural transmission in this phenomenon. To more directly assess the role of noradrenergic transmission in impaired sensory gating, the presynaptic alpha$_2$ adrenergic antagonist, yohimbine, was administered. Several doses were injected, i.p., and the effect on gating of the N40 waveform was determined at various times post injection. At a dose of 1 mg/kg, both 20 and 45 minutes post injection, the ratio of the second to the first response was elevated compared to controls. This disruption was achieved by both a decrease in the response to the first click and an elevation of the response to the second. To determine if dopaminergic blockade could effect this alteration in gating, the selective D1 antagonist, SCH23390, was co-administered with yohimbine. Preliminary data indicate that, rather than ameliorating the effects of yohimbine, D1 blockade actually exacerbates the gating deficit. (Supported by P50 MH44212-02)

575.10

YOHIMBINE CAUSES A TRANSIENT IMPAIRMENT IN P50 AUDITORY SENSORY GATING. L.E. Adler, L. Hoffer*, H.T. Nagamoto, M.C. Waldo*, R. Perkins*, and R. Freedman. Department of Psychiatry, University of Colorado Health Sciences Center, and Veterans Affairs Medical Center, Denver, CO 80262

Previous research using the auditory P50 response in a paired click paradigm has shown that schizophrenics have a chronic inability to gate auditory sensory stimuli. That is, the electrophysiological response to the second click is not reduced compared to the first click, as it is in normal individuals. This is taken as an indication of reduced sensory gating. Manic patients share this deficit when acutely ill, but the disruption is transitory and returns to normal when they are euthymic. Similar transient disruptions of gating of the P50 waveform in humans and the N40 waveform in rats can be induced by amphetamine. However, it is unclear whether this is due to increasing CNS noradrenergic and/or dopaminergic neuronal transmission

This pilot study used yohimbine, a presynaptic alpha$_2$ adrenergic antagonist to increase CNS noradrenergic neuronal transmission in 6 normal control subjects. Yohimbine caused a transient impairment of P50 auditory sensory gating during the first 30 minutes post ingestion, followed by a rapid return to baseline levels. The data suggest that increasing CNS noradrenergic neuronal transmission may transiently impair P50 auditory sensory gating in human subjects. This work was supported by grants 5K01 MH00728-03 and P50 MH44212-02.

575.11

INCREASED PHOSPHORYLATION OF GAP-43 AND OTHER SYNAPTIC PROTEINS IN SCHIZOPHRENIC BRAINS. N. I. Perrone-Bizzozero, L.I. Benowitz, E.D. Bird, S.E. Lewis*, L. Boling*, P. Holtzman*, S. Matthysse* and S.L. Rogers Department of Biochemistry, University of New Mexico, Albuquerque, N.M., 87131 and McLean Hosp., Harvard Univ., Belmont, MA, 02178.

The growth of axons and formation of synaptic connections are associated with the expression of the neuronal membrane phosphoprotein, GAP-43. To examine whether GAP-43 levels were altered in conditions of abnormal brain function such as in schizophrenia, we compared the levels of GAP-43 and other synaptic proteins in postmortem brain tissues derived from 5 control and 5 schizophrenic subjects. Following *in vitro* phosphorylation reactions in the presence of either Ca^{2+} or cAMP, synaptic phosphoproteins from primary visual (A17), and visual association (A20) areas were analyzed by two-dimensional gel electrophoresis and fluorography. Quantitative analysis revealed that levels of phosphorylation of GAP-43 in both areas 17 and 20 of schizophrenic brains were about 3-fold higher than in control brains. A similar increase was observed for other Ca^{2+}-dependent and cAMP-dependent synaptic phosphoproteins, whereas the levels of phosphorylation for tubulin and another major synaptic protein showed no change. Preliminary analysis of the frontal cortex (A10/11) in schizophrenic brains also showed increased levels of GAP-43 phosphorylation, suggesting that the anomaly observed in the previous studies is not limited to visual cortical areas. Finally, the fact that haldol-treated rats did not show any significant increase in GAP-43 phosphorylation suggests that our findings in schizophrenic brains are not the result of neuroleptic treatment and that an altered expression of GAP-43 and other synaptic proteins may correlate with some aspect of the disease. *Supported by the Scottish Rite Schizophrenia Research Program*

575.12

^{3}H-DTG BINDING IN POST-MORTEM BRAINS OF SCHIZOPHRENICS. H.Shibuya, M.Mori* and M.Toru*. Dept. of Neuropsychiatry, Sch. of Med., Tokyo Medical and Dental Univ., Tokyo 113 JAPAN

The pharmacological profile of sigma receptor in Wistar male rat and human were studied using ^{3}H-1,3,-(2-o-tolyl)guanidine(^{3}H-DTG). Futhermore, sigma receptor binding in the postmortem brains of 10 controls and 12 schizophrenics were assayed. None-specific binding was determined in the presence of 10^{-5}M haloperidol. 100mM Na^+ significantly decreased ^{3}H-DTG binding in rat brain homogenate but not in human brain cortex. K^+, Mn^{++}, Mg^{++}, Ca^{++} made no obvious difference in the binding between two species. 1μM Glu, Asp or Gly significantly increased ^{3}H-DTG binding in the human brain cortex but not in the rat brain.

In schizophrenics, sigma receptors in the frontal cortices, the temporal cortices, the parietal cortices and the occipital cortices showed no obvious difference compared to those in controls. There was no significant difference in sigma receptor binding between on-drug groups and off-drug groups in schizophrenics. These findings suggest that sigma receptor might not play important roles in the pathogenesis of schizophrenia.

575.13

REGIONAL CORTICAL SURFACE AREA MEASUREMENTS IN MONOZYGOTIC TWINS DISCORDANT FOR SCHIZOPHRENIA SUGGEST A LEFT HEMISPHERE BASIS FOR THE DISEASE. R.L. Green*, M.J. Tramo, W.C. Loftus*, C.E. Thomas*, P.J. Brown*, J.B. Weaver*, and M.S. Gazzaniga, Program in Cognitive Neuroscience and Dept. of Psychiatry, Dartmouth Med. School, Hanover, NH 03756

In order to consider whether behavioral differences in monozygotic twins discordant for schizophrenia (MZDS) are associated with neuroanatomical differences, we measured the surface area of 27 cortical regions via three-dimensional computer-generated reconstructions of magnetic resonance (MR) images. Four pairs of female MZDS were studied. Patients met DSM-III-R criteria for the diagnosis of schizophrenia. Monozygosity was determined by red blood cell marker assays and a standardized questionnaire. Contiguous 3mm-thick T1-weighted MR images were obtained in the coronal plane. For each of the 27 regions of interest (ROIs), differences in surface area (SA) within twin pairs and differences in SA between unrelated twin pairs were compared using a repeated measure ANOVA and Bartlett's test to compare variances. In a prior study (Gazzaniga et al. 1991), 15 of 27 ROI SAs in the left hemisphere of five normal female MZ twin pairs showed significantly less variance. In the MZDS group, only one ROI showed significantly less variance in the left hemisphere. In marked contrast, the right hemisphere of the five normal MZ twin pairs showed only four areas of significantly less variance. In the MZDS group significantly less variance was seen in three of these same regions.

These preliminary results are consistent with the view that: 1) schizophrenia reflects disease of the left hemisphere; and 2) the pathogenesis of the disease goes beyond abnormalities of neurotransmitter function and involves disorganization of cortical structures. (Supported by NARSAD, and ONR N00014-89-J-3035.)

575.14

SENSORIMOTOR GATING OF THE STARTLE REFLEX (SR) IS MEDIATED BY VENTRAL PALLIDAL EFFERENTS TO THE PEDUNCULOPONTINE NUCLEUS. N.R. Swerdlow*, S.B. Caine and M.A. Geyer. Departments of Psychiatry and Neuroscience, UCSD School of Medicine, La Jolla, CA 92093

The SR is inhibited or "gated" when a startling stimulus is preceded 30-500 msec earlier by a weak click or "prepulse". This "prepulse inhibition" (PPI) provides an operational measure of sensorimotor gating. PPI is impaired in schizophrenic patients, and this loss of startle gating may be a psychophysiologic correlate of deficient inhibitory processes that cause sensory and cognitive "flooding" in schizophrenia.

Our previous findings indicate that PPI is modulated in rats by neural circuitry connecting the hippocampus, nucleus accumbens and ventral pallidum (VP). Davis (J Neurosci 1982) reported that the "primary" control of the SR occurs in a 5-neuron circuit that links the sensory receptor through the pontine reticular formation to the motor effector. We examined how limbic/motor circuitry that modulates PPI might influence activity in this "primary" startle circuit.

In a replication of our published work, infusion of picrotoxin into the VP disrupted PPI of acoustic startle. PPI, but not startle itself, was profoundly impaired in rats after electrolytic lesions of VP efferent terminal regions in the pedunculopontine nucleus (PPN). PPI was not significantly impaired by electrolytic lesions of the dorsomedial thalamus. Limbic/motor information that modulates PPI appears to access the "primary startle circuit" via ventral pallidal efferents to the PPN. By linking forebrain and reticular formation substrates that modulate the SR, we have identified circuitry that controls a specific quantifiable behavioral abnormality in schizophrenia.

575.15

ABNORMALITIES IN NAAG AND NAALADase LEVELS IN SCHIZOPHRENIC BRAIN. G. Tsai, B.S. Slusher, R.E. Carter*, L. Passani, F. Casanova, J. Kleinman, J.T. Coyle. Depts. of Psychiat., Pharmacol., Neurosci., Johns Hopkins School of Med., Balto. MD 21205 and Neuroscience Center at St. Eliz., Washington, D.C. 20032.

Recent studies suggest that disturbed glutamatergic neurotransmission may play a role in schizophrenia. An important contribution to this hypothesis is the observation that phencyclidine (PCP), a potent psychotomimetic drug, acts at the NMDA-type glutamate receptor. The endogenous neuropeptide, N-Acetyl-Aspartyl-Glutamate (NAAG), has been proposed to act as a mixed agonist-antagonist at the NMDA receptor. To determine whether NAAG is involved in the neurochemical alterations of schizophrenia, NAAG and its catabolic enzyme, N-Acetylated Alpha-Linked Acidic Dipeptidase (NAALADase), were measured in eight different regions of freshly frozen schizophrenic, neuroleptic control and normal control brains. Preliminary results indicate abnormalities in NAAG levels and NAALADase activity in schizophrenics compared to normal controls. NAAG levels were increased in frontal and parietal cortices, but decreased in putamen. NAALADase activity was decreased in temporal and frontal cortices, hippocampus and amygdala. The alterations of NAAG in the putamen and of NAALADase in the frontal cortex, hippocampus and the amygdala were also observed in the neuroleptic control group, suggesting that these changes may be the effects of drug treatment. These results support the hypothesis that glutamate may be involved in schizophrenia and underscore the importance of studying the metabolism of NAAG, a potential NMDA ligand, in the pathophysiology of schizophrenia.

575.16

IN VIVO ^{1}H MAGNETIC RESONANCE SPECTROSCOPY (MRS) OF THE HIPPOCAMPUS/AMYGDALA COMPLEX IN SCHIZOPHRENIA H.A. Nasrallah, T.S. Skinner *, P. Schmalbrock*, A. Obringer* and P.M.L. Robitaille*, Departments of Psychiatry and Radiology, Ohio State University, Columbus, Ohio 43210

Several postmortem and brain imaging studies provide evidence for pathology of the medial (limbic) temporal lobe in schizophrenia. This includes hypoplasia and histoarchitectural abnormalities in the hippocampus of schizophrenic patients, suggestive of neurodevelopmental disruption of neuronal migration. We conducted an in vivo MRS study of the medial temporal lobe in schizophrenics and controls to generate hypotheses about the putatively abnormal neurochemical functions of this region in schizophrenia.

Eleven schizophrenic patients (by DSMIII-R) and six healthy volunteers consented to participate in the study. ^{1}H MRS was conducted using a GE Signa (1.5T).

Proton spectra were acquired from a 2 X 2 X 3 cm^3 voxel localized in the right/left hippocampus regions, using a modified STEAM sequence with a TE of 50 msec. Signal intensities of N-acetyl aspartate (NAA), creatine, and choline relative to a standardized noise measure were obtained. Preliminary analysis reveals no significant differences in the intensities of the creatine or choline resonances between the controls and schizophrenics bilaterally. However, mean NAA intensities from the right (but not the left) hippocampus were 30% lower in the schizophrenic group (p .05). Implications of these findings for the neurobiology of schizophrenia will be discussed.

575.17

REDUCED SEN (VISUAL ERP) INDEXES PROCESSING IMPAIRMENTS IN SCHIZOPHRENIC ADULTS. R.J. Strandburg, J.T. Marsh, W.S. Brown*, R.F. Asarnow*, D.Guthrie*, J. Higa* Dept. of Psychiatry, UCLA, Los Angeles CA 90024-1759.

ERPs were recorded from 19 normal and 18 schizophrenic adults during performance of a visual discrimination task, the Span of Apprehension (Span) which may detect vulnerability factors in schizophrenia. An RT condition preceded the Span. Identical stimuli (12 letter arrays) were presented in both conditions. Subjects responded to the visual arrays in the RT condition and discriminated between the presence of either a T or F in the Span condition. EEG was recorded from 17 scalp sites and ERPs spanned a 2 second epoch. Difference potentials (Span-RT) were computed to isolate Span endogenous negativity (SEN) associated with Span discrimination. Topographic fields were computed at the peak of SEN.

Schizophrenics produced significantly less SEN than normals. SEN differed in topography and time course from the exogenous components (N1, P2) it overlaps. SEN is similar to NA (Ritter et al 1983), which is associated with attentional effort during pattern recognition. Results suggest thatschizophrenics are impaired in allocation of adequate attentional resources for Span processing. This deficit is manifest early (220 msec) in discriminative processing. SEN results are similar to those obtained from normal and schizophrenic children.

575.18

CHARACTERIZATION OF A GROWTH PROMOTING AGENT FOR HUMAN NEUROBLASTOMA CELLS DERIVED FROM SCHIZOPHRENIC CSF. S.Shirabe*[1,2], J.P.Schwartz[2], and J.R.Stevens[1].1) NIMH, St. Elizabeths Hosp., Washington DC 20032, & 2) CNB, NINDS, NIH, Bethesda MD 20892.

We have identified a factor in schizophrenic (Sch) but not control CSF which promotes the growth of the human neuroblastoma cell line SH-EP. Once transformed, the cells themselves produce the factor. Further characterization has shown that the biological activity is destroyed by treating with $CHCl_3$, heat (at 56°C, 60°C or 90°C), and protease (proteinase K or trypsin), but not destroyed by 24 hr incubation at 4°C or RNAse. Growth of the cells for 24 hr in serum-free medium increased the biological activity approx. 100-fold compared to regular 10% FCS containing media. On a Percoll density gradient of culture media, the activity migrated to 1.081 -1.107 g/ml, using a gradient designed to fractionate viruses and subcellular particles, but not proteins or nucleic acids. Two additional changes in the properties of Sch-transformed cells include 1) decreases of vimentin and neurofilament by Western blotting (in agreement with previous immunohistochemistry results) and 2) loss of response to EGF stimulation.

AFFECTIVE ILLNESS AND RELATED DISORDERS

576.1

ANXIETY RESPONSES TO IV MCPP IN HEALTHY SUBJECTS AND PATIENTS WITH PANIC DISORDER. A.W. Goddard, D.S. Charney, M. Germine, G.R. Heninger, and S.W. Woods. Yale Univ. Dept. of Psychiatry, New Haven, CT 06519.

Patients with panic disorder (PD) have been reported to have an increased sensitivity to oral doses of the 5-HT agonist, m-chlorophenylpiperazine (MCPP). However a previous study by our group failed to find patient vs healthy subject behavioral differences following administration of higher doses of intravenous (IV) MCPP. The current study used a lower dose of IV MCPP, 0.05mg/kg, to determine whether the different findings were due to a dose effect. METHOD: Twenty nine PD patients and 22 healthy subjects participated in two study days, separated by at least one week. They received either a bolus infusion of normal saline or a solution of 0.05mg/kg MCPP, according to random assignment. Within challenge measurements included visual analog scale (VAS) anxiety, DSM III-R somatic panic symptoms, and blood pressure and heart rate. RESULTS: Baseline VAS anxiety on the active challenge day was 41±28 mm for the PD group and 4±11 mm for the healthy subject group. These baseline values were virtually the same for the placebo study day. Inspection of the data revealed that the peak anxiety response occurred at 30 min. post infusion. The MCPP minus placebo change from baseline anxiety at 30 min was 26±30 mm for the PD group and 17±27 mm for the healthy group. This difference was not significant on student t-test analysis, $p<0.3$. DISCUSSION: These data replicate the results of our previous study, suggesting that MCPP fails to discriminate between PD and healthy subjects on measures of anxiety. While 5-HT may play an important role in mediating human anxiety responses in general, it is unlikely that anxiety associated with PD specifically is the result of 5-HT receptor hypersensitivity. The effects of different routes of MCPP administration remain to be investigated.

576.2

EFFECTS OF SODIUM LACTATE INFUSIONS ON CISTERNAL LACTATE AND CARBON DIOXIDE MEASURES IN NONHUMAN PRIMATES. Jeremy D. Coplan, M.D. Leonard A. Rosenblum, PhD, Steven Friedman PhD, Trina B. Bassoff, MA, Jack M. Gorman, M.D. From: New York State Psychiatric Institute,Biological Studies Unit, Columbia University and Primate Behavioral Facility, SUNY HSCAB. To further understand lactate induced panic in patients with panic disorder, we evaluated the central effects of sodium lactate infusions as measured by cisternal lactate and gas measurements in a sample of nonventilated nonhuman primates under ketamine anesthesia. Despite the development of the characteristic peripheral biochemical effects of infused sodium lactate, no increases of central lactate or carbon dioxide was observed. The study therefore supports previous findings of non permeability of the blood brain barrier to lactate but conflicts with another nonhuman primate study suggesting a central increase in lactate during infusions. These discrepant data may be accounted for by type of anesthetic agent used, ventilation effects and concentration of lactate infused. Available studies, however, are consistent regarding the absence of central hypercarbia during lactate infusions, a factor hypothesized to be an important trigger of an abnormally sensitive "suffocation alarm mechanism" in panic disorder. The study thus supports theories of lactate panic based on cognitive and/or brainstem misevaluation of peripheral somatic sensations.

576.3

INCREASED SENSITIVITY OF THE HYPOTHALAMIC-PITUITARY-ADRENAL (HPA) AXIS TO GLUCOCORTICOID FEEDBACK INHIBITION IN A TRANSGENIC MOUSE MODEL OF DEPRESSION TREATED WITH ANTIDEPRESSANTS.

N. Barden, M-C. Pepin and F. Pothier* Molecular Psychogenetics, CHUL Research Centre and Laval University, Ste-Foy, Québec, Canada G1V 4G2

We have created transgenic mice which bear a glucocorticoid receptor mRNA antisense transgene as an animal model for depression. These animals have decreased brain glucocorticoid binding activity and elevated serum cortisol and ACTH levels. Depressive illness is often characterized by an increased cortisol secretion which is refractory to glucocorticoid suppression. This apparent lack of glucocorticoid feedback inhibition could be explained by decreased glucocorticoid receptor (GR) gene expression in the brain. Normalization of HPA axis regulation follows successful antidepressant (AD) therapy of depression. To test the hypothesis that ADs might restore glucocorticoid sensitivity to brain areas by increasing GR gene expression, we have treated transgenic mice with desipramine (20mg/kgBW) and observed the effects on GR mRNA, glucocorticoid binding and corticosterone secretion. The glucocorticoid binding activity of different tissues from AD-treated and control transgenic mice was measured using [^{3}H]-dexamethasone. When transgenic mice were treated with desipramine, we observed up to a 4 fold increase in the glucocorticoid binding activity of different brain regions. The GR mRNA concentration of different tissues was determined by Northern blot analysis using a ^{32}P-labelled RNA probe complementary to a 1.8 kb fragment of the GR cDNA and a ß-actin cRNA probe for standardization. The GR mRNA concentration in the brain of AD-treated transgenic mice was twice that of non-treated transgenic animals.. Moreover, the serum corticosterone of AD-treated transgenic mice was within the concentration range of normal mice. These results suggest that ADs can restore the sensitivity of the HPA axis to glucocorticoid feedback inhibition by increasing the concentration of brain GR.

576.4

[^{3}H]-IMIPRAMINE BINDING IN PLATELETS OF NEVER-MEDICATED DEPRESSED PATIENTS. J.A. Franks*, D.L. Knight, K.R.R. Krishnan, T.A. Slotkin and C.B. Nemeroff. Duke Univ. Med. Ctr., Durham, NC 27710.

The serotonin uptake site in brain is labeled by [^{3}H]-imipramine with binding characteristics virtually identical to the imipramine binding site in platelets. Several previous studies demonstrated that the number of [^{3}H]-imipramine binding sites in platelets is decreased in drug-free depressed patients. However a recent study has suggested that the reduction in platelet [^{3}H]-imipramine binding sites found in these studies is due to prior treatment of patients with antidepressant drugs (Mellerup and Plenge, Acta Psychiatr. Scand. 1990). To test this hypothesis, platelet [^{3}H]-imipramine binding was evaluated in 12 depressed patients who had never received antidepressant drug therapy, and compared to that of age- and sex-matched controls. Patients fulfilled DSM-IIIR criteria for major depression and ranged in age from 22 to 81 years. Total [^{3}H]-imipramine binding was assayed at seven concentrations (0.25-8 nmol) of drug. Nonspecific binding was defined as binding persisting in the presence of 50 µmol desipramine. The number of platelet [^{3}H]-imipramine binding sites was significantly decreased by 33% in the depressed group vs controls. Bmax for the depressed group was 753.8 ± 78.2 vs control Bmax of 1120.9 ± 63.6. The Kd values were not significantly different between the two groups. This decrease in platelet [^{3}H]-imipramine binding site number observed in depressed patients is not the result of prior antidepressant drug therapy. (Supported by NIMH MH-40159).

576.5

LACK OF SIGNIFICANT MONTHLY VARIATION IN PLATELET [^{3}H]-IMIPRAMINE BINDING KINETICS IN CONTROLS OR PATIENTS WITH MAJOR DEPRESSION. D.L. Knight, K.R.R. Krishnan and C.B. Nemeroff. Depts. Psychiatry & Pharmacology, Duke Univ. Med. Ctr., Durham, NC 27710.

Specific high affinity binding sites for [^{3}H]-imipramine (IMI), that label the serotonin transporter, have been described in brain and platelets. In studies reported by our group and others, drug-free depressed patients have exhibited a reduced number (Bmax) of platelet IMI binding sites when compared to normal controls. Other groups have reported a seasonal variation in platelet IMI binding kinetics that masked any group differences in IMI Bmax. With our large database (104 controls, 178 depressed patients, 88 non-depressed psychiatric patients), we examined the possibility of monthly variations in platelet IMI binding site density or affinity using one-way ANOVA. We observed no significant monthly variation in platelet IMI binding Bmax or Kd in controls or patients with major depression. There were small statistically insignificant peaks of IMI binding in Febuary and August with the lowest binding found in July. Bmax values for depressed patients were significantly below the monthly mean of normal control Bmax values at every time point examined. There were no differences betwcen control and depressed Kd values in any month. The present results, by demonstrating no monthly variation in controls or depressed populations, indicates that seansonality is not a confound in the use of platelet IMI binding as a biological marker of major depression. (Supported by NIMH MH-40159).

576.6

CLONIDINE STIMULATION OF GROWTH HORMONE AND CORTISOL IN OBSESSIVE-COMPULSIVE DISORDER. W.A. Hewlett*, K. Martin, and S. Berman. Department of Psychiatry, Stanford University, Stanford, CA 94305.

Clonidine stimulates the release of growth hormone via an alpha-2 adrenergic mechanism. Altered alpha-2 adrenergic functioning has been suggested to occur in Obsessive Compulsive Disorder (OCD) and the growth hormone response to clonidine stimulation has been reported as blunted in the setting of OCD. In an attempt to replicate this finding, a clonidine challenge test was conducted on 19 subjects with a DSMIIIR diagnosis of OCD and 18 control subjects. Two baseline blood samples were obtained prior to IV administration of clonidine (2 μg/kg.) Blood samples were then collected at 15 minute intervals for one hour and subsequently at 2 and 4 hours post administration. Plasma samples were frozen at -70°C prior to assay for growth hormone. Cortisol levels were also assayed as a nonspecific endocrine measure of stress. Clonidine induced a six-fold increase in plasma growth hormone levels in both patients and controls. There was no difference in the amount of stimulation between these populations. Cortisol levels dropped by 15% during the period of clonidine-induced growth hormone stimulation and there was no significant difference between patients and controls in this decrement. These results would indicate that there is no difference in.alpha-2 adrenergic functioning between OCD patients and control subjects.

576.7

EFFECTS OF LITHIUM ON TYROSINE HYDROXYLASE GENE EXPRESSION IN PC12 CELLS. **G. Allan and N.G. Bazan. LSU Eye Center and Neuroscience Center, New Orleans, LA 70112.**

Lithium is an effective drug for the treatment of some manic disorders, but its precise mechanism of action has not yet been determined. It has been shown to affect the inositol lipid cycle via inhibition of inositol-1-phosphatase, and to affect the coupling of G-proteins. Recent evidence showing that lithium augments c-*fos* expression in PC12 cells has led to the suggestion that lithium might also act at the level of gene expression. The control enzyme of catecholamine biosynthesis, tyrosine hydrolase (TH), has been shown to be a target of the AP-1 translational activator complex. PC12 cells were treated with nerve growth factor (NGF) and/or lithium and TH mRNA levels were determined by Northern analysis. Lithium (0.1 mM and 1 mM) elevated TH message levels in NGF-treated cells after 5 and 6 days, but not after 8 days. TH mRNA levels in undifferentiated cells were not affected. Ten millimolar lithium decreases TH mRNA levels with respect to controls in both differentiated and undifferentiated cells after 5 and 8 days of exposure. These data suggest that chronic lithium treatment can affect TH gene expression in this cell line, but, at the highest concentration, might have nonspecific effects. We are currently studying this system in more detail, since it provides a model to study its action in neural cells. (Supported by NINDS NS23002).

576.8

AN HYPOTHESIS OF THE NEUROBIOLOGY OF THE ATTENTION DEFICIT HYPERACTIVITY DISORDER (ADHD) SYNDROME. **W. A. Weinberg and R. A. Brumback. Dallas Children's Hospital, Dallas, TX and University of Oklahoma, Oklahoma City, OK.**

Children and adolescents not doing well in school and/or home are often labeled as suffering from ADHD. However, ADHD is a symptom complex resulting from multiple specific etiologies. We have developed an hypothesis in which ADHD syndrome is explainable as genetically-based dysfunction of specific brain areas. Depression relates to loss of right parietal-temporal cortex control of locus ceruleus and median raphé nuclei with resultant reduced bioamine neurotransmission to cerebral cortex bilaterally. Disturbed control of hypothalamic circadian functions results in associated abnormalities of biologic rhythms and sleep. Primary disorder of vigilance relates to loss of controlling modulation by inferior parietal lobule on reticular activating system resulting in dearousal sleep. In narcolepsy, inferior parietal lobule control is intact, but genetically-abnormal reticular activating system aberrantly influences both cerebral hemispheres with resultant sleep attacks and hypovigilance. Although abnormal functioning of left mesial temporal cortex can alone present as mania, more commonly interactions between hemispheres determine clinical symptoms of affective illness. Disorder of right hemisphere not only generates depression, but its influence on left hemisphere can result in mixed symptoms (depression and mania) or (less commonly) pure mania. Occasionally, primary dysfunction of left mesial temporal (limbic) cortex can induce right cerebral hemisphere dysfunction presenting clinically as mixed symptoms or as pure major depression. Individual variation in higher cortical cognitive functions may be apparent as learning disabilities. In right parietal cortex learning disability syndrome, stressing dysfunctional cortical areas in an unfavorable learning environment results in inattention, secondary hypovigilance, and promotion of affective illness. Although most symptoms of conduct disorder are secondary to underlying affective illness, dysfunction of anterior-inferior right frontal cortex results in primary conduct disorder (sociopathy). Comorbidity and concurrent diagnoses are explainable as a consequence of interactions between affected brain centers.

576.9

LYMPHOCYTE DNA-SYNTHESIS AND GLUCOCORTICOID-RECEPTOR PLASTICITY IN MAJOR DEPRESSIVE DISORDER. N.Wodarz*, C.Konradi, R.Rupprecht*, J.Kornhuber*, P.Riederer*. Dep. of Psychiatry, Clin. Neurochem., Univ. of Würzburg, Füchsleinstr. 15, W-8700 Würzburg, Germany.

Abnormalities of hypothalamic-pituitary-adrenal (HPA) axis integrity, as well as alterations in immune function have repeatedly been associated with the syndrome of depression. We investigated in 12 drug-free patients with severe major depressive disorder (according to DSM-III-R) and 13 age-matched non-hospitalized healthy individuals the lectin-induced lymphocyte DNA-synthesis (Concanavalin A, Phytohemagglutinin A, Pokeweed Mitogen), i.e., an in-vitro measure of cell-mediated immunity, and the sensitivity of the lymphocyte glucocorticoid receptor. In contrast to previous reports, the basal lectin-driven lymphocyte DNA-synthesis did not differ between patients and controls. But a weaker sensitivity to in-vitro adrenocortical hormones might be indicative of an impaired plasticity of glucocorticoid receptor regulation in a subgroup of depressed patients. The elucidation of the exact mechanism may yield insights into the pathophysiology of major depressive disorder.

576.10

ADRENAL ACTIVITY DOES NOT MEDIATE ALARM PHEROMONE REACTION IN THE FORCED SWIM TEST. P.J. Bilitzke and E.L. Abel. Depts. of Psychology, Ob/Gyn and Fetal Alcohol Research Center, Wayne State Univ., Detroit, MI 48201.

Rats tested in fresh water in a forced swim test are more immobile than rats tested in soiled water which presumably contains an alarm pheromone. This study was done to see if the adrenal glands are somehow involved in this phenomenon. Male Long-Evans rats, 110-120 days of age, were tested in the forced swim test after adrenalectomy or after SHAM surgery. Plexiglas cylinders were filled to a height of 25cm with fresh tap water (26±1°C.) ADX did not affect the behavior of rats in fresh or soiled water nor did it affect production of alarm pheromone or reaction to it. The results suggest that adrenal function does not mediate the response to, or production of alarm pheromone.

Supported by grant P50 AA07606.

576.11

AN ANIMAL MODEL OF PANIC DISORDER: PSYCHOPHARMACOLOGIC EVIDENCE. J.H. Hannigan, E.L. Abel and P.J. Bilitzke. C.S. Mott Center, Wayne State Univ., School of Medicine, Detroit, MI 48201.

Rats forced to swim with no possibility of escape initially swim vigorously but soon show considerable immobility. In contrast, animals tested in water soiled by another rat exhibit almost no immobility. We hypothesized that behavior in soiled water resembles a panic-like response which will respond to drugs with anti-panic effects. The present results supported this hypothesis by showing that buspirone, diazepam and imipramine had no significant effects on immobility when rats were tested in fresh water. In soiled water, diazepam was still ineffective; imipramine increased immobility slightly; and buspirone increased immobility to the level of animals tested in fresh water. Since the selective serotonin agonist buspirone has relatively greater anti-panic effects clinically than diazepam or imipramine, we concluded that our variation of the forced swim test may be a useful animal model for studying mechanisms of panic and treatment of panic disorder.

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576.12

ABSENCE OF HABITUATION TO ALARM PHEROMONE IN THE FORCED SWIM TEST. E.L. Abel and J.H. Hannigan. C.S. Mott Center, Wayne State Univ., School of Medicine, Detroit, MI 48201.

Rats were tested for 8 consecutive days in a forced swim test in either fresh water or soiled water. On day 9, some rats previously tested in fresh water were tested in soiled water, and vice versa. On days 10 and 11, all rats were tested in their original water condition. On day 12, escape from a water maze was measured for all rats, plus a group of naive animals. Rats were more immobile in fresh than in soiled water on all days. Rats tested repeatedly in fresh but not soiled water increased immobility over days. When switched between water conditions, rats behaved like rats that had been tested previously in each condition, with no evidence that prior testing influenced immobility in either condition. Rats that had been tested continuously in fresh water had the longest escape latencies and were the only group to differ from naive controls.

Supported by P50 AA07607 and R01 AA06721.

NEUROTOXICITY II

577.1

CALCIUM CHANNEL ANTAGONISTS INDUCE NEURONAL DEATH IN CORTICAL CULTURE.

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Organic calcium channel antagonists (OCCA) have been shown to protect neurons from ischemic or excitotoxic injury, possibly by limiting Ca influx through the voltage-gated calcium channels. However, the important possibility that OCCAs alone may possess neurotoxic potential has not been examined. In the present study, we report that 2 d exposure of mouse cortical cultures to nifedipine (10-100 μM), verapamil (3-30 μM), diltiazem (10-100 μM), or flunarizine (1-10 μM) produces selective neurotoxicity in a concentration-dependent manner. Interestingly, neurotoxicity produced by nifedipine or verapamil is largely attenuated by 1-10 μM cycloheximide (CHX), an inhibitor of protein synthesis. Furthermore, extensive neuronal degeneration developing in low Ca media is also similarly blocked by CHX. Although other possibilities cannot be excluded, these findings suggest that a decrease in intraneuronal Ca levels may trigger synthesis of proteins mediating neuronal death, similar to what has been proposed to occur in growth factor deprivation neuronal injury. Regardless of the exact mechanisms involved, more study regarding neurotoxicity of OCCAs seems warranted since these compounds are currently clinically used in patients with certain cardiovascular problems.

577.2

POSTMORTEM STABILITY OF mRNA FOR GLUCOCORTICOID AND MINERALOCORTICOID RECEPTOR IN RODENT BRAIN.

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In preparation for experiments with human tissue we determined the relative postmortem stability of the respective mRNA's for glucocorticoid (GR) and mineralocorticoid (MR) receptor in rodent brain using semi-quantitative *in situ* hybridization (ISH). Groups of 3 rats were killed by CO_2 asphyxiation and their brains removed immediately (O hr) or following, 12 hr or 24 hr delays. Rat carcasses in the 12 hr group were maintained at RT until brain removal; rat carcasses in the 24 hr group were maintained for 12 hours at RT followed by an additional 12 hr at 4°C. Specific hybridization of GR anti-sense ^{35}S-RNA-probe to tissue mRNA encoding the (GR) was detected using film and emulsion autoradiography. The most intense labelling was in the dentate gyrus followed by the CA1 hippocampal region. Less, but still detectable, signal was apparent over CA3-CA4 pyramidal cell regions. Positive hybridization of the MR anti-sense ^{35}S-RNA-probe was detected throughout the CA1-4 pyramidal cell fields of the hippocampus and the granular cells of the dentate gyrus. Film images demonstrated that even in the 24 hr postmortem delay group intense specific signal is present in sections hybridized with both anti-sense GR and MR probes. The distribution of the detected mRNAs changes little with postmortem time and there is no overall increase in non-specific background. These initial experiments with rat brain demonstrate that the mRNA for both GR and MR, as detected with ISH, are stable up to 24 hours following death. (Supported by NIH grants AG03644 and NS20832).

577.3

CORTICOSTERONE EXACERBATES HIPPOCAMPAL CALCIUM-DEPENDENT SPECTRIN PROTEOLYSIS IN VIVO. E Elliott[1] P Vanderklish*[2] J Chang*[1] G Lynch[2] and R Sapolsky[1]. [1]Dept Biol Sci, Stanford Univ, Stanford, CA 94305 and [2]CNLM, Univ of CA, Irvine, CA 92715.

Corticosterone (CORT), a steroid with enhanced secretion during stress, increases hippocampal neuronal vulnerability to excitotoxins, hypoxia-ischemia, and metabolic poisons. One hypothesis for this CORT endangerment is an exacerbation of the glutamate/NMDA/calcium cascade. This would result in the activation of calcium-dependent proteases such as calpain, the preferred substrate of which is spectrin, a prominent component of the membrane cytoskeleton. We investigated whether CORT increases hippocampal spectrin proteolysis induced by the excitotoxin Kainic Acid (KA).

Adrenalectomized (ADX) rats, half with daily CORT replacement (3 mg/100 g bw), were infused with KA in the hippocampus and sacrificed 6 hours later. Spectrin and its proteolytic fragments were compared in the 2 groups by immunoblot assay. CORT treatment increased KA-induced hippocampal spectrin proteolysis 124%. This may be energetic in nature as CORT decreases hippocampal neuronal glucose transport. To test this, ADX and ADX+CORT rats were injected ip with 0.75 gm mannose every 3 hr for 24 hr before and after KA infusion. This mannose supplementation eliminated the CORT-induced increase in spectrin proteolysis. The CORT exacerbation of spectrin degradation would be expected to disrupt membrane-associated events and intracellular transport and could explain the hippocampal neuronal vulnerability and toxicity evident following CORT exposure.

577.4

SELECTIVE DEGENERATION OF THE RAT OLFACTORY NERVE AND OLFACTORY-RELATED CORTICAL AREAS DURING THE DEVELOPMENT OF ETHANOL DEPENDENCE

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Adult male rats were maintained at high blood ethanol concentrations (300-500mg%) for 4 days via an indwelling stomach catheter (adaptation of the Majchrowicz dependence technique) and then sacrificed. Using the deOlmos cupric-silver stain, neuronal degeneration was histologically found in the dentate gyrus (granule layer), the entorhinal cortex (layer 3), the perirhinal cortex (layer 3), the piriform cortex (layer 2) and the olfactory amygdala, confirming the findings of Switzer et al. (Anat. Rec. 1982: 202:186a). In addition, we detected neuronal degeneration in the insular cortex (layers 2&3), the lateral orbital cortex (layer 2), and striking degeneration of the olfactory nerve terminals in the olfactory bulb. We have also found that adding a 36 hour withdrawal did not increase the observed degeneration (cell counts), implying that the degeneration was a direct effect of the ethanol and not secondary to withdrawal seizures.

577.5

BRAIN SAM-DEPENDENT N-METHYLATIONS OF β-CARBOLINES AND THEIR TETRAHYDRO FORMS: POTENTIAL BIOACTIVATION ROUTES TO NEUROTOXINS.

M.A. Collins, E.J. Neafsey and K. Matsubara*, Biochem. and Anatomy Depts., Loyola Univ. Med. Center, Maywood, Ill. 60153.

S-Adenosylmethionine (^{3}H-SAM)-dependent N-methylation of β-carboline (BC) and 1,2,3,4-tetrahydro-BC (THBC) alkaloids was examined in undialyzed subfractions of guinea pig and rat brain, using HPLC/radiodetection. Simple THBCs were methylated solely on the 2[β]-nitrogen by cytosolic activity possibly identical to aza-heterocyclic N-methylating activity reported by others. However, simple BCs such as harman or norharman underwent both 2[β]- and 9[indole]-N-methylations in sequential fashion by activity in the nuclear subfractions. Importantly, the 2,9-dimethyl-BC products displayed dopaminergic neurotoxic effects that surpassed the 2-methyl-BCs and approached MPP+, the parkinsonian "street drug" toxin. Since THBCs and BCs are endogenous constituents, their respective N-methylations by differing brain methyl-transferases could lead ultimately to cationic 2,9-[indole]-methylated BC toxic factors which are trapped within the brain. Supported by NS23891 and LUMC.

577.6

SPINAL KINETICS OF LIPOSOME-ENCAPSULATED 1-ß-D-ARABINO-FURANOSYL CYTOSINE IN RATS.

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Maintenance of therapeutic concentrations of anti-neoplastic agents in the CNS for extended periods of time is efficient method for the delivery of the cell cycle specific antimetabolites in cancer chemotherapy. Multivesicular liposomes composed of bilayer lipid membrane have been reported as slow release reservoirs for hydrophilic drugs.

We studied the intraspinal kinetics of liposome-encapsulated (liposomal) 1-ß-D-arabino-furanosyl cytosine (Ara-C) in rats. Liposomes were observed in lumbar and cisternal CSF for 7 days after intrathecal (IT) injection of liposomal Ara-C (1mg/50ul) through a chronic lumbar IT catheter. The IT T1/2 of the liposomal Ara-C was 154 hr in contrast to T1/2 of 0.3 hr for free Ara-C. T1/2 of IT inulin-C^{14} in CSF was 0.5 hr. This suggests liposomes are cleared more slowly than bulk CSF flow. No toxicity on general behavior with IT liposomes was observed.

CSF sample	Ara-C		T2/1(hr)	R
Lumbar	free		0.28	.96
	liposomal	pellet	154*	.97
		super	80*	.84
Cisternal	liposomal	pellet	58.3*	.9
		super	54*	.85

R = Correlation coefficient, * = Second order kinetics, free = unencapsulated drug, pellet = drug in liposome, super = free drug in supernatant.

577.7

COLCHICINE-INDUCED DEGENERATION OF CHOLINERGIC SEPTOHIPPOCAMPAL NEURONS AS DEMONSTRATED BY THE LOSS OF NGFr-IMMUNOREACTIVITY.

Sheryl R. Ginn and Gary M. Peterson. Department of Anatomy and Cell Biology, East Carolina University School of Medicine, Greenville, NC 27858-4354.

Neuronal somata in the medial septum (MS), which are immunoreactive for choline acetyltransferase (ChAT), are also immunoreactive for the nerve growth factor receptor (NGFr). Similarly, the pattern of innervation of the hippocampus by cholinergic afferents is matched by the pattern of NGFr-immunoreactive (NGFr) fibers. Thus, NGFr-immunoreactivity appears to be a reliable marker for cholinergic somata and axons in the septohippocampal system. The present study was conducted to determine if ICV injections of colchicine, which reduce the number of ChAT-immunoreactive somata in the MS and the numbers of acetylcholinesterase (AChE)-positive fibers in the hippocampus, produce a similar change in the numbers of NGFr-immunoreactive somata and fibers. Colchicine (2.5 μl of 0.2%) was injected into each lateral ventricle of female Sprague-Dawley rats; the rats were allowed to survive for 1 week before aldehyde perfusion. The brains were cut through the MS and the hippocampus, and sections in these areas were stained immunocytochemically for NGFr using 192 IgG (generously provided by Eugene M. Johnson, Jr). ICV colchicine resulted in a 48% reduction in the number of NGFr-immunoreactive somata in the MS, compared to control. Within the hippocampus the intensity and numbers of NGFr-immunoreactive fibers were greatly reduced. These results are similar to what has been reported for ChAT-immunoreactive somata in the MS and AChE-positive fibers in the hippocampus, and support the conclusion that ICV colchicine induces degeneration of cholinergic neurons in the septohippocampal system.

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577.8

NEUROTOXIC EFFECTS OF CHRONIC GABA INFUSION INTO THE NUCLEUS BASALIS WERE NOT ATTENUATED BY CO-INFUSION OF THE $GABA_A$ RECEPTOR ANTAGONIST, SR95531.

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Previous studies have demonstrated that unilateral GABA infusion into the Nucleus Basalis (NB) of rats produces pronounced gliosis in the NB and various surrounding structures, as well as cholinergic deafferentation in the ipsilateral frontoparietal cortex (Will et al., 1988; Majchrzak et al., 1990). This investigation was undertaken to examine whether the neurotoxic effects of chronic GABA infusion are directly mediated by the $GABA_A$ receptors. A total of 42 male Long-Evans rats (250-300g) was randomly divided into 6 treatment groups with 7 animals per group. Each rat received a unilateral, intra-NBM infusion of either "SR-0.5" (i.e., SR95531; 0.5 μg/μl/h), "SR-0.05" (0.05 μg/μl/h), "GABA100" (100 μg/μl/h), GABA100 + SR-0.5 (0.5 μg SR + 100μg GABA per μl/h), GABA100 + SR-0.05 (0.05 μg SR + 100μg GABA per μl/h), or the vehicle (ultrafiltered saline; 1 μl/h) for a period of 24 hours. It has been demonstrated that SR95531 (i.e., "GABAzine") is a potent competitive $GABA_A$ receptor antagonist. Animals were monitored for alterations in spontaneous activity (i.e., actography) and spontaneous turning (i.e., bucket rotation). Rats were sacrificed 8 days following the initiation of infusions. Brain sections were examined using AChE and cresyl violet histochemistry, and GFAP immunocytochemistry. These results demonstrate that co-infusion of SR-0.5 or SR-0.05 with GABA100 did not attenuate GABA-induced neurotoxicity and/or behavioral abnormalities. On the contrary, it was observed that SR alone or co-infused with GABA produced a similar or greater extent of cortical deafferentation, gliosis and GFAP immunoreactivity than GABA alone. It is concluded that GABA-induced neurotoxicity and behavioral abnormalities are not abolished or even attenuated by co-infusion of the $GABA_A$ antagonist, SR95531, at least not at the dosages employed.

577.9

SERIAL EVALUATION OF THE NEUROPATHY ASSOCIATED WITH THE ANTI-AIDS COMPOUND ddC IN THE RABBIT.

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The nucleoside, 2',3'-dideoxycytidine (ddC) is a potent inhibitor of HIV but has been associated with a dose-dependant peripheral neuropathy in clinical trials. Previous studies in the rabbit have established that high doses (50 - 250 mg/kg/day) of ddC, administered for 18 weeks, produced a profound neuropathy characterized by both myelin and axonal dysfunction. This severe neuropathy most likely reflected an end stage process clinically characterized by paralysis in the hindlimbs and paresis in the forelimbs. The present study was designed to establish the earlier stages and progression of ddC neuropathy. We studied 40 New Zealand white rabbits that were equally divided into a control and a treated group. ddC was administered by gavage at a dose of 35 mg/kg/day. Histopathology was serially examined at 8, 12, 16, 20, 24 wks (electrophysiologic measures were recorded at these timepoints plus at baseline).

Beginning at wk 16, the proximal segments of the sciatic nerve and the associated ventral roots showed myelin splitting and intramyelinic edema. At 20 wks, clinical signs of weakness appeared. At 24 wks, a significant reduction in axonal cross sectional diameter was evident in the sciatic and distal tibial nerves. The principal electrophysiological change was seen at 24 wks in the peroneal F-Wave, which was delayed by approximately 45% of it's baseline latency. These findings confirm a ddC induced proximal motor fiber myelinopathy and a distal axonopathy, and demonstrate the effectiveness of the rabbit as a model for ddC neuropathy.

577.10

BEHAVIORAL DEVELOPMENT OF INTACT AND NEONATALLY CAPSAICIN TREATED RATS.

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Capsaicin (CAP) neonatally injected damages small primary sensory neurons related with thermo-nociceptive activity. This study analyzes the development of scratching, still, grooming, rearing, sniffing, digging, and searching behaviors in intact and neonatally (day 2pp) CAP treated (50mg/Kg s.c.) rats. The frequency of these behaviors was scored every other day from day 3 to day 90 of life. Eye opening day, body weight and physical appearance were also recorder. RESULTS: Control: Still behavior frequency (BF) prodominated over others BFs during the first 7 days of life and progressively diminished until day 13th, when rearing and digging BFs started to increase. Sniffing, grooming and searching BFs suddenly increased from day 9, however, the latter showed a fast decrement on day 13th. Scratching BF was slightly apparent showing a gradual, small increment. On days 14-15 eye opening occurred. CAP-treated: The frequency of all behaviors but scratching was similar as controls. Scratching BF increased significantly on day 13th and was maintained at a higher level than control throughout the study. Eye opening occurred before (days 10-14) and these rats presented head wounds. CONCLUSION: Thermo-nociceptive stimuli (activity carried by CAP sensitive fibers) influence the time of eye opening and the intensity of scratching behavior.

SEP C91 (P.P.); SEP C91, CONACyT D111-903850 (J.M.).

577.11

SUPPRESSION OF HIPPOCAMPAL SLICE EXCITABILITY BY 2-, 3-, AND 4-METHYLPYRIDINE. S.B. Fountain and T.J. Teyler, Dept. Psychol., Kent State Univ., Kent, OH 44242 and Dept. Neurobiol., N.E. Ohio Univs. Coll. of Med., Rootstown, OH 44272.

Neurophysiological assessments in rats have shown that 2-, 3-, and 4-methylpyridine have effects on sensory function and cerebral excitability, causing effects similar to those produced by depressant agents. The present study assessed the effects of 2-, 3-, and 4-methylpyridine on rat hippocampal slice excitability. Slices were prepared using standard techniques and maintained at the interface of a pool of artificial CSF and an O_2/CO_2 atmosphere. Stimulating and recording electrodes were positioned in the Schaffer collaterals and CA1 cell body layer, respectively. When a stable waveform was recorded, an input/output (I/O) profile was obtained by administering a series of increasing stimulus intensities. Paired-pulse tests of inhibitory processes were also conducted. Tests of excitatory and inhibitory systems in area CA1 of the hippocampal slice were conducted over a period of 3 hr postexposure. Following exposures of 100 μM 2-, 3-, or 4-methylpyridine, evoked population EPSP and population spike responses recorded in the cell body field of hippocampal area CA1 were slowly suppressed over the course of 3 hr, whereas no effects on local inhibitory processes or latency of evoked responses were detected. No significant differences were observed between agents.
(Supported by US EPA and Johns Hopkins Center for Alternatives to Animal Testing.)

577.12

EFFECTS OF BILIRUBIN ON TRANSMEMBRANE ELECTRICAL AND PROTON GRADIENTS OF RAT CORTICAL SYNAPTOSOMES, AND ON RECONSTITUTED NICOTINIC ACETYLCHOLINE RECEPTOR FUNCTION. E.L.M. Ochoa, T. Nguyen*, R.P. Wennberg*, and T.Takashima*, Neonatology, University of California at Davis, Davis CA. 95616.

Bilirubin IXa, 4Z, 15Z (BR) depolarizes synaptosomes, releasing trapped tetraphenylphosphonium$^+$ ion (TPP$^+$). The intervening mechanisms (unknown) are acutely relevant to neonatal bilirubin encephalopathy. Equilibrium distribution values of ouabain-sensitive $^{86}Rb^+$ transport, and TPP$^+$ influx were monitored in rat cortical synaptosomes isolated by iso-osmotic Percoll-sucrose gradient centrifugation. Potassium (35 mM) or 4 μM human serum albumin (HSA) / 40 μM BR produced a rapid (1-2 min) efflux of both trapped cations. Steady-state pH gradients were estimated using the weak base methylamine in two media: (a) Krebs-Ringer, (b) Ca^{2+} and Na^+-free, 1 mM ouabain. Potassium released trapped methylamine in both conditions, but HSA/BR only showed this effect in synaptosomes incubated in Krebs-Ringer. Possible post-synaptic effects of BR were explored using purified *T.californica* nicotinic acetylcholine receptor (nAchR) reconstituted into Asolectin or dioleoylphosphatidylcholine-phosphatidic acid-cholesterol vesicles (60:20:20 molar ratio). BR (10-40 μM) did not modify: (1) the passive or the carbamylcholine (Carb)-stimulated (20-100 μM) $^{86}Rb^+$ influx, (2) the 5 - 10 μM Carb-induced first-order decrease in the Carb-stimulated $^{86}Rb^+$ transport, (3) the cation efflux from $^{86}Rb^+$-loaded *pure* Asolectin vesicles, (4) the equilibrium binding of ^{125}I-α-bungarotoxin, or, (5) the affinity shifts associated to nAChR desensitization. BR (50-100 μM) decreased the rate binding of toxin by 20% with respect to controls, and 10 μM pigment shortened (3-fold) the time course of receptor desensitization to 5 μM Carb. We conclude that BR truly depolarizes the synaptosomal plasma membrane, since intra-synaptosomal mitochondria have little or no Rb^+ permeability, and their membrane potential is independent of $[K^+]_{out}$. The pigment requires media containing Na^+, and Ca^{2+} to dissipate proton gradients, suggesting a BR–induced release of transmitter-filled vesicles (known to accumulate the base). Finally, BR displays little interaction with the nAChR. The observed effects on rate of toxin binding and receptor desensitization may be mediated through BR–vesicular lipid matrix interactions. *Supported by NIH Grant R01HD25241-01A2.*

577.13

MICROWAVE-INDUCED BRAIN HYPERTHERMIA ALTERS LOCAL CEREBRAL GLUCOSE UTILIZATION (LCGU). B.L. Cobb, G.A. Mickley and T.J. Nemeth, Armstrong Laboratory, Brooks AFB, TX 78235-5301

Alterations in LCGU in response to hyperthermia induced by fever or ambient temperature rise have been examined in some detail (Morimoto et al., Brain Res., 381, 1986, 100-106; McCulloch et al., J. Neurochem., 39, 1982, 255-258). Less is known about the patterns of LCGU produced by microwave radiations. In order to map these patterns, rat's basal brain temperature (Mean = 38.1$\pm$.1^{o}C) was raised 2^{o}C ($\pm$ <0.1^{o}C), in 9.4$\pm$1.97 minutes, through either head-only exposure to 5.6 GHz microwaves or hot-moist air (temperature control group). Hyperthermia was maintained for 45 minutes during which LCGU was assessed using the autoradiographic 2-Deoxyglucose method of Sokoloff et al. (J. Neurochem., 28, 1977, 897-916). Other rats were sham irradiated, and therefore experienced no brain temperature rise. Compared to normothermic controls, both microwaves and hot-moist air generally raised the LCGU of most brain areas sampled (e.g., somatosensory cortex and selected thalamic nuclei). However, microwave exposure produced rates of LCGU sometimes higher (e.g., in septal nuclei) or lower (e.g., in hypothalamic nuclei) than those recorded in hyperthermic controls. These data suggest that, while raising brain temperature generally enhances LCGU, distinct nuclei-specific alterations in metabolism may be observed during brain hyperthermia produced by 2 different sources. These phenomena may reflect either variable temperature gradients within the brain or unique cerebral functional alterations that vary with the source of thermal energy.

577.14

EFFECTS OF ^{60}Co IRRADIATION ON LOCOMOTOR ACTIVITY IN THE RAT. H.D. Davis, S.B. Kandasamy, V. Bogo and A.H. Harris. Armed Forces Radiobiology Research Institute, Behavioral Sciences Department, Bethesda, Maryland 20889-5145.

Male Sprague-Dawley albino rats were exposed to bilateral doses of 0 Gy (control), 0.5 Gy, 1.0 Gy and 3.0 Gy ^{60}Co radiation at 1.0 Gy/min. Irradiation occurred with 4 hours of light remaining in the animals' 12:12 hour light-dark cycle. Immediately after irradiation, rats were placed in automated monitors that measured locomotor activity (ambulation and vertical activity) for the next 24 h. The 0.5-Gy and 1.0-Gy irradiated rats showed about 75% of the cumulative ambulation of control rats throughout the experiment, while the 3.0-Gy group showed about 55% of cumulative ambulation of control rats. Deficits were significant during the periods of time when control rats showed enhanced activity: the first hour of exposure to the monitor (introduction to a novel environment) and the dark portion of the light-dark cycle (the normal period of high activity in the rat). Deficits in ambulation were not significant during periods when control rats showed low activity. Measurements of vertical activity showed a similar pattern of radiation-induced decrements. Future studies will examine the roles of neural and peripheral mechanisms in radiation-induced behavioral decrements.

577.15

HIGH ENERGY ELECTRON AND PROTON IRRADIATION OF RAT BRAIN INDUCES DEGENERATION DETECTABLE WITH THE CUPRIC-SILVER STAIN. R.C.Switzer III, V. Bogo*[1], and G.A. Mickley[1]. Dept. of Pathology Univ. of Tennessee Med. Ctr., Knoxville, TN 37920, [1]Armed Forces Radiobiol. Res. Inst., Bethesda, MD. 20889.

Different types of ionizing radiation can produce behavioral effects in laboratory animals but neuronal morphologic changes that correlate with these effects have not been detected using conventional histopathologic techniques. Since the silver-degeneration staining methods (eg Nauta, Fink-Heimer, and deOlmos' cupric-silver (CuAg)) impregnate disintegrated cell components that exist long after the cell has died, we used the CuAg method to detect degeneration induced by proton irradiation and levels of electron radiation that are known to influence behavior (Radiat. Res., 118: 341-352, 1989). Male rats (521g mn wt.) were irradiated with either 18.6 Mev electrons (N=20) or 155 Mev protons (N=16) at doses of 22-100 Gy @ 20 Gy / min (1Gy=100 rad). Six other rats, 319 g mn wt., were irradiated with 2-8 Gy of protons. All rats were sacrificed 4 days after exposure. CuAg stained sections from both electron and proton irradiated rats displayed diffuse and wide spread astrocyte-like degenerative profiles (DP) which were counted from 29 different cerebral areas for each brain to yield a total score that included the few neuronal profiles observed. A linear dose response was found for the low dose proton irradiated rats while the plot for the higher doses for both the electron & proton groups suggests a plateau effect with about the same DP scores. Control rats (6) averaged 1/5th of the median DPs found in those irradiated. It is concluded that: 1) the CuAg stain is able to reveal neuronal / glia degeneration due to these radiation levels; 2) degeneration vs dose is linear in the range of 2 - 8 Gy of protons.

577.16

BRAIN GLUTATHIONE S-TRANSFERASE (GST) ACTIVITY AND ISOENZYME PROFILES IN THE GUNN RAT. J.A. Johnson*, A. El Barbary*, S.E. Kornguth, J.F. Brugge and F.L. Siegel, Depts. of Pediatrics, Physiological Chemistry, Neurology and Neurophysiology, the Environmental Toxicology and Waisman Centers, University of Wisconsin, Madison, WI 53706.

GSTs are a multifunctional group of dimeric enzymes (α, μ or π-class) involved in the metabolic detoxication of electrophilic compounds and the intracellular binding and transport of hydrophobic molecules. GSTs were isolated from cerebrum, cerebellum and brainstem of Jj (non-jaundiced) and jj (jaundiced) Gunn rats, and the level of each subunit was determined by reverse-phase HPLC. Subunit 7 (π) was the major subunit in brainstem, whereas in the cerebrum, subunits 6 (μ) and 7 were expressed equally. The GST subunit pattern and GST activity in cerebrum and brainstem were the same in both Jj and jj rats. The primary subunits present in cerebellum were 3 (μ), 7, 6 and 11 (μ). In jj cerebellum, there was a 4-fold increase in subunits 2 (α) and 4 (μ) and a 2.5-fold increase in 8 (α) and 11 compared to Jj rats. There was a corresponding 1.5-fold increase in the total cytosolic GST activity in jj cerebellum. Immunohistochemical studies on cerebellum of normal and Jj animals showed that subunit 3 was present in Bergmann glia, but heterogeneously distributed among other astrocytes and Purkinje cells. Purkinje cells were strongly GST-positive in the flocculus and lateral hemispheres, but lightly-stained or GST-negative in the vermis. Thus, the increased GSTs in jj cerebellum could result from induction and/or toxicity-induced selection for GST-containing cells. These data imply that GSTs may be important in modulating the neurotoxicity of bilirubin. (Supported by NIH grants DC00398, NS24669, HD03352.)

578.1

ALTERATIONS IN PROTEIN SYNTHESIS BY ISOLATED ASTROCYTES EXPOSED TO LEAD. L. Shemancik*, D. Cory-Slechta and J.N. Finkelstein*, Env. Health Sci. Center and Strong Children's Res. Center Univ. of Rochester, Rochester NY. 14642

Prenatal and perinatal lead exposure has long been associated with significant behavioral impairments. The cellular and molecular mechanisms of these effects and the specific interactions between individual cell types in the CNS and added Pb have not been completely elucidated. The current study was undertaken to determine the nature of the response of isolated astroglial cells to exogenously added Pb. The effects of Pb and other metals were studied in 14-18 day primary astrocytes isolated from neonatal rat cerebral cortex. The cultures were >95% GFAP positive. These cells were then incubated with varying concentrations of lead acetate for up to 7 days. At varying times, cells were pulse labeled with ^{3}H-leucine and analyzed by SDS-PAGE followed by autoradiography. During the first 24 hours, cells exhibited protein synthesis patterns consistent with a classical stress response. After 12 hours, Pb exposed cells began to synthesize a unique protein species with an apparent molecular weight of 23-24 kDa. This protein persisted throughout the 7 day treatment period. The synthesis of this protein was dependent on the dose of Pb in the culture media and was not observed when cells were cultured with other metals including Zn, Cd, Mn, or Ca^{++} controls. Subcellular fractionation suggested that this protein was localized to the cytosolic fraction of cells. The relationship between this astroglial protein and those observed in whole brain or kidney from lead-exposed animals remains to be elucidated. (Supported in part by ES 07026, ES 01248, and HL 36543)

578.2

LACK OF EFFECT OF CHRONIC LEAD TREATMENT ON BIOAMINE METABOLITE CONCENTRATIONS IN RHESUS MONKEY CEREBROSPINAL FLUID (CSF). S. A. Ferguson*, G. W. Kraemer*, R. E. Bowman, M. H. Ebert*, and D. E. Schmidt. National Center for Toxicological Research, Division of Reproductive and Developmental Toxicology, Jefferson, AR; Harlow Primate Laboratory, University of Wisconsin, Madison; Vanderbilt University School of Medicine, Nashville, TN.

Concentrations of norepinephrine (NE), 3-methoxy-4-hydroxyphenylglycol (MHPG), 3,4 dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 5-hydroxy-indoleacetic acid (5-HIAA) were measured in the CSF of developing rhesus monkeys treated orally with moderate levels of lead acetate (n=4) or sodium acetate (n=4). Blood lead levels peaked at 62 μg/dl at 1.5 months of age and were maintained at 14 μg/dl from 20-56 months of age. Cisternal CSF samples were collected monthly from 5-41 months of age and every 1-3 months until 56 months of age. Bioamine metabolite concentrations were assayed by high-performance liquid chromatography. As is typical of rhesus monkeys, HVA and 5-HIAA concentrations decreased with age. NE and MHPG levels remained fairly stable across time. Concentration of DOPAC began to decrease at approximately 40 months of age. However, no measure interacted with lead treatment and overall concentrations for the five measures were not significantly different between control and lead-treated monkeys. As measured in cisternal CSF, bioamine metabolites were not altered by chronic low-level lead treatment.

578.3

Sensitive Measurements of Lead Toxicity in Cultured Astroglia by Interactive Laser Cytometry. M.E. Legare*, R. Barhoumi,* R.C. Burghardt* and E. Tiffany-Castiglioni. Department of Veterinary Anatomy and Public Health, Texas A&M University. College Station, TX 77843.

Low levels of lead (Pb) are proposed to damage cells at subcellular sites, including several calcium (Ca^{2+}) homeostatic pathways, mitochondria, and gap junctions. Sensitive measurements for damage at these sites were carried out with an ACAS 570 Interactive Laser Cytometer. Astroglial primary cultures were prepared from neonatal rat cerebral hemispheres. After 10-20 days in culture the cells were treated daily with medium containing 1 μM or less of Pb acetate and were subcultured before examination with the laser cytometer. Intracellular calcium ($[Ca^{2+}]_i$) levels were measured with an Indo-1 label. In preliminary experiments astroglia responded to ionomycin by a rapid 50% increase in $[Ca^{2+}]_i$ followed by partial recovery towards basal levels. Pb-treated astroglia showed a blunted Ca^{2+} spike in response to ionomycin. In a control experiment, cells were bathed with Ca^{2+}-free medium. Astroglia not treated with Pb had no response to ionomycin. By contrast, Pb-treated astroglia showed a marked Ca^{2+} spike followed by recovery. This finding indicates that Pb alters the handling and storage of $[Ca^{2+}]_i$. Other parameters measured included cell-cell communication via gap junctions, which was unaffected by Pb treatment, and mitochondrial permeability, which was reduced after one week of Pb treatment but not after three weeks.

578.4

SELECTIVE TOXICITY TO CENTRAL CATECHOLAMINERGIC NERVOUS SYSTEM IN POSTNATALLY LEAD EXPOSED RAT. J.H. Ryu*, J.H. Cheong*, J.H Jung*, U.T. Oh and K.H. Ko. Department of Pharmacology, College of Pharmacy, Seoul National University, Seoul 151, Korea.

Possibility whether postnatal lead ingestion can cause selective toxicity to central catecholaminergic nervous system in rats was tested. Three groups of Wistar rats; 1) Control, 2) Low dose and 3) High dose groups, were prepared. Right after parturition from dams rat pups received drinking water containing either 0% (control), 0.05% (low dose) or 0.2% (high dose) of lead acetate. At 2, 4, 6 and 8 weeks of age, tyrosine hydroxylase (TH) activity and Na-K ATPase activity were measured in 4 areas of rat brain; Telencephalon, Diencephalon, Midbrain and Pons-Medulla. TH activities were assayed by modified method of Reinhard *et al.* (1986) using ^{3}H-[3,5]-L-tyrosine as substrate. TH activity was determined as a criterion of lead poisoning to central catecholaminergic nervous system and ATPase activity as a criterion of nonspecific lead poisoning to any kinds of tissues. In lead exposed rats, TH activities were higher but Na-K ATPase activities were lower than those observed in age-matched control animals. Selective toxicity of lead poisoning to central catecholaminergic nervous system was evaluated by the changes of TH activities without concomitant changes of ATPase activities. Brain areas where selective toxicity of lead seems to be induced were pons-medulla (2 weeks of age) and telencephalon (6 weeks of age) in low dose group, and midbrain (4 and 6 weeks of age) in high dose group.

578.5

THE EFFECTS OF ALUMINUM INGESTION ON SPATIAL MEMORY IN THE RAT. T.L. Brown, P. Stewart*, J.P. Ryan. Department of Psychology, State University of New York at Plattsburgh, Plattsburgh, NY 12901

The study tested the hypothesis that spatial memory deficits in laboratory rats may be induced by oral administration of a daily solution comprised of aluminum chloride and sodium citrate. Aluminum (AL3+) is capable of bonding with citrate to form a neutral AL3+ citrate complex that may pass through internal membranes including the blood-brain barrier. Thirty-six Long-Evans hooded rats were divided randomly into one of three experimental conditions for sixteen weeks. The control group received a daily oral aqueous solution comprised of water and sodium citrate. The low and high groups received either 100 or 1,000 ppm aluminum chloride, dissolved in a sodium citrate solution. Each animal was rotated regularly into metabolic cages for a twenty-four hour period. The animals were then behaviorally tested in the Morris water maze to assess spatial memory impairments. Brain aluminum content (BAC) was analyzed by Electrothermal Atomic Absorption Spectrophotometry. Aluminum in the urine and feces was analyzed by Flame Atomic Absorption Spectrophotometry. There was a significant difference of BAC in neuronal tissue between the high group and control group, but no significant differences in spatial learning and memory between groups. Although aluminum can enter the brain in the presence of citrate there appears to be no significant correlation between BAC, other variables, and behavioral deficits. Long-term intake of aluminum-citrate and subsequent behavioral testing of the aged rat may clarify the role of AL3+ in AD.

578.6

EFFECTS OF ALUMINUM ON THE PHOSPHATASE ACTIVITY OF RAT SYNAPTOSOMAL $(Na^+ + K^+)$-ATPase. M.L. Caspers, P.S. Jacques* and M.J. Fu* Dept. of Chem. Univ. of Detroit Mercy, Detroit, MI 48221.

The presence of aluminum in the CNS has been linked to several neurological disorders, including Alzheimer's disease. $AlCl_3$ inhibits the release of ^{32}P from ATP catalyzed by the $(Na^+ + K^+)$-ATPase of cerebral cortical synaptosomes in a dose dependent manner. At 25 μM $AlCl_3$, the enzyme from adult rats is inhibited 14.5% and this increases to 46.8% at 100 μM $AlCl_3$. Sodium citrate (100 μM), added after pre-incubation with $AlCl_3$(100 μM), fails to reverse the inhibitory effect of $AlCl_3$, however it can prevent inhibition of $(Na^+ + K^+)$-ATPase when added simultaneously with $AlCl_3$. Lineweaver Burk analysis of enzyme activity with varying concentrations of ATP indicates a reduction in the V_{max} but not the K_M of the $(Na^+ + K^+)$-ATPase in the presence of $AlCl_3$, suggestive of noncompetitive inhibition. A comparison of the enzyme in synaptosomes prepared from young or aged rats shows that the V_{max} is significantly elevated in aged rats. The susceptibility of the $(Na^+ + K^+)$-ATPase to 100 μM $AlCl_3$ is greater (72% inhibition) in the aged group. Disruption of the $(Na^+ + K^+)$-ATPase in neurons may underlie, at least in part, the neurotoxicity associated with an elevated aluminum content in the CNS. (Supported by Alzheimer's Disease Research of the Amer. Health Assist. Found. and a gift from J. Rose.)

578.7

ALUMINUM-INDUCED NEURODEGENERATION IN THE RABBIT RETINA. K.R. Fry, A.L. Chapman, K.A. Shaw and C.B. Watt. Alice R. McPherson Laboratory of Retina Research, Center for Biotechnology, Baylor College of Medicine, The Woodlands, TX 77381.

Study of the effects on the nervous system of long-term exposure to aluminum has been hampered by the unavailability of an appropriate animal model. Recent studies in this laboratory (Fry et al, Neurosci. Lett. 124:216-220, 1991) have examined aluminum-induced neurofibrillary changes in rabbit retinal ganglion cells as a potential long-term model system for aluminum neurotoxicity. The current study examines the dose-response and temporal relationships of the disorder. Adult New Zealand White rabbits were injected intravitreally with concentrations of $AlCl_3$ ranging from 0.05% to 2.0% and allowed to survive for periods ranging from 1-3 months. The retinas were then prepared for light level immunocytochemistry using either a ganglion cell-specific or a neurofilament-specific antibody. The results of the study indicated that the extent of neurofibrillary change is directly related to both the dosage of aluminum and the time post-injection. At low aluminum dosages (<1%), the vast majority of ganglion cells undergoing neurofibrillary degeneration were located in the retinal periphery suggesting a specific susceptibility of ganglion cells in these regions to aluminum. At higher aluminum dosages (1% and above), the distribution of ganglion cells undergoing neurofibrillary degeneration more closely followed the normal total ganglion cell distribution, suggesting a more generalized toxicity. Examination of total ganglion cell populations indicated that there is a loss of ganglion cells which also appears to be directly related to the dosage of aluminum as well as the time post-injection. Interestingly, the loss of ganglion cells occurs primarily in the central retina, suggesting that some mechanism other than the neurofibrillary disorder may be responsible for ganglion cell death. Supported by the American Health Assistance Foundation and The Retina Research Foundation.

578.8

NEUROBEHAVIORAL EFFECTS OF SUBCHRONIC DIETARY ALUMINUM IN MICE. M. S. Golub, C.L. Keen*, M.E. Gershwin*. Dept Internal Medicine, Univ. California, Davis Sch. Med. Davis, CA 95616.

Female Swiss Webster mice (n=12/grp) were fed purified diets containing 25 (control) or 1000 µg Al/g diet (Al as Al lactate). Weight, food intake and gross neurotoxic signs were monitored regularly. At 45 and 90 days a modification of the NTP neurotoxicity screening battery was administered and spontaneous motor activity was determined using automated method for 1 24-hr period. At necropsy, brain and liver Al and trace metals (Fe, Mn, Cu, Zn) concentrations were determined by AAS and lipid peroxidizability was measured by generation of thiobarbituric acid reactive substances (TBARS) in vitro. Al was associated with a slight acceleration of weight gain during the second half of the 90-day period; no effect on food intake and no mortality or morbidity were observed. Relative to controls, mice fed 1000µg Al/g diet had lowered locomotor activity (p=.015), hindlimb grip strength (p=.012) and air puff startle responsiveness (p=.024) at the 90 day time point. Temperature sensitivity (tail flick test), negative geotoaxis, forelimb grip strength and footsplay were not affected. Al was increased 3-fold in both brain and liver (p<.001) but not in femur. Essential trace elements were not altered and there was no effect on TBARS measures. These results demonstrate a specific pattern of neurobehavioral deficit caused by subchronic dietary exposure to Al lactate and suggest the value of further testing using more sensitive endpoints and/or longer exposure periods and higher doses. (Supported by ES04190).

578.9

EFFECT OF $ZnCl_2$ ON ^{54}Mn-UPTAKE BY SYNAPTOSOMES OF MOUSE BRAIN. J. Estévez*, R. Katiyar*, E. Bonilla, H. Martínez. Instituto de Investigaciones Clínicas. Universidad del Zulia. Apartado 1151. Maracaibo, Venezuela.

The accumulation of Mn in brain decreases when given together with zinc in mice (Khandelwal S. et al. Bull Environ Contam Toxicol 32:10,19-84). We studied the in vitro effect of Zn^{+2} on ^{54}Mn uptake by synaptosomes. Male albino mice were killed by cervical dislocation and their brains removed immediately. Striatum (ST), olfactory bulb (OB) and hypothalamus (HT) were dissected out. Synaptosomes were obtained by the technique of Hajos F.(Brain Res 93:485,1975). Isolated synaptosomes were suspended in 0.3M sucrose and assays were performed in two different ways as follows: a) 200ul of the synaptosomal preparation were added at 37ºC for 15 min to a solution containing 0.05 M tris-buffer, pH 7.4, 0.02 uCi of $^{54}MnCl_2$ and increasing concentrations of $ZnCl_2$ (10^{-1} to 10^{-4}M). The final volume of the incubation mixture was 0.5 ml. b) On other assays $ZnCl_2$ was added after a 15-min preincubation of the above mixture. Incubations were ended by suction through a Skatron cell harvester. The radioactivity contained in the filters were counted in a gamma counter. With a concentration of 10^{-4}M $ZnCl_2$ the ^{54}Mn uptake was completely inhibited in OB. For ST and HT a concentrations of 10^{-3}M was needed to obtain a similar inhibition. On the contrary, after preincubating the synaptosomes with ^{54}Mn the maximun concentration of Zn^{+2} used (10^{-1}M) displaced only 49.5±3.9%, 33.7±4.7% and 31.7±3.7% of the ^{54}Mn taken up by OB, ST and HT, respectively. The inhibitory effect of Zn^{+2} on Mn uptake by synaptosomes seems to be due to a higher binding affinity for Zn^{+2} than Mn. The results obtained after preincubation seem to indicate that ^{54}Mn is apparently bound in an insoluble or high affinity state to intra synaptosomal structures(i.e.synaptic vesicles) and/or molecules(i.e.ATP).

578.10

MANGANESE TOXICITY: MUSCARINIC RECEPTOR BINDING IN THE MOUSE BRAIN. E. Bonilla, V. Villalobos*, F. Castro*, J. Estévez*, J.O. Dávila*. Instituto de Investigaciones Clínicas, Universidad del Zulia and Inbiomed-Fundacite, Apartado 1151, Maracaibo, Venezuela.

Cholinergic neurons seem to be resistant to the toxic effect of manganese, since the activity of choline-acetyltransferase was not affected in any of 7 brain regions studied in rats after eight months of treatment with manganese. We now report the effect of chronic administration of manganese on the binding parameters of ^3H-quinuclidinyl benzilate (^3H-QNB) in different brain regions of mice. Experiments were carried out on male albino mice divided into two groups. Mice in one group were injected i.p. with manganese chloride (5mg Mn/kg/day) five days per week during nine weeks. The control group was treated similarly with saline. Mice were killed by cervical dislocation two days after the end of the intoxication. Brains were extracted immediately and the following regions dissected out: striatum, hippocampus and frontal cortex.

The binding of ^3H-QNB to homogenates of mouse brain was performed according to the method of Yamamura and Snyder (Proc. Nat. Acad. Sci USA 71:1725, 1974). The Bmax values (means ± S.E.M.) of ^3H-QNB in the three brain regions of controls and Mn-treated mice were as follows: a) striatum: 344.2 ± 45.2 and 371.0 ± 90.8 fmol/mg protein; b) frontal cortex: 194.5 ± 21.1 and 232.0 ± 13.3; c) hippocampus: 219.1 ± 23.6 and 239.0 ± 30.8. The Kd's found were: a) striatum: 1.46 ± 0.3 and 1.32 ± 0.45 nM; b) frontal cortex: 0.80 ± 0.20 and 0.71 ± 0.15; c) hippocampus: 0.86 ± 0.10 and 0.93 ± 0.17.

The results of the present study clearly show that chronic manganese administration does not alter the binding constants of ^3H-QNB to the muscarinic cholinergic receptors in mouse brain.

578.11

AN IMMUNOCYTOCHEMICAL STUDY OF THE EFFECTS OF TRIMETHYLTIN ON PEPTIDERGIC NEURONS WITHIN THE HIPPOCAMPUS. L. L. Addington*, K. LeVee*, R. H. Baisden and M. L. Woodruff. J. H. Quillen Col. of Med. East Tenn. St. U., Johnson City, TN 37614.

It is well established that trimethyltin (TMT) causes loss of projection neurons in the CA3c and CA1 subfields of Ammons horn and the temporal part of the dentate gyrus. The effects of TMT on hippocampal neurons containing specific neuropeptides has been less well documented. Immunocytochemistry visualized vasoactive intestinal polypeptide (VIP), somatostatin (SS), and cholecystokinin (CCK) in the hippocampus of male Long-Evans rats exposed to 6 mg/kg TMT. When compared to controls cell counts revealed a significant loss of neurons showing VIP- and SS-like immunoreactivity in rats exposed to TMT. CCK appeared to be less affected by the toxin. These effects resemble those produced by cerebral ischemia (Johansen et al., Acta Neuropathol. 73:110-114, 1987) and may be interpreted to indicate that loss of SS neurons, which presumably excite inhibitory GABAergic neurons, may contribute to loss of CA1 pyramids after TMT. (Supported by NIH Grant ES 04070-05 to MLW)

578.12

TRIMETHYLTIN (TMT) AND GLUTAMINE-EVOKED RELEASE OF GLUTAMATE FROM HIPPOCAMPAL AND CAUDATE SLICES OF RAT BRAIN. A.C. Scallet, G.W. Lipe*, J.C. Matthews[1] and J.F. Bowyer. National Center for Toxicological Research, Jefferson, AR 72079 and [1]University of Mississippi, University, MS 38677

We have previously reported that although the hippocampal neurotoxin trimethyltin (TMT) produced comparable increases of hippocampal glutamine in both 7 and 17 month old rats, it produced passive avoidance deficits, hippocampal neuronal necrosis and gliosis only at 17 months. Glutamate levels measured in whole hippocampal extracts were reduced in the older rats, while their glutamate receptors (KA sub-type) were increased. Although TMT had little effect on total glutamate content "in vivo", there might be a selective effect on extracellular release. Therefore, we superfused glutamine and TMT through hippocampal and caudate slices and measured glutamate in the effluent. Glutamine (up to 1mM) produced a dose-related increase in glutamate release of at least 15-fold, while addition of TMT (up to 13 uM) only slightly increased this release. Since potassium increased the glutamine effect, but in a non-concentration-related fashion, we are at present uncertain of the exact mechanism of the glutamine effect. However, our results are consistent with the possibility of an indirect action of TMT to produce neurotoxicity secondary to glutamine-evoked release of glutamate.

578.13

BISMUTH DISTRIBUTION IN MOUSE CNS AFTER INTRAPERITONEAL DOSING. M.R. Poston, J.F. Ross and G.T. Lawhorn*. Miami Valley Laboratories, Procter & Gamble Co., Box 398707, Cincinnati, OH 45239-8707.

Routes of entry and distribution of metals into the CNS have toxicologic significance. To determine the distribution of bismuth (Bi), adult female Swiss-Webster mice were injected ip once with dH2O (n=10) or 2500 mg/kg Bi subnitrate (n=22) as a 10% suspension in dH2O to establish a Bi depot. Four weeks later, mice were anesthetized and perfused with aldehyde fixatives; brain and spinal cord were processed for frozen and paraffin sectioning. Bi was coated with silver using autometallography and visualized as silver grains. Sections were counterstained with toluidine blue.

Control sections had negligible Bi. In dosed mice, somata of brainstem upper motor neurons (in red, lateral vestibular, gigantocellular reticular and lateral reticular nuclei) had dense intracytoplasmic granulation. Similar, but less dense granulation was observed in somata of cranial and spinal motor nerves and large projection neurons (mitral and purkinje cells). Small cells in primary projection zones of spinal and cranial sensory nerves had lighter, intracellular accumulations. Gray matter adjacent to circumventricular organs had multifocal aggregates not clearly associated with neuronal somata. White matter had few grains except for fornix and corpus callosum near the subfornical organ. Choroid plexus epithelium had diffuse grains throughout, while meninges had both diffuse grains and aggregates. There were several gradients of Bi, including a ventral (higher)/ dorsal (lower) gradient in olfactory bulb, and a ventral/dorsal gradient in cerebellum. These patterns suggest mechanisms for entry and distribution of Bi into CNS that may apply to other metals.

578.14

REGIONAL BISMUTH LEVELS AFTER PARENTERAL DOSING IN MICE AND FERRETS. J. F. Ross and G. T. Lawhorn*. Miami Valley Laboratories, Procter & Gamble Co., Box 398707, Cincinnati, OH 45239-8707.

Bismuth (Bi) neurotoxicity (encephalopathy) occurs rarely during long term oral treatment with Bi-containing medications. Signs of neurotoxicity (hyperreactivity, myoclonus, ataxia, tremors, convulsions) like those in humans are produced by ip injection(s) of Bi subnitrate (BSN) into mice (Ross et al., NeuroToxicology 9(4):581-6, 1988). Here we measured regional brain Bi levels of animals under different subchronic exposure conditions.

BSN or the sodium salt of Bi subgallate (NaBSG) or dH2O (n=4-6) was injected ip into female mice (BSN, NaBSG) or ferrets. Parts of CNS and PNS were dissected and Bi level measured by atomic absorption spectrometry/hydride generation after wet ashing with acid. BSN (2500 mg/kg) was dosed once and regions analyzed 28 days later (mice; n=9) or dosed (100-200 mg/kg) up to four times during eight weeks (ferrets; n=11). NaBSG was dosed (20 mg/kg/day) to mice (n=10) five days/week for 13 weeks. Regional Bi levels were indexed to the hypothalamic Bi level and compared by analysis of variance followed by Student's t-test or a rank sum test adjusted for multiple comparisons.

Bi distribution was independent of type of Bi dosed, the dosing regimen or the species tested. In the CNS, levels were highest in meninges (15 ppm). Relatively high levels of Bi were also found in lumbosacral spinal cord (10 ppm), hypothalamus (6-9 ppm), olfactory bulb (4-7 ppm) and brainstem (2-3 ppm). Significantly lower levels were found in cerebellum, striatum and cerebral cortex (all ~1-2 ppm). The data complement autometallographic studies (see M. R. Poston et al., this meeting) and indicate that Bi is inhomogeneously distributed in the nervous system, and concentrates in sites consistent with the signs of neurotoxicity.

578.15

ORGANIC AND INORGANIC MERCURY ALTER SYNAPTOSOMAL CALCIUM CONCENTRATIONS. M.F.Hare, M.F.Denny*, W.D.Atchison. Dept. Pharm./Tox. & Neurosc. Prgm., Mich. State Univ., E. Lansing, MI 48824.

Methyl mercury (MeHg) increases the release of neurotransmitters and intracellular calcium ($[Ca^{2+}]_i$) in mammalian nerve terminals. The Ca^{2+} pool(s) responsible for the elevated $[Ca^{2+}]_i$ and transmitter release are unknown. Less is known about the effects of inorganic mercury (Hg^{2+}) on nerve terminal $[Ca^{2+}]_i$ and transmitter release. Ruthenium red (RR), a polysaccharide dye, decreases MeHg-induced acetylcholine release from synaptosomes and $^{45}Ca^{2+}$ efflux from isolated rat brain mitochondria. We therefore examined the ability of RR to prevent elevations in synaptosomal $[Ca^{2+}]_i$ by MeHg and Hg^{2+}. In fura-2 loaded synaptosomes, MeHg and Hg^{2+} (10 μM each) increased $[Ca^{2+}]_i$ by 55 and 121 nM respectively in media containing 200 μM Ca^{2+}. Neither effect was altered by pretreatment with 20 μM RR. This concentration of RR had no effect on $[Ca^{2+}]_i$. Alterations in $[Ca^{2+}]_i$ and transmitter release by MeHg and Hg^{2+} may be mediated by an increase in the permeability of the plasma membrane to Ca^{2+}. To test this possibility we took advantage of the effect of Mn^{2+} on fura-2 fluorescence. Mn^{2+} decreases fura-2 fluorescence upon binding. Since synaptosomes contain no Mn^{2+}, decreases in the intrasynaptosomal fluorescence signal must result from divalent cation influx. Addition of 1 mM Mn^{2+} decreased the fluorescence signal at the 340 nm excitation wavelength by 70.9% after 4 min. This decrease was not altered by pretreatment of the synaptosomes for 6 min with either 10 or 25 μM MeHg. Pretreatment with 10 μM Hg^{2+} prior to Mn^{2+} addition decreased the 340 excitation signal by 79.9%. These preliminary data suggest that increases in $[Ca^{2+}]_i$ by MeHg may result from an action on intracellular, RR insensitive Ca^{2+} pool(s), whereas Hg^{2+} may increase $[Ca^{2+}]_i$ by actions on both the plasma membrane and intraterminal Ca^{2+} pools. Supported by NIEHS grant ES03299.

578.16

ALUMINUM TREATMENT IMPAIRS THE ABILITY OF ASTROGLIA TO PROTECT MOTONEURONS AND CORTICAL NEURONS FROM GLUTAMATE EXCITOTOXICITY. J.B. Sass* and B.H.J. Juurlink. Dept. of Anatomy, University of Saskatchewan, Saskatoon, Canada, S7N 0W0.

Aluminum has been implicated as a neurotoxin in a number of neurological disorders, including Amyotrophic Lateral Sclerosis, Alzheimer's disease, and senile dementia. Glutamate may, under pathological conditions, act as an excitotoxin. In experiments designed to examine synergistic interactions between aluminum (Al) and glutamate we demonstrated that Al-treatment of astrocytes impaired their ability to protect neurons from glutamate excitotoxicity. Astroglial cells were isolated from newborn CD1 mice and cultured on coverslips for 2 weeks. Motoneurons were isolated from E15 CD1 mouse embryos and cultured on coverslips for six days. Astroglial and neuronal cultures were then treated with aluminum citrate at a final concentration of 100 µM for 3 days. Control or Al-treated astrocytes were co-cultured with the control or Al-treated motoneurons; these co-cultures were treated with glutamate at a final concentration of 50 µM. Cell viability was determined 24h later using the fluorescein diacetate-propidium iodide fluorescent technique (Jones and Senft. 1985. J Histochem Cytochem, 33:77). Glutamate did not affect the viability of control or Al-treated neurons when co-cultured with control astrocytes; however, glutamate caused death of all neurons (both control and Al-treated) when neurons were co-cultured with Al-treated astrocytes. These experiments have been repeated using cortical neuron cultures with similar results. Our observations demonstrate that sublethal levels of aluminum will severely impair the ability of astroglial cells to protect neurons from glutamate excitotoxicity, rendering normally subtoxic levels of glutamate lethal to neurons. This suggests that impaired astroglial function may play a causal role in diseases involving neuronal degeneration. We are currently comparing glutamate uptake in control and Al-treated astrocytes.

578.17

AN *IN VITRO* ASSAY OF IRON INDUCED NEURONAL MEMBRANE DAMAGE: PROTECTION BY ANTIOXIDANT LAZAROIDS. H.M.Scherch*, P.F.Vonvoigtlander, and E.D.Means. CNS Diseases Research, The Upjohn Company, Kalamazoo, MI 49001.

Membrane damage related to lipid peroxidation has been implicated in the pathophysiology of a variety of diseases. The Upjohn Company has developed a series of compounds, known as the lazaroids, aimed at preventing the propagation of the lipid peroxide chain reaction. Several of these compounds have been tested in an *in vitro* model of Fe^{++} induced lipid peroxidation.

Fetal mouse spinal cord neuronal cultures were treated with various lazaroids at concentrations ranging from 0.3 to 100 µM. The cultures were then exposed to 200 µM ferrous ammonium sulfate for 30 min to induce lipid peroxidation. Membrane damage and cell viability were determined by ^{3}H-α aminoisobutyric acid uptake. Of the 21-aminosteroids, U-74006F showed significant improvement above control, and U-74500A was highly efficacious with an 86.5% increase in viability above control at 10 µM. The 2-methylamine chroman, U-78517F, was the most potent of the compounds tested. It displayed a maximum increase of 98% above control at 1 µM, and was used as an internal standard for all compounds tested.

This assay provides a neuronal culture method of detecting compounds that protect against Fe^{++} induced lipid peroxidation and subsequent membrane dysfunction and death.

578.18

SCAVENGER ENZYMES IN SUPEROXIDE DISMUTASE TRANSGENIC MICE. **S. Przedborski, V. Jackson-Lewis, V. Kostic, E. Carlson, C. J. Epstein & J.L. Cadet. Columbia University, Dept. of Neurology, New York, N.Y. 10032**

Oxygen-based free radicals have been implicated in the pathogenesis of several neurological disorders. Transgenic (Tg) mice with increasedCu/Zn-superoxide dismutase (SOD) activity, one of the key enzymes of the cellular scavenging system, is an animal model of critical importance to test the effects of free radicals in vivo. In the present study, we measured Mn-SOD, catalase and glutathione peroxidase (G-Px) activities in cytosolic and particulate fractions in various brain regions of SOD-Tg and their non-Tg littermates. Cytosolic fractions exhibited a 3-fold increase in Cu/Zn-SOD activity and a 2-fold increase in catalase activity in Tg compared to non-Tg littermates; the activities of both enzymes correlate significantly ($r=.71$, $df=29$, $p=0.0001$). In contrast, there was no change in G-Px in SOD-Tg mice compared to non-Tg animals. Particulate fractions showed a 4-fold increase in Cu/Zn-SOD activity which correlated significantly ($r=.80$, $df=29$, $p=0.0001$), with a 1.8-fold increase in Mn-SOD activity in Tg compared to non-Tg mice. In contrast, the two groups of mice showed no significant differences in catalase and G-Px activities. These findings indicate that increases in brain Cu/Zn-SOD activity in Tg mice are associated with significant increases in other scavenger enzymes. These results provide more information on the free radical scavenging capacity of the SOD-Tg mice and indicate that SOD-Tg mice may be able to buffer any excess H_2O_2 produced in response to enhanced dismutation of superoxide radicals in these animals.

578.19

CEREBELLAR GRANULAR CELL MODELS FOR TESTING OF PROTECTION AGAINST NEUROTOXICITY. G.J.Fici*, J.S. Althaus, and P.F.VonVoigtlander. The Upjohn Company, Kalamazoo, MI 49001

A novel *in vitro* method for testing various lipid peroxidation inhibitors' (lazaroids) ability to protect cerebellar granular cells has been developed. These neurons possess excitotoxin receptors and have been used in studies involving excititory amino acids. We have postulated that they would be susceptible to injury induced by buthionine sulfoximine (BSO), an inhibitor of γ-glutamylcystein synthetase, and ferrous ammonium sulfate (FAS), an initiator of lipid peroxidation. This study reveals BSO (10-1000μM) after 24 hrs produces a dose-dependent reduction of glutathione (GSH) in the intact cells, as determined by HPLC with electrochemical detection, along with a reduction of cell viability as measured by amino-isobutyric acid (AIB) uptake. Data will also be presented which shows the importance of glutathione in FAS toxicity. In addition, our results show that pretreatment with lazaroids can block these toxic insults. It is hoped that the described toxicity models will reveal mechanisms of lazaroid-mediated neuronal protection.

578.20

LIPID HYDROPEROXIDES IN CEREBRUM AND CEREBELLUM OF RAT BRAINS. Eli Kaplan. Research Service, VA Medical Center Minneapolis, MN 55417.

As a prelude to studies on the metabolism of arachidonic acid in the brains leading to the formation of lipid hydroperoxides, studies were done to determine if rapidly frozen tissues could be used as a control. Male, S/D rats, 150-200g, were fasted overnight prior to perfusion with 5% heparin in K-R bicarbonate buffer, pH 7.6. Cerebrum and cerebellum were quickly excised, dropped into liquid N_2 and kept at -70°C for 5 days. In the 2nd set of experiments the brain tissues were quickly excised, homogenized in same buffer and lipids extracted using Bligh-Dyers method. The frozen tissue was homogenized and lipids extracted in a similar manner. The lipid classes were separated by SPE, and individual lipid hydroperoxides identified and quantitated by reverse phase HPLC column chromatography using acetonitrile:0.02 M PO_4 buffer as mobile phase. The eluant was monitored at 235 and 215 nm. 15-, 12-, 9-, and 5- hydroperoxy-eicosatetraenoic acid (HPETE) were observed in both sets of experiments. The quantity of all the HPETEs in the frozen group was 3 times greater than in the freshly homogenized cerebrum and cerebellum. 12- and 9-HPETEs were found in the largest quantity in both groups. The quantity of HPETEs in the cerebellum appeared to be greater than the cerebrum. It is apparent that tissues, frozen in liquid N_2, produce large quantitites of HPETEs, and can not be used as a control.

578.21

REACTIVE OXYGEN SPECIES DETECTED BY MICRODIALYSIS IN BRAIN EXTRACELLULAR FLUID INCREASE DURING SEIZURES. M.E. Layton*, T.L. Pazdernik, S.R. Nelson and F.E. Samson. Dept. of Pharm., Tox. and Therap. and R.L. Smith Res. Ctr., Kansas Univ. Med. Ctr., Kansas City, KS 66103.

Reactive oxygen species (ROS) such as hydroperoxides (ROOH) may form free radicals responsible for excitotoxic neuropathology. Free radicals damage critical components of membranes, DNA and proteins when normal cellular defenses are overwhelmed. Intracerebral microdialysis sampling permits the analysis of biochemical constituents in the brain cell microenvironment in awake animals. To determine if hydroperoxides are present in brain extracellular fluid, we analyzed the microdialysis perfusates from the piriform cortex of rats (N=6) prior to and after a systemic injection of kainic acid (16 mg/kg; i.p.). Perfusates were collected during control and seizure phases and analyzed in a hydroperoxide-dependent chemiluminescence assay. Hydroperoxides were detected under control conditions and increased substantially during the seizure episode. Pretreatment of perfusate samples with catalase (20 U/ml) reduced chemiluminescence to blank levels, indicating that hydrogen peroxide (HOOH) was the major hydroperoxide detected in brain extracellular fluid during seizures. These results suggest that the conspicuous brain damage in the piriform cortex following kainic acid-induced seizures may be mediated by free radicals. Supported in part by U.S. Army DAMD 17-90-C-0041 and ESO7079.

NEUROTOXICITY: ENVIRONMENTAL

579.1

EFFECT OF HYDROGEN SULFIDE ON MEMBRANE AND SYNAPTIC CONDUCTANCES IN DORSAL RAPHE *IN VITRO*. Samuel B. Kombian, R.J. Reiffenstein and W.F. Colmers. Dept of Pharmacology, Univ. of Alberta, Edmonton, AB, Canada T6G 2H7.

Hydrogen sulfide (HS^-) is suspected to cause asphyxiation and death by an action on respiratory neurons in the brainstem. Dorsal raphe (DR) nucleus neurons share many properties with these respiratory neurons. We studied the effects of HS^- on DR cells in transverse slices of brainstem *in vitro*.

Intracellular voltage and current clamp recordings were made with 2M KCl-filled microelectrodes. Focal (bipolar) stimulation of the nucleus evoked synaptic responses: fast, excitatory amino acid (EAA) and $GABA_A$ currents were followed by an IPSC (5-HT_{1A}), then by a slower EPSC (α_1 adrenoceptor). HS^- produced a dose-dependent inhibition of both IPSC (ED_{50}≈105 μM) and EPSC (ED_{50}≈50 μM). 200 μM HS^- inhibited the IPSC by 88.5±6.8% (n=4), while completely abolishing the EPSC. Four different responses to HS^- were observed: 1) an outward current or hyperpolarization caused by an increase in membrane conductance over the voltage range -115 to -40 mV (E_{Rev}=85.5±3.8 mV, n=4), partially blocked by 100 μM Ba^{++} (n=1) and completely blocked by Ba^{++} plus 2 mM Cs^+ (n=3); 2) a voltage-independent inward current (n=6) which was sensitive to strophanthidin (3-10 μM, n=6) [Cs^+ and Ba^{++} unmasked this in cells having an outward current]; 3) a biphasic response (n=8), first an inward current (sensitive to strophanthidin, n=1) then an outward current (sensitive to Ba^{++}+Cs^+, n=1); 4) no response (n=4). After washing out HS^-, a voltage-independent, strophanthidin-sensitive outward current appeared briefly.

These results indicate that in DR, HS^-: 1) activates a potassium conductance in some cells, 2) inhibits the Na^+-K^+ ATPase in most cells, 3) removal of HS^- allows the pump to reactivate, and 4) inhibits synaptic transmission. Hydrogen sulfide's effects on respiration may be caused by any or all of these actions.

Supported by MRC (Canada). SBK is an MRC/AHFMR student. WFC is an AHFMR scholar.

579.2

EFFECTS OF VARIOUS PCB DOSES ON THYROID STATUS AND CHOLINE ACETYLTRANSFERASE (ChAT) ACTIVITY IN 15 DAY OLD RATS. M. Sharma, L.M. Juarez de Ku, M.A. Baratian, R.B. Amiri and L.M. Meserve. Dept. Biol. Sci., BGSU, Bowling Green, OH 43403-0212.

Polychlorinated biphenyl (PCB) is a common pollutant, the ingestion of which is harmful to a number of physiological systems in adult animals. Previous studies in our lab have found addition of 250ppm (PCB (Arocohlor 1254), to the maternal diet during pregnancy and lactation to depress both thyroid status and choline acetyltransferase (ChAT) activity in the hippocampus and basal forebrain of 15 day old rats. The present study was done to determine whether the maternal ingestion of lesser amounts of PCB (125, 62.5ppm) results in a proportionally lesser depression of thyroid status and ChAT activity. Thyroid status was determined by measuring circulating T_4 and T_3 levels with RIA. ChAT activity was estimated quantitatively by the ability of a homogenate to incorporate ^{14}C-labelled acetyl-CoA into acetylcholine and qualitatively by immunohistochemistry. All PCB doses significantly depressed thyroxine (T_4) levels but had insignificant effects on triiodothyronine (T_3). Quantitation of ChAT activity revealed a linear dose response with less ChAT activity in the hippocampus and basal forebrain as the PCB doses increased. Fewer neuronal processes of PCB fed animals stained positively for ChAT than in controls. Thus, there seems to be a significant physiological influence of maternal PCB ingestion at doses lower than 250ppm (125, 62.5ppm).

579.3

USE OF NEUROTOXICITY DATA TO SET REGULATORY STANDARDS FOR ENVIRONMENTAL TOXICANTS Eve Andersen, Clement Intl. Fairfax VA.

Animal and human experimental data have been used by government agencies such as the Environmental Protection Agency (EPA) to determine safety limits for human exposure to chemicals in the everyday environment.

The methodology for deriving these limits is well established. The literature is reviewed to determine the study which shows the effects at the lowest dose level. The effects can be behavioral, developmental, structural, or biochemical. When available, human experimental data are preferred, otherwise animal data are used. Ideally an acceptable study will reveal no observable adverse effects at one dose level (NOAEL), and lowest observable adverse effects (LOAEL) at a slightly higher dose level. When necessary, the experimental doses are converted to account for chronic daily exposure. Uncertainty factors, when appropriate, are applied to account for interspecies differences between animals and humans, sensitive human subpopulations, and other factors.

This methodology has been used to set environmental standards for metals (chromium, manganese, mercury), solvents (bromomethane, hexane, toluene, xylene), pesticides (cresol, diazinon, endrin, parathion) and other substances. The methodology can only be used when experimental data exist that show a positive dose-response effect with a lower limit, so some neurotoxicants, such as lead, are not regulated with this system. The methodology can also be used to set standards for chemicals which are not neurotoxic.

Regulatory limits are mandated by the Toxic Substances Control Act, Resource Conservation and Recovery Act, Safe Drinking Water Act, Clean Air Act etc. Other agencies such as the National Institute for Occupational Safety and Health, the Occupational Safety and Health Administration, and the American Conference of Governmental Industrial Hygienists also set standards and regulations for occupational settings using less rigorous methodologies.

579.4

THE NEUROTOXICITY OF CHEMICALS FROM ACUTE EXPOSURES. R. M. Mastrangelo* and C. Rabe. Clement Int., Fairfax, VA 22031.

A review was conducted to determine what neurotoxic effects occur from short-term exposures to chemicals and to evaluate the use of short-term exposure neurotoxicity data as criteria for adding chemicals to or deleting chemicals from the Section 302 SARA Title III Extremely Hazardous Substance (EHS) list.

Effective doses for chemicals including organophosphates and pesticides were compared to their Level of Concern (LOC) and/or acute exposure limit (AEL) values. LOCs, which have been derived specifically for chemicals on the EHS list, and AELs are levels at which acutely exposed individuals should not experience adverse effects. Acute exposure effective doses were compared to these values only for chemicals with neurotoxic effects that impair a person's ability to escape in an emergency, inflict injuries that impair escape, or that are serious and irreversible. Based upon these comparisons, it was found that humans exposed to either hydrogen sulfide, lindane, or endrin, at their current LOC values, are not protected against such neurotoxic effects. The pesticides diazinon, dieldrin, Nexion™, methamidophos, and tri-o-cresyl phosphate also exhibit these effects, but are not on the EHS list. Finally, bromotrifluoromethane, carbon dioxide, fluorocarbon 12, heptane, hexane, isophorone, perchloroethylene, and petroleum distillates each have AELs that are protective against these neurotoxic effects but those for carbon monoxide, toluene and trichloroethylene are not protective. None of these chemicals with AELs are on the EHS list.

The results show that the EHS list can be revised using acute exposure neurotoxicity data and that this endpoint is more sensitive than acute lethality, which is the current toxicity criterion used to include chemicals on the list and to derive their LOC values.

579.5

IN VITRO TOXICITY OF N-BUTYL BENZENESULFONAMIDE: A NEWLY DISCOVERED NEUROTOXIN. V.R. Nerurkar*, I. Wakayama*, T. Rowe*, R. Yanagihara* and R.M. Garruto. Laboratory of Central Nervous System Studies, NINDS, NIH, Bethesda, MD 20892.

N-Butyl benzenesulfonamide (NBBS), a plasticizing agent used in the production of plastic resins and in the synthesis of an agricultural herbicide, produces a dose-dependent, progressive spastic myelopathy characterized by neuroaxonal degeneration in New Zealand white rabbits (Strong M.J. et al., Lancet, 330:640, 1990; Strong M.J. et al., Acta Neuropathol, 81:235, 1991). Using conventional assays for cell viability (trypan blue dye exclusion, LDH, MTT) and cell growth and function (^{3}H-thymidine incorporation, immunostaining), we studied the effects of NBBS on C6 glioma and Neuro-2a, two continuous cell lines of glial and neuronal origin, respectively. Micromolar (μM) concentrations of NBBS inhibited cell growth and produced morphological changes and altered cell function in these cell lines. A lower concentration of NBBS was required to inhibit DNA synthesis in Neuro-2a cells (10 μM) than in C6 cells (100 μM), suggesting that neuronal cells are more sensitive than glial cells to NBBS toxicity. Thymidine incorporation by subconfluent, actively growing C6 glioma cells was affected more than confluent cell monolayers. The effect of NBBS on DNA synthesis in C6 cells occurred as early as 24 hrs at a concentration of 10 μM (18% inhibition of thymidine incorporation), with 30% inhibition at 72 hrs. At an NBBS concentration of 250 μM, DNA synthesis was strikingly inhibited (70%) at 72 hrs. C6 cells exposed to 100 μM and 250 μM NBBS exhibited markedly reduced or absent immunoreactivity with antibodies against S-100 protein and glial fibrillary acidic protein. Our data indicate a cell type-specific neurotoxicity of NBBS and support our in vivo studies in rabbits. Studies are underway to determine the effects of NBBS on primary cultures of neuronal cells.

579.6

TRIADIMEFON SELECTIVELY INHIBITS DOPAMINE UPTAKE, IN VITRO. Q.D. Walker, M.H. Lewis, and R.B. Mailman. Curriculum in Toxicology, Brain and Development Research Center, University of North Carolina School of Medicine, Chapel Hill, NC

Triadimefon [1-(4-chlorophenoxy)-3,3-dimethyl-1-(1H-1,2,4-triazol-1-yl)-2-butanone] is an agricultural triazole fungicide that induces "stimulant-like" effects when administered to rodents. Previous reports from this laboratory indicated that low doses of triadimefon caused increased locomotion and rearing in rats. At higher doses, more intensely stereotyped behaviors (e.g., head weaving and backward locomotion). We have also reported that triadimefon administration generally decreases dopamine concentrations in striatum, and increases them in olfactory tubercles. The fungicide was found not to have direct effects on D_1 or D_2 dopamine receptors. These initial investigations led to the hypothesis that triadimefon acts like an indirect-acting dopamine agonist, either inhibiting dopamine uptake or inducing dopamine release. The present studies investigated this further.

The first experiment examined whether triadimefon would inhibit the uptake of dopamine, serotonin, or norepinephrine. This assay was run in crude synaptosomal preparations and measured uptake of each radiolabeled neurotransmitter. Using striatal synaptosomes, triadimefon dose-dependently inhibited the uptake of ^{3}H-dopamine with an IC50 = 20 μM, about one-fifth the potency of GBR 12909, a selective blocker of dopamine uptake. Triadimenol, a mammalian metabolite of triadimefon and a commercial triazole fungicide, was 2-3 fold less potent than triadimefon in its ability to inhibit the uptake of ^{3}H-dopamine. Triadimefon did not alter the uptake of either ^{3}H-serotonin or ^{3}H-norepinephrine into synaptosomes prepared from frontal cortex (IC50's > 100 μM). The second experiment used slices of rat striatum to assess the ability of triadimefon to release dopamine. Amphetamine (1 μM), used as a positive control, doubled the basal efflux of preloaded ^{3}H-dopamine. Conversely, 31 μM triadimefon had no effect on release. Together, these data suggest that triadimefon is a relatively selective inhibitor of dopamine uptake with potency sufficient to explain its behavioral and neurochemical effects.

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579.7

ACRYLAMIDE ALTERS THE DISTRIBUTION OF MAP1 AND MAP2 IMMUNOREACTIVITY IN RAT BRAIN. N.B. Chauhan*, M.I. Sabri, P.S. Spencer. Center for Research on Occupational and Environmental Toxicology, Oregon Health Sciences University, Portland, OR 97201.

Microtubule-associated proteins (MAPs) play an important role in the formation and maturation of neurites. Two MAPs (kinesin and dynein) mediate microtubule-based fast axonal transport which is retarded in rodents receiving the occupational chemical acrylamide (AC), a cause of axonal degeneration and cerebellar dysfunction in animals and humans. To study actions of AC on brain MAP immunoreactivity, rats were given 0.03% AC in drinking water for two weeks, systemically perfused with paraformaldehyde, brains dissected and 10-μm-thick paraffin sections prepared for MAP immunocytochemical localization according to Sternberger's PAP technique. Control MAP1 immunoreactivity was observed in neuronal perikarya, axons and dendrites of cortical pyramidal and Purkinje cells; in hippocampus, staining was limited to axons. AC caused significant loss in the immunoreactivity of hippocampal axons and of Purkinje cells, while staining in the cortical pyramidal cells was less affected. Control MAP2 immunoreactivity labeled axons and dendrites of cortical and hippocampal pyramidal cells, Purkinje cell processes and the cerebellar granular layer. AC reduced MAP2 immuno reactivity in cortical dendrites, cerebellum and hippocampal neurites. In summary, AC differentially disrupts the specific spatial distribution of MAP1 & 2 immunoreactivity in regions of rat brain involved in motor and memory functions. [Supported by NIH Grant, NS 19611]

579.8

EFFECTS OF PERINATAL EXPOSURE TO SPECIFIC POLYCHLORINATED BIPHENYL (PCB) CONGENERS ON LOCOMOTOR ACTIVITY OF RATS. S.L. Schantz and D.K. Ness*. Institute for Environmental Studies and Dept of Veterinary Biosciences, Univ of Illinois at Urbana-Champaign, Urbana, IL 61801.

Individual PCB congeners vary greatly in toxic potency. Those with 0 or 1 *ortho* chlorines, 2 *para* chlorines and at least 2 *meta* chlorines can assume a coplanar configuration similar to TCDD and are extremely toxic. Those with 2 or more *ortho* chlorines cannot assume a coplanar configuration and are believed to be considerably less toxic. However, recent evidence suggests the structure-activity relationship may be different for neurotoxicity. That is, *ortho*-substituted PCBs may be more neurotoxic than coplanar PCBs. Perinatal exposure to low-doses of complex PCB mixtures has been shown to alter activity in rodents, monkeys and humans. Therefore, we compared the effects of perinatal exposure to a coplanar PCB, an *ortho*-substituted PCB and a complex PCB mixture on activity in rats. Time-pregnant Sprague-Dawley rats were treated orally with 33',44'-tetrachlorobiphenyl, 22',55'-tetrachlorobiphenyl, Aroclor 1242 or corn oil vehicle on days 10-16 of gestation. Litters were culled to eight pups each on day two and weaned on day 21. One male and one female from each litter were tested for horizontal motor activity in an automated open-field. Activity of animals exposed to the high dose of the coplanar PCB or the high dose of the PCB mixture was suppressed compared to that of controls ($p < .01$) and compared to that of their respective low dose groups ($p < .05$). Activity of animals exposed to the noncoplanar PCB was not suppressed.

579.9

EFFECTS OF EXPOSURE TO TRIPHENYL PHOSPHITE (TPP) ON THE CNS OF THE CHICK (GALLUS DOMESTICUS) D. Tanaka, Jr., S.J. Bursian and E. Lehning, Depts. of Anatomy and Animal Science and Institute for Environmental Toxicology, Michigan State University, East Lansing, MI 48824.

Although it is clear that chicks are resistant to Type I organophosphorus delayed neurotoxicants for as long as 8-10 weeks post-hatch, it has not been as well established that they are similarly resistant to Type II neurotoxicants. In order to assess the neuropathological effects of a Type II compound on the developing CNS, we injected subcutaneously 1184mg TPP/kg body weight in 1-, 2-, and 3-week-old chicks. The chicks were killed after 21 days and their brains processed with the Fink-Heimer silver method for degenerating axons and terminals. The brains of 1-week-old chicks contained no degeneration. In 2-week-old chicks the amount of degeneration varied, ranging from small numbers of degenerating axons in the lower brainstem to large numbers of degenerating axons and terminals in medullary, cerebellar, midbrain, and forebrain regions. In 3-week-old chicks degeneration was also noted at all brain levels. These results indicate that the CNS of the chick is susceptible to TPP and that adult-like degeneration patterns may result from exposure as early as 2 weeks post-hatch.

Supported by the Michigan Agricultural Experiment Station and by BRSG funds awarded to the College of Veterinary Medicine.

579.10

Interactions Between Chronic Physostigmine and Repeated Soman Exposure in Rats and Monkeys. S. A. Miller[1*], D. W. Blick[2], S. Z. Kerenyi[1*], M. R. Murphy[1], and S. L. Hartgraves[1]. [1]Directed Energy Div., Armstrong Laboratory and [2]Systems Research Laboratories, Inc. Brooks AFB, TX 78235.

We previously showed that chronic infusion of pyridostigmine (PYR) did not increase the toxicity (symptoms, lethality) of daily exposure to the irreversible cholinesterase inhibitor, soman, but did have a small, but reliable, protective effect against soman-induced Primate Equilibrium Platform (PEP) performance deficits in monkeys.

In contrast to PYR, physostigmine (PHY) readily crosses the blood-brain barrier, and thus may be more efficacious in protecting against the CNS effects of repeated soman exposure. In rats with and without chronic PHY infusion, we measured weight, symptoms, brain acetylcholinesterase (AChE), and lethality as a result of five daily repeated soman injections. In monkeys, we tested the protective effects of PHY infusion at several different rates on 5-day repeated soman-induced changes in serum cholinesterase (ChE) and PEP performance.

We found that, like PYR, chronic PHY had a small protective effect against lethality (rats), and performance decrements (monkeys) induced by repeated daily exposure to soman. No adverse interaction between the PHY and repeated soman was seen, even when PHY alone produced serum ChE inhibition levels averaging 60%.

This work was supported in part by the U.S. Naval Medical Research and Development Command.

579.11

Behavioral Toxicity of Organophosphate Nerve Agents: Efficacy of Exogenous Cholinesterases as Blood-borne Scavengers in Primates. D. W. Blick[1], B. P. Doctor[2*], A. D. Wolfe[2*], S. A. Miller[3*], G. C. Brown,[1*] and M. R. Murphy[3] [1]Systems Research Laboratories, [2]Walter Reed Army Institute for Research, and [3]USAF Armstrong Laboratory, Brooks AFB, TX 78235

We previously showed that adding an anticonvulsant (diazepam) to the therapy for high-dose soman exposure after pyridostigmine pretreatment increased survival and reduced deficits in Primate Equilibrium Platform (PEP) performance in rhesus monkeys during the first few days after such exposure. However, all of these chemical countermeasures failed to protect against complete prostration lasting 1.5 to 4 hrs, and delayed/residual deficits lasting up to 3 weeks.

We have now shown that high levels of exogenous cholinesterases (fetal bovine serum acetylcholinesterase, AChE, or equine serum butyrylcholinesterase,BChE) can provide complete protection against both symptoms and performance decrements produced by soman dosages 4-5 times the LD_{50}, even in the absence of any other pretreatment or therapy. Four monkeys were pretreated with each esterase. Soman (27-32 μg/kg) was given in 3 spaced injections and performance tested for 30 min after each injection. Serum esterase assays verified the stoichiometric inhibition caused by the soman and allowed titration of the next injection. No PEP deficits were seen in animals pretreated with AChE; however, 3 animals with BChE showed minor PEP deficits after the 3rd soman injection. No delayed/residual deficits were seen in any animal.

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579.12

EFFECTS OF SCOPOLAMINE (SCP) ON STRIATAL NEUROCHEMICAL LEVELS IN RATS INTOXICATED WITH SOMAN. B.R. Capacio*, T.-M. Shih, L. Cook*, T. Koviak*, T. Sewell* and N. Adams*. U.S. Army Med. Res. Inst. Chem. Def., APG, MD 21010

The antimuscarinic compound SCP, which is known to affect striatal neurochemistry, has been reported to prevent convulsions induced by the cholinesterase inhibitor soman. Evidence suggests that convulsions associated with soman intoxication contribute to brain neuropathology as a result of excitotoxic mechanisms. The purpose of this investigation was to determine the effects of SCP (0.125 mg/kg, im) on striatal amino acid and catecholaminergic parameters in rats intoxicated with a neurotoxic dose of soman (100 μg/kg, sc). The time course (0-2 hr) for concentrations of aspartate (ASP), glutamate (GLU), γ-aminobutyric acid (GABA), glycine (GLY), lysine (LYS), norepinephrine (NE), dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) were determined after soman, SCP and soman + SCP. Soman administration produced decreases in ASP and LYS concentrations which returned to control levels by 30 min. Soman + SCP blocked the decrease in ASP and produced a 30% increase in LYS which returned to control levels at 30 min. Soman did not significantly affect levels of GLU, GABA or GLY. Striatal concentrations of NE and DA were not altered by soman, but, the DA metabolites (HVA and DOPAC) increased to twice that of control. SCP blocked the effects of soman on DOPAC and HVA levels. The limited effects of soman on amino acid levels support findings that soman produces less damage in the striatum than in other brain areas. Furthermore, the data suggest that the anticonvulsant effects of SCP may be partially attributed to its influence on DA metabolism in addition to its well known anticholinergic activity.

579.13

DOWN REGULATION OF MUSCARINIC RECEPTORS IN RATS DUE TO EARLY POSTNATAL EXPOSURE TO PARATHION. C.L. Dvergsten*, R.B. Meeker, and James F. Howard, Department of Neurology, University of North Carolina, Chapel Hill, NC 27599.

Parathion (PTN), an organophosphorous pesticide, is a potent inhibitor of acetylcholinesterase (ACHE). We are currently investigating the acute and long-term effects of ACHE inhibition in the developing brain due to parathion. Acute studies indicated: 1) a dose-dependent and transient inhibition of brain ACHE and 2) an increased susceptibility of the brains of 12-day-old rats (IC_{50}=1.5 mg/kg) versus adult rats (IC_{50}=2.5 mg/kg) to the anti-ACHE properties of PTN. Based on these observations we produced a prolonged dose-dependent inhibition of brain ACHE by giving rat pups daily injects of PTN from postnatal days 8-21. Growth retardation was dose dependent and varied between 6-30%. Growth was inhibited only during the first 5-6 days of exposure, thereafter the growth rate paralleled controls. Daily injections of 1.5 mg/kg PTN reduced whole brain ACHE activity by 50-70% throughout the exposure period. ACHE histochemistry indicated ACHE was inhibited in a variety of brain regions including the caudate-putamen, hippocampus and cerebral cortex. ^{3}H-QNB binding in whole brain homogenates from 21-day-old rats injected daily for 13 days with 1.5 mg/kg PTN showed that PTN reduced B_{max} by about 20% but the K_D was unchanged. Hill analyses of competition binding studies with acetylcholine indicated agonist binding properties were unaffected by PTN. Thus, prolonged exposure of young rats to PTN and the resulting inhibition of ACHE produced a decrease in the number of muscarinic receptors but did not influence the binding of acetylcholine to the receptor.

Supported by US EPA.

579.14

METABOLISM OF THE NEUROTOXIC PYRROLIZIDINE ALKALOIDS FROM *TRICHODESMA INCANUM*. S.L. Glowaz*, R.J. Bowers*, K. Kashihara and R.J. Huxtable, Department of Pharmacology, Arizona Health Sciences Center, Tucson, AZ 85724.

Contamination of grain with the seeds of the pyrrolizidine alkaloid-containing plant, *Trichodesma incanum*, caused an outbreak of human poisoning in central Asia in 1950. Poisoning in both humans and animals differ from other pyrrolizidine alkaloid intoxications inasmuch as *T. incanum* has been reported to have a primary neurotoxic action. *T. incanum* contains the alkaloids, trichodesmine and incanine. These alkaloids are structurally similar to the *Crotalaria spectabilis* alkaloid, monocrotaline. However, monocrotaline is associated primarily with hepatic and pulmonary toxicities rather than neurotoxicity. Its toxicity results from hepatic metabolism to reactive pyrrole intermediates. We have compared the metabolism of the pyrrolizidine alkaloids, heliotrine (hepatotoxic only), monocrotaline (hepatotoxic and pneumotoxic), and trichodesmine and incanine (neurotoxic) by isolated rat liver microsomes. Incubation with 0.5 mM monocrotaline for 10 min yielded 9 ± 2 nmol.mg^{-1} protein of a highly reactive alkylating pyrrole, as measured by trapping through thioether bond formation with thiopropyl sepharose resin, and 19 ± 5 nmol.mg^{-1} protein of nonalkylating pyrroles. By comparison, 0.5 mM trichodesmine/incanine mixture (3:1 ratio) yielded 21 ± 4 nmol.mg^{-1} protein of alkylating pyrrole and 23 ± 2 nmol.mg^{-1} protein of nonalkylating pyrrole. No alkylating pyrrole was trapped from incubations with heliotrine. It appears, therefore, that the organ-specific toxicity of pyrrolizidine alkaloids correlates with the quantity of reactive alkylating pyrrole generated, as determined by an *in vitro* trapping system.

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579.15

ACUTE EFFECT OF Karwinskia humboldtiana (Kh) ON SOME MOTOR REGIONS OF THE CENTRAL NERVOUS SYSTEM. G.G. Ortiz*, I. González-Burgos*, G. Tapia-Arizmendi and A. Feria-Velasco. Morphology Lab., Fac. Cienc. Biol., Univ. de Guadalajara; and Exptl. Path. Div., Unidad Invest. Biomed. Occte., I.M.S.S. Guadalajara, Jal.

Kh is a shrub that grows in Mexico and U.S.A. Ingestion of Kh fruit produces ascendent flaccid paralysis and death. Distal peripheral demyelination is observed in large motor nerves which degenerate. Structural alterations have been reported in cerebellum, pons and spinal cord. With the purpose to explore the effect of Kh in other brain regions, 2g/Kg of Kh endocarp were orally administered to 24 male Wistar rats, and after 6,12,48 and 72 hr, their brains were fixed with buffered-2% paraformaldehyde by intracardiac perfusion under general anesthesia. Sections of sensory-motor cortex (Cx), CA1 region of hippocampus (Hp) and caudo-putamen (CP) were studied under a light microscope, after being stained with aniline dyes. Widening of Virchow-Robin spaces and shrinkage with hyperchromasia of pyramidal cells were seen in Cx throughout the study. Neuronal death was observed in all regions since 6 hr after Kh administration, increasing at 12 and 48 hr, mainly in Hp, where the lesions remained until 72 hr after Kh administration. Hp was the most affected region.

It is concluded that Kh has an acute neurocytotoxic effect on Hp and to a lesser extent on Cx and CP. These findings could be related in part, to the non-paralytic motor disturbances observed in the first week after Kh ingestion.

579.16

3-NITROPROPIONIC ACID - EXCITOTOXIC LESION OF THE HIPPOCAMPUS INDUCED BY AN INHIBITOR OF CHEMICAL ENERGY METABOLISM. M. Riepe*, A.C. Ludolph*, M.I. Sabri, P.S. Spencer, C.N. Allen. Center for Research on Occupational and Environmental Toxicology, Oregon Health Science University., Portland, Oregon 97201

3-Nitropropionic acid (3-NPA), a widespread fungal and plant toxin, and inhibitor of mitochondrial Complex II is of pathogenetic significance in human and animal diseases. The pattern of damage in the basal ganglia and the hippocampus *in vivo* mimics excitotoxic lesions. In mouse cortex explant cultures, 3-NPA induces vacuolization and chromatin clumping, which are preceded by cellular energy depletion and partly attenuated by glutamate antagonists (MK-801). In contrast, 3-NPA (0.1 to 10 mM) does not induce currents mediated by ionotropic glutamate receptors in whole-cell patch clamp recordings of cultured hippocampal neurons. Diazoxide (0.1 mM), an agonist of ATP-dependent potassium channels, induced a hyperpolarizing current in the same system. We propose that the hyperpolarization observed in cells treated with 1 mM 3-NPA alone is due to an increased potassium conductance following impaired energy metabolism similar to observations in ischemic cells. A further energy depletion by 3-NPA possibly causes a depolarization of the cell membrane that gates the toxic action of endogenous excitatory agents. (Supported by NG 19611 and grants from the Deutsche Forschungsgemeinschaft and the Medical Research Foundation of Oregon).

579.17

LINDANE INHIBITS NEURITE INITIATION AND ELONGATION AND DECREASES INTRACELLULAR FREE CALCIUM ION CONCENTRATIONS IN CULTURED RAT HIPPOCAMPAL NEURONS AND MOUSE N1E-115 NEUROBLASTOMA CELLS. C. Ferguson*, L. Cabell-Kluch*, D. Shugarts*, T. Audesirk and G. Audesirk. Biology Department, U. Colorado at Denver, Denver, CO 80217-3364.

Several studies suggest that neurite initiation and elongation may be modulated by intracellular free calcium ion concentrations and/or calcium fluxes across the plasma membrane. Departures from optimal intracellular calcium concentrations, both increases and decreases, appear to lead to decreased neurite initiation (J. Frank abstract, this meeting), as does blocking calcium influx through voltage-sensitive calcium channels (Audesirk, et al., 1990). The organochlorine pesticide lindane has been reported to increase intracellular calcium concentrations in short-term experiments (less than an hour). We cultured embryonic rat hippocampal neurons and mouse N1E-115 neuroblastoma cells in lindane concentrations ranging from 0.1 to 100 uM. Neurite initiation and elongation were measured after 48 hours in culture. Lindane significantly inhibited neurite initiation at concentrations as low as 1 uM (hippocampal neurons) and 0.5 uM (N1E-115 cells). Lindane significantly inhibited neurite elongation at concentrations as low as 25 uM in both cell types. Contrary to previous reports using short-term exposure, digital imaging microscopy with fura-2 showed that long-term exposure to lindane (24 hours or more) decreased intracellular free calcium ion concentrations in a dose-dependent manner.

CLINICAL CNS NEUROPHYSIOLOGY

580.1

MAGNETIC COIL STIMULATION OF STRAIGHT AND BENT PERIPHERAL NERVE IN-VITRO: EFFECT OF CHANGING STIMULUS PROFILE AND POLARITY. P.J. Maccabee, V.E. Amassian, L.P. Eberle* R.Q. Cracco and A.P. Rudell. SUNY-Health Science Center at Brooklyn, Brooklyn, N.Y. 11203

A 9-11 cm segment of amphibian nerve was placed in a relatively large flat-bottommed plastic trough containing Ringer's solution. The distal end of the nerve emerged from the solution for monophasic recording. A 5 x 10 cm figure '8' magnetic coil [MC] was positioned beneath the trough, with the long axis of the junction centered under and parallel to the nerve. The direction and waveform of the energizing pulses [polyphasic, phase II 80% of phase I; biphasic, phase II 20% or 35% of phase I] was selectively changed by a mechanical switch.

Suprathreshold biphasic pulses elicited a single response corresponding to the (-) going spatial derivative of the electric field, just distal to the anterior divergence of the coils. Reversing the current shifted the latency by 0.65-1.0 ms, corresponding to approximately 30 mm at the measured conduction velocity. This second site at the figure '8' is posterior to its middle region. These locations were confirmed by direct electrical stimulation. For the two current directions, the responses were stable in latency at all stimulus output intensities.

Polyphasic MC pulses of either polarity resulted in stimulation at the spatial derivative site closest to the recording electrodes; the responses differed in latency by approximately 200 μsec possibly reflecting the period between 1st and 2nd phases. Presumably, excitation by the 1st or reversed polarity 2nd phase of the polyphasic pulse at this site leads to collision with impulses generated at the more distant site.

Bending the nerve from 30° to 90° or more created a low threshold stimulation site at or near the bend.

These findings may help explain MC excitation of axons in the CNS.

580.2

EFFECTS OF CONTROLLED AMBIENT LIGHT AND CIRCADIAN FACTORS ON THE PUPILLARY LIGHT REFLEX IN HUMANS. W.B. Pickworth, J.S. Fosnaugh* and E.B. Bunker*, NIDA, Addiction Research Center, Baltimore, MD 21224

Measures of pupillary size and the light reflex are safe and noninvasive methods to quantify and characterize the mechanism and site of drug action. In this study, we measured the effect of variations in the ambient light level and time of measurement on pupil diameter and the dynamic light reflex. Light levels of: >.1,4,40,100 and 200 ftcd were used every 3 hr between 6 AM and 9 PM in 14 adult males. Measurements were made with a Fairville Medical Optics pupilometer. The diameter of the dark-adapted pupil at >.1 ftcd averaged 6.4 mm, whereas the diameter in the 200 ftcd condition averaged 2.3 mm. Time of day effects were significant but small and a significant interaction between light level and time suggests that the pupil size is influenced differently by the ambient light over the course of the day. Constriction amplitude decreased with ambient light from 2.1 mm (>.1 ftcd) to .2 mm (200 ftcd) while constriction and dilation velocities decreased from 7.7 to 2.8 and 4.3 to 2.8 mm/sec respectively. A patch over the counterlateral eye increased the size of the pupil and measures of the light reflex. Such baseline data may be useful in describing the normal variations in these increasingly popular measures.

580.3

NONINVASIVE MEASUREMENT OF NERVE ELECTRICAL MEMBRANE PROPERTIES IN HUMANS USING VARIABLE PULSE-WIDTH MAGNETIC STIMULATORS. D. Rudiak* & E. Marg, Sch. of Optometry, U. Calif., Berkeley, 94720.

Using strength-duration protocols, magnetic stimulators can make nerve membrane measurements, but without the pain of electrical stimulation. If threshold units $\propto$ induced charge density (e.g., $\sqrt{\text{Energy}}$), threshold is linearly related to pulse width. The membrane time constant (T) can be computed from the slope (m) and y-intercept (b) of a linear regression through the data. T = .582 b/m and is typically 125-150 μsec in peripheral nerve and motor cortex. Assuming "typical" values for membrane capacitance (e.g., 1 $\mu F/cm^2$) and threshold depolarization voltage (15-20 mV), membrane leakage resistance and current density may also be computed, yielding 125-150 ohm-cm^2 and .6-.9 A/m^2. Another possibly useful quantity, the ratio of membrane to total current density, may be computed from absolute thresholds. Typical values are .25-.50. This ratio is relatively uniform in cortex but highly variable in peripheral nerve. Variability is related to tissue inhomogeneity, e.g., conductance changes. Studies in normal vs. pathological populations are now needed to establish the clinical utility of these measurements.

Funded, in part, by the Minerva Foundation, Berkeley, California.

580.4

NEW INTRAMUSCULAR ELECTRODES AND MICROPROCESSOR CONTROL OF ELECTRICAL STIMULATION TO IMPROVE HEMIPARETIC GAIT. A. Prochazka, L. Davis* & M. Gauthier*, Div. Neurosci., University of Alberta, Edmonton AB, T6G 2S2, CANADA

There are two major impediments to the widespread use of functional electrical stimulation to improve motor function in paralysed patients: i) unreliability of electrodes; ii) unreliability of sensors and controllers. We have tested a robust intramuscular electrode similar to that developed recently in Cleveland by J.T.Mortimer. Two Cooner 632 insulated stainless steel wires are coiled tightly around a length of 4/0 prolene monofilament and terminated with a knotted monofilament barb which acts as an anchor. The last 25 mm of each wire is bared, so that 50 mm of wire coiled into a 10 mm length interfaces with muscle. Implantation is done with a jacketed translumbar aortogram needle under fluoroscopy control. 5 electrodes implanted in psoas, gluteus medius muscles and peroneal nerve in 2 incomplete quadriplegics have remained electrically stable and functional for a year. We have also developed a portable microprocessor controller which uses signals from underfoot force sensors to control multichannel stimulation during gait. The system compensates for transducer drift and can be monitored and parametrically adjusted from an IBM AT host. *Supported by Canadian MRC.*

580.5

TEMPERATURE CHANGES IN DEPTH ELECTRODE DURING MR SCANNING. J. Zhang, C.L. Wilson, M. Levesque, E. Behnke* and R. Lufkin,* Brain Research Institute and Department of Neurosurgery and Department of Radiology, UCLA, Los Angeles, CA 90024.

Surgical therapy for complex partial epilepsy requires precise identification of the seizure focus. Magnetic resonant imaging (MRI) compatible depth electrodes have been implanted stereotaxically in the temporal lobes for prolonged stereoelectroencephalography (SEEG) recording to assist in focus identification. Post-implantation MRI scans offer accurate localization of electrode sites; however, eddy currents in a metallic electrode induced by the radiofrequency magnetic field applied by the MRI scanner may heat the electrode. Electrode heating may raise the local brain tissue temperature, possibly to a dangerous extent. We therefore examined electrode temperature changes in a 1.5-T MRI scanner.

A bundle of five Nickel-Chromium electrodes (NiCr, Nickel 80%, Chromium 20%) were attached around a miniaturized precision thermistor (model 44003A, YSI Inc.) which was connected to a ohmmeter by copper wires. Masking tape was wrapped around the electrode bundle to prevent heat dissipation. The bundle of electrodes was then placed between two saline bags in a GE Signa 1.5-T MRI scanner with the electrode axes in parallel with the scanner axis to obtain maximal heating effect. The electrode temperature increased by 0.07°C after scanning for 3 minutes with multi-echo sequences (TR/TE = 800/20).

We conclude that MRI scanning will not cause a significant NiCr electrode temperature rise.

580.6

RESPONSE OF ESSENTIAL WRIST TREMOR TO SINUSOIDAL SOMATOSENSORY FEEDBACK. R. J. Elble and C. A. Higgins*. Southern Illinois University School of Medicine, P.O. Box 19230, Springfield, IL 62794-9230.

Sinusoidal forcings at 4 to 15 Hz were delivered by a computer-controlled torque motor to the wrists of 11 normal adults and 10 patients with advanced essential tremor. Wrist angle and forearm EMG were recorded. Our normal adults exhibited a second-order underdamped wrist response with a natural frequency of 5.5 Hz. The wrist response of our patients exhibited 66% greater resonance at forcing frequencies of 4 to 6 Hz, which was the frequency range of their tremor. The increased resonance in our patients was attributable to intermittent frequency entrainment of essential tremor by the mechanically-induced somatosensory feedback. The wrist response of our patients and controls did not differ at frequencies greater than 7 Hz. The marked entrainment of motor units by essential tremor precluded normal reflex modulation of forearm EMG by wrist forcings at frequencies greater than 6 Hz. Consequently, the forearm EMG response of our patients was less than control values at frequencies greater than 6 Hz. Furthermore, forcings at frequencies greater than 12 Hz tended to suppress essential tremor. Essential tremor is a centrally generated oscillation that can be enhanced or suppressed by somatosensory feedback. (Supported by NINDS grant NS20973.)

580.7

PATTERNS OF MUSCLE ACTIVATION DURING GAIT IN SUBJECTS WITH DOWN SYNDROME (DS). M.Cioni, A.Cocilovo*, F.Comeau*. OASI Inst.(IRCCS) Troina, Inst. Physiology, Catania Univ.,Italy Neurobiology Res. Centre, Hop. Enfant J., Québec, Canada.

In order to explore the basic mechanisms underlying the disturbed and delayed motor development in DS, we have analyzed the muscle activations and movement patterns during free gait of 12 young subjects of different age with DS as well as of 10 normals subjects. Gait movements have been monitored by an electrogoniometer and surface EMG was recorded from four leg muscles (hamstrings, quadriceps, tibialis ant., triceps s.).Three patterns of muscle activation have been identified: the first one showed EMG characteristics similar to those observed in the normals, the second pattern was characterized by the precocious activation of triceps surae and was observed in most DS subjects, the third pattern was mainly represented by the activation of the triceps surae at the beginning (0-35%) and at the end (70-100%) of the gait cycle.Our results show the presence of a different phenotypical expression of walking patterns in DS and of a specific delay in the functional development of the triceps surae.Moreover they suggest a key role for this muscle and its neural control in the delayed development of gait observed in this syndrome.

580.8

TREMOR REDUCTION BY MICROINJECTION OF LIDOCAINE INTO THE THALAMUS OF MOVEMENT DISORDER PATIENTS. G.D. Sher*, J.O. Dostrovsky, R.R. Tasker*, A. Lozano, A. Parrent* and F.E.B. Wells*. Departments of Physiology and Neurosurgery, University of Toronto, Toronto, Ontario, M5S 1A8, Canada.

Thalamotomy has long been used in the treatment of intractable tremor. The optimal site for the lesion is believed to be in the region immediately anterior to the tactile relay in the thalamic ventrobasal complex where cells respond to joint movement and/or fire in synchrony with the tremor (tremor cells) and where microstimulation blocks or reduces the tremor. However, it is unclear whether these criteria predict the optimal lesion site and also whether stimulation-induced tremor reduction (TR) is due to disruption and block of neural activity or to activation of neural elements. The present study utilized microinjections of the local anesthetic lidocaine hydrochloride (1-2ul of 1 or 2% solutions) in order to obtain further information related to these points. Injections of lidocaine, at sites where joint activated units and/or tremor cells were recorded and/or where microstimulation evoked TR, produced marked or complete TR in 2 Parkinsonian and 4 other movement disorder patients; TR occurred within 3 min., was maximal at 5 and persisted for up to 20 min. The TR effects were confirmed by marked reductions of EMG recordings of 4 muscle groups on the contralateral forearm. In all cases speech and voluntary movements were unaffected during lidocaine block, and there was no loss of tactile sensibility. These findings suggest that stimulation-induced TR may be due to block or disruption of neural activity. Furthermore, since lidocaine block mimics the effects of a lesion, it allows one to determine the sequelae of lesioning prior to permanent ablation and thus may serve as a very useful additional tool in functional stereotactic neurosurgery. (Supported Canadian MRC and Parkinson Foundation)

580.9

ABNORMAL BLOOD FLOW RESPONSES TO VIBRATION IN PATIENTS WITH WRITER'S CRAMP. L.W. Tempel, J.S. Perlmutter. Departments of Neurology and Radiology, Washington Univ. Sch. of Med., St. Louis, MO 63110.

We evaluated sensorimotor processing in patients with writer's cramp using positron emission tomography (PET) and $H_2^{15}O$. We studied 6 right-handed patients (3 female) 24-72 yrs of age (mean 50). All had unilateral writer's cramp. These were compared to 8 right-handed normals (6 female) 20-72 yrs of age (mean 41). Data were analyzed using subtraction image analysis. Vibration produced a consistently localized and robust peak response (ml/(100gmin) +/- SD) in primary sensorimotor cortex (S1) contralateral to hand vibration in normals (right hemisph: 13.11 +/- 3.25;left hemisph: 12.57 +/- 1.18). This response was significantly reduced in patients with writer's cramp ($p < .001$) whether vibrating the unaffected (7.87 +/- .62) or affected (10.40 +/- 2.07) hand. Coordinates for the SMA locus were determined from the averaged responses in an independent data set of 14 normals 20-69 yrs of age (mean 39). It was in high medial frontal cortex contralateral to the vibrated hand. This locus then was used to objectively search the data from the patients and the other 8 normals. Right and left SMA responses in normals were not significantly different from each other (right hemisph: 6.52 +/- 1.47; left hemisph: 6.46 +/- 1.45). There also was no significant right-left (or "unaffected-affected") difference in writer's cramp patients (unaffected hemisph: 4.30 +/- 1.70; affected hemisph: 4.66 +/- 1.09). Both SMA responses were significantly less than in normals ($p < .01$). These data indicate that patients with unilateral writer's cramp have bilateral brain dysfunction. Abnormal responses in S1 and SMA are consistent with dysfunction of the cortico-striato-pallido-thalamo-cortical circuit.

580.10

ELECTROMAGNETIC MAPPING OF DYNAMIC BRAIN ACTIVITY: SPATIO-TEMPORAL ANALYSIS AND ANATOMICAL CONSTRAINTS. J. Mosher*, J.S. George, P.S. Lewis*, R. Leahy*, M. Singh*. Los Alamos National Lab., M-715, Los Alamos, NM 87545 & Univ. Southern California, Los Angeles, CA

Neural electromagnetic measurements offer the prospect of high resolution, noninvasive mapping of dynamic patterns of activity of the human brain. However, localization of neural sources from external magnetic field or potential measurements requires the solution of an ill-posed inverse problem. We have developed an approach based on MUSIC (MUltiple SIgnal Classification) algorithms that systematically exploits spatio-temporal correlations in the measurement matrix. The method also incorporates anatomical information, derived from volumetric magnetic resonance imaging, to constrain the locations of candidate sources. The *signal subspace* of the electromagnetic measurements is estimated by performing an eigenvalue decomposition on the correlation matrix of the time series data. Because theoretical and experimental evidence suggests that the dominant sources observed in noninvasive electromagnetic measurements are cortical dendritic currents, in our application the *source region* is limited to cortex. A scan metric is computed at each spatial location in the source region by projecting the field of a current element at that location onto the signal subspace, to identify sources that produce fields that lie within the signal subspace. Locations that produce a peak in the 3-D distribution of the metric are accepted as probable neural sources. If greater accuracy in localization or a description of the timecourse of component sources is required, sources identified by the algorithm may be used as location estimates for multiple dipole fitting procedures. We have applied these procedures to experimental neuromagnetic data from somatosensory studies to resolve the sources activated by electrical stimulation of the thumb, ring finger or the simultaneous stimulation of both. We have also resolved neuromagnetic sources associated with early cortical activation in response to a small sinusoidal grating presented to single quadrants of the visual field. Our MUSIC-based algorithm provides a powerful and systematic approach to estimate a spatio-temporal ensemble of sources, while incorporating anatomical information to improve the accuracy and efficiency of electromagnetic imaging of brain function.

580.11

BEHAVIOR-RELATED CHANGES IN HIPPOCAMPAL THETA ACTIVITY RECORDED FROM OCCIPITAL TRAJECTORY DEPTH ELECTRODES. **J.L. Thompson, K.J. Meador, D.W. Loring*, G.P. Lee*, D.W. King*, B.B. Gallagher, A.M. Murro*, J.R. Smith* and H.F. Flanigin*. Departments of Neurology and Surgery, Medical College of Georgia, Augusta GA 30912.**

In previous studies of temporal lobe epilepsy (TLE) patients, we have shown that hippocampal theta activity recorded from vertex trajectory depth electrodes changes as a function of behavior. In this study of nine patients with unilateral TLE, theta activity recorded from an occipital trajectory hippocampal depth electrode (contralateral to the seizure focus) was compared across four behavioral conditions: resting eyes closed (RC), resting eyes open (RO), eyes open with auditory word activation (AW), eyes open with visuospatial activation (AP). Theta activity decreased significantly during AW and AP compared to RC ($p=0.0195$& $p=0.002$,respectively) and during AP compared to RO ($p=0.0195$).

In contrast, vertex trajectory patients showed a significant decrease in theta during RO compared to AW, and no significant differences between AW & RC or AP & RO. The differences between the vertex and occipital groups in behavior-related theta activity changes may be due to: group differences, small size of the occipital group, or an electro-physiologically important difference in electrode placement. Occipital trajectory electrodes allow comparison between contact points along a line from the amygdala through anterior hippocampus to posterior hippocampus. It is interesting to note that no significant behavior-related changes in theta activity were observed in the amygdala and, that theta changes within the hippocampus varied from anterior to posterior suggesting that precise electrode placement is an important consideration.

580.12

ENHANCED CORTISOL RESPONSE TO FENFLURAMINE IN MAJOR DEPRESSION. R.T. Malison*, L.H. Price, P.L. Delgado, D.S. Charney and G.R. Heninger. Department of Psychiatry, Yale University School of Medicine., New Haven, CT 06519.

Alterations in central serotonergic neurotransmission have been implicated in the pathophysiology of major depression. We studied central serotonin (5HT) function in major depression by comparing the neuroendocrine response to pharmacologic challenge with the potent 5HT releasing agent fenfluramine (FEN) both between depressed (n=21) and healthy (n=17) subjects and within a depressed subset (n=12) later treated with desipramine (DMI). Serum cortisol (COR), prolactin (PRL), and growth hormone (HGH) were measured at baseline, and at 30 min intervals between 2 and 5 hrs after an oral dose of FEN (60 mg). Results showed a significantly ($p<.05$) increased mean (± SEM) peak change in COR in depressed (6.61 ± .91 µg/dl) as compared to healthy (2.64 ± 1.62 µg/dl) subjects. This enhanced COR response to FEN was significantly attenuated and appeared to normalize following DMI treatment (6.94 ± 1.42 vs 3.33 ± .85 µg/dl, $p<.05$). No significant differences in PRL or HGH responses were noted. These findings lend support to the hypothesis of altered 5HT function in depression which normalizes with antidepressant treatment.

580.13

ELECTROPHYSIOLOGIC ANALYSIS OF CEREBRAL CORTICAL DYSFUNCTION IN FELINE MODELS OF NEURONAL STORAGE DISEASE. S.U. Walkley and C.E. Schroeder, Dept. Neuroscience, Kennedy Center, Albert Einstein College of Med., Bronx, NY 10461.

The pathogenetic mechanisms underlying brain dysfunction in Tay-Sachs disease and other neuronal storage disorders are poorly understood. Two abnormalities of neurons known to commonly occur in cerebral cortex in gangliosidosis and to a lesser degree in other storage disorders are (i) ectopic dendritogenesis and associated asymmetrical synapse formation on axon hillocks of pyramidal neurons, and (ii) axonal spheroids predominating on GABAergic neurons. We evaluated the relative contributions of these cytopathologic features to cortical dysfunction using feline models of GM1 gangliosidosis (GM1) and Niemann-Pick disease type C (NPD). To date we have studied 1 GM1 cat (9 mo. old), 1 NPD cat (7 mo. old), and 4 normal cats (9 mo. and older). Laminar profiles of field potentials, current source density, and multiunit activity, elicited by 90 dB, 100 µsec, contralateral clicks, were sampled from primary auditory cortex under pentobarbital anesthesia using multicontact electrodes. In the GM1 cat, response latencies in laminae III-IV were elevated by 2-7 ms and response durations by 4-10 ms over normal values, whereas in the NPD cat elevations were minimal. In both diseases the amplitude and spatial extent of supragranular activation was greater than normal. Attenuation of cortical response amplitudes in lamina III at high stimulation rates was 20-40% greater than normal at rates above 10 Hz; 100% attenuation was reached at 40 Hz, vs. 200 Hz for normals. Control data suggested a minimal contribution from subcortical pathology and neural fatigue processes. While there is clear evidence for neurophysiologic abnormalities in the cerebral cortex of GM1 and NPD cats, the relative contributions of increased excitatory and decreased inhibitory influences remain to be elucidated. (NS18804, MH06723, NS10967, AR37095)

580.14

EXPERIMENTAL THERAPY OF HUMAN GLIOMA BY MEANS OF A GENETICALLY ENGINEERED VIRUS MUTANT. R. L. Martuza[1], A. Malick[1*], J. M. Markert[1*], K. L. Ruffner[2*], D. M. Coen[2*]. [1]Molecular Neurogenetics Laboratory, Dept. Surgery (Neurosurgery) Mass. General Hospital, Boston, MA 02114; [2]Dept. Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115.

Malignant gliomas are the most common malignant brain tumors and are almost always fatal. A thymidine kinase negative mutant of herpes simplex virus-1 (*dl*sptk), that was attenuated for neurovirulence, was tested as a possible treatment for gliomas. In cell culture, *dl*sptk killed two long-term human glioma lines and three short-term human glioma cell populations in a dose-dependent fashion. In nude mice with implanted subcutaneous and subrenal U87 human gliomas, intraneoplastic inoculation of *dl*sptk caused significant growth inhibition. In nude mice with intracranial U87 gliomas, intraneoplastic inoculation of 10^3 and 10^5 plaque forming units (pfu) dlsptk prolonged survival in a dose-dependent fashion ($p<0.05$). Moreover, at the highest tested dose (10^5 pfu), 2/7 (29%) animals were alive, healthy, and neurologically normal at 19 weeks. After sacrifice, histological sections revealed no residual tumor. We are testing genetically engineered viruses like *dl*sptk and others that are attenuated for neurovirulence for use as novel antineoplastic agents for malignant brain tumors.

582

SYMPOSIUM. "LIPID MEDIATORS" IN SYNAPTIC TRANSMISSION AND SIGNAL TRANSDUCTION OF NEURONAL CELLS: PHYSIOLOGICAL AND PATHOLOGICAL IMPLICATIONS. G.Z. Feuerstein, SmithKline Beecham Pharmaceuticals and N. Bazan, Louisiana State University School of Medicine (Chairpersons); D. Piomelli*, Institut National de la Sante et de la Recherche Medicale; T.V.P. Bliss*, National Institute for Medical Research.

Lipids are the major constituent of all plasma membranes and possess vast diversity of composition and dynamic plasticity in structure and function. While much of our past efforts have been spent on understanding the structural significance of membrane lipids, their supportive role in enzyme and receptor function and a source of autocrine and paracrine autacoids (e.g., prostaglandins, leukotrienes), recent evidence emerges to suggest that certain lipids might have a role in preparation of external signals across the cell membrane toward intracellular targets. Activated PLA_2 via voltage or receptor operated membrane signals results in phospholipid remodeling and production of platelet-activating factor and selected eicosanoids which results in intracellular events such as elevated $[Ca^{2+}]_i$, IP_3, PKC and immediate gene-expression. Functional consequences of archidonate metabolites such as those produced by the 5 or 12-lipoxygenase enzyme include modulation of neurotransmitter release. At the neural system function, evidence has been raised in support of a role for eicosanoids in the evolution of the phenomenon of long term potentiation and its physiological and pharmacological implications. While this field is clearly at its infancy, it provides new dimensions for basic research and new therapeutic strategies in modulation of normal and pathological aspects of brain function.

583

SYMPOSIUM. NEURAL GRAFTING AND PARKINSONS DISEASE. A. Björklund, Univ. of Lund, Sweden (Chairperson); M.R. De Long, Emory University; S.B. Dunnett, Univ. of Cambridge, U.K.; D.M. Gash, Univ. of Rochester; O. Lindvall, Univ. of Lund, Sweden.

The session will deal with recent progress in the understanding of the pathophysiology of the dopamine-denervated striatum and the mechanisms by which neural grafts can ameliorate symptoms in experimental parkinsonism in primates and in Parkinsons disease in man. M.R. De Long will focus on changes in neuronal activity in the primate basal ganglia following MPTP treatment, which results in an increased tonic and phasic output from the globus pallidus and excessive inhibition in the thalamus. S.B. Dunnett will review recent studies on the behavioral recovery induced by dopamine-rich nigral grafts in 6-hydroxydopamine-lesioned marmosets, and the topographic and behavioral specificity of these effects. D.M. Gash will deal with trophic effects, and trophic substances, elaborated by neural grafts or by the host brain in response to the grafting process, and the ability of such trophic mechanisms to rescue damaged neurons or stimulate sprouting in the nigrostriatal system. O. Lindvall will summarize recent data emerging from ongoing clinical trials using fetal nigral transplants, and discuss the shortcomings of the present techniques and the problems which have to be addressed in order to develop the neural transplant approach into a useful clinical therapy in PD patients.

VISUAL SYSTEM: CORTICAL MECHANISMS

585.1

SPONTANEOUS CALCIUM TRANSIENTS IN DEVELOPING NEOCORTICAL NEURONS.

L. C. Katz and R. Yuste. Dept. of Neurobiology, Duke University, Durham, NC 27710 and Lab. of Neurobiology, Rockefeller University, NY, NY 10021.

Spontaneous activity in the developing visual system plays a critical role in the activity-dependent modification of cortical circuits. Since synaptic activation causes postsynaptic calcium influx, video-based optical recording of neuronal $[Ca^{2+}]_i$ can be used to monitor activity over extensive cortical regions, with single cell resolution (Yuste & Katz, *Neuron* 6:333, 1991). Using fura-2, we have now recorded spontaneous $[Ca^{2+}]_i$ changes in slices of somatosensory and visual cortex of neonatal rats. During the first postnatal week the majority of neurons in all cortical layers exhibit spontaneous transients that result in 5-10 fold increases in $[Ca^{2+}]_i$. These events are non-periodic and occur in complex spatial and temporal patterns. Individual transients last 3-20 sec and occur at intervals ranging from 12 sec to 10 min.

Transients are totally blocked by 1mM Ni^{2+}, indicating that the entry of extracellular Ca^{2+} is probably involved. Although glial cells also show calcium transients (Cornell-Bell *et al.*, *Science* 247:471, 1990), and we cannot exclude their involvement, most of the events we observed occurred in neurons, as distinguished by their apical dendrites and large cell bodies. Transients are blocked by 100 µM APV but are unaffected by 20 µM CNQX. Their pharmacology suggests that they probably represent NMDA receptor-mediated responses to spontaneously released glutamate.

$[Ca^{2+}]_i$ transients have been associated with intracellular regulation of other messenger systems and the control of gene expression. In addition, optical recording of these spontaneous calcium events and others changes in $[Ca^{2+}]_i$ elicited by different stimulation paradigms can be used to unravel the spatial and temporal dynamics of the activity of neuronal networks.

Supported by the Lucille P. Markey Charitable Trust.

585.2

NEURONAL GROUPS REVEALED BY OPTICAL RECORDING OF CALCIUM TRANSIENTS IN SLICES OF DEVELOPING NEOCORTEX.

R. Yuste, A. Peinado and L. C. Katz. Lab. of Neurobiology, Rockefeller University, NY, NY 10021 and Dept. of Neurobiology, Duke University, Durham, NC 27710.

Correlations in the spontaneous activity of neighbouring neurons may play a critical role in the developmental refinement of cortical connectivity. By imaging slices of rat visual and somatosensory cortex with the Ca^{2+} sensitive dye fura-2, during the first postnatal week, we have observed frequent spontaneous calcium transients in individual cells (see previous abstract). When larger areas (~1 mm^2) were imaged, we found that discrete regions of neurons frequently changed $[Ca^{2+}]_i$ in synchrony, defining spherical, multicellular groups of 50-100 µm in diameter. These synchronous transients last 4-8 sec, appearing without any clear temporal relation to one another. Groups occur in both tangential and coronal slices, in visual and somatosensory cortices, and in all cortical layers, being more frequent in the upper layers. In tangential slices, groups of co-active cells tile the cortex without significant overlap. Groups are present at birth, and remain similar in size and properties throughout the first postnatal week.

To explore possible relationships between co-active groups and patterns of thalamic innervation, we combined Ca^{2+} imaging with fluorescent anterograde labelling of the "barrel field" in slices of somatosensory cortex. Rhodamine was injected in the Ventrobasal nucleus of the thalamus and the resulting pattern of "barrels" imaged in a tangential slice through layer 4. In preliminary experiments, groups, which are smaller than individual barrels, occur both inside and outside the barrels without any clear relationship to them.

In numerous cases, groups were observed at identical locations several minutes apart. This, combined with the lack of overlap with neighbouring groups, suggests that they may represent modular units in the developing brain.

Supported by the Lucille P. Markey Charitable Trust.

585.3

INTRACELLULAR STUDY OF VISUAL RESPONSE PROPERTIES AND OSCILLATORY BEHAVIOR OF KITTEN AREA 17 NEURONS AT THE PEAK OF THE CRITICAL PERIOD.

D. DEBANNE*, V. BRINGUIER*, D. SHULZ*, A. BARANYI & Y. FREGNAC. (Spon:A.Baranyi) Lab. de Neurobiologie et Neuropharmacologie du Développement Université Paris XI, 91405 Orsay Cedex, FRANCE

Intracellular recording techniques were used *in vivo* in anesthetized (Altesine) and paralyzed (Flaxedil) kittens to study the physiological properties, visually evoked PSP's underlying functional selectivity, and oscillatory behavior of visual cortical neurons.

77 cells have been recorded intracellularly (10 to 110 min.) in 7 to 15 week-old kittens, with 70 MΩ glass micropipettes filled with K+methyl-sulfate 2 M. Stability of recordings was improved by minimal craniotomy (1 to 2 mm), bilateral pneumothorax and eventually cystema drainage.

1) The membrane potential (-63 +/- 7 mV), spike amplitude (54 +/- 11 mV), and membrane resistance (25 +/- 9 MΩ) were similar to those usually found in adult visual cortex. However, the rather high proportion of bursting neurons which exhibit Ca2+ spikes (28 %) contrasts with their relative low representation in *in vitro* slices of kittens of the same age (Frégnac et al., 1990).

2) "Simple" (n=24) and "complex" (n=19) cells were visually characterized before and during impalement with static and dynamic stimuli. Most of the recorded simple cells exhibit an EPSP-IPSP sequence in response to the extinction of the stimulus in the ON subzone, and to the presentation of the stimulus in the OFF subzone of the RF. This suggests that the spatial segregation of ON and OFF subzones in simple cells is achieved by selective inhibition applied to a "complex" excitatory connexion scheme.

3) 8 to 30 Hz subthreshold oscillations of the membrane potential were observed in many cases during dynamic stimulation and less often during static stimuli. In the absence of visual stimulation, depolarization of the same neurons did not elicit such subthreshold oscillatory patterns. These observations suggest that in most cells, oscillatory behavior in kitten visual cortex is not an intrinsic voltage dependant property but could be mediated by synaptic input of either cortical or subcortical origin.

This work was supported by HFSP to Y.F.

585.4

POSTSYNAPTIC MEMBRANE POTENTIAL REGULATES POTENTIATION AND DEPRESSION OF VISUALLY EVOKED SYNAPTIC POTENTIALS IN KITTEN CORTICAL NEURONS RECORDED IN VIVO.

A. BARANYI, D. DEBANNE*, D. SHULZ* & Y. FREGNAC (SPON:Y. Frégnac) Lab. Neurobiologie du Développement, Univ. Paris XI, 91405 Orsay, FRANCE

It has been shown from in vivo extracellular recordings in cat visual cortex that changes in covariance between pre- and postsynaptic activity can potentiate or depress visual responses (Frégnac et al. Nature 333: 367, 1988). The effects of pairings between visual triggered PSP's with concomitant depolarizing or hyperpolarizing current injection in the target cell were studied to study the role of membrane potential of the postsynaptic neuron in inducing changes in synaptic efficacy.

77 stable intracellular recordings were made in area 17 of 7-15 week old kittens, using 2M K+methyl sulfate filled glass micropipettes. Visual compound PSP's in response to ON and OFF stimulation in 2 different positions of the receptive field (RF)were recorded in "simple" and "complex" cells of area 17. 24 cells were submitted to 40 low frequency (0.25 Hz) pairings where short depolarizing (S+, +1.5 to +4 nA) or hyperpolarizing pulses (S-, -1 to -4 nA) were associated to the presentation (ON) or extinction (OFF) of an optimal stiumulus in a given position of the RF. Visual responses averaged on 20-50 trials were compared before and after pairing at the same postsynaptic membrane potential. An increase (by more than 20%) of the paired compound PSP amplitude was observed in 8 out of the 12 S+ pairings. A decrease (by more than 20%) in PSP's amplitude was observed in 14 out of the 28 S- pairings. Input resistance and resting potential were usually unchanged. Spatial input specificity and temporal associativity were assessed by studying other parts of the receptive field which were stimulated out of phase from the paired position. After pairing, the distribution of the PSP's amplitude of the paired characteristic was significantly different from that of the unpaired characteristics in both positions of the RF.

These results demonstrate that the same visually evoked compound PSP can be potentiated or depressed by the temporal association of afferent stimulation with a local control of the membrane potential of the postsynaptic cell.

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585.5

SIMULATING EXERIENCE-DEPENDENT DEVELOPMENT OF ORIENTATION AND SPATIAL FREQUENCY TUNING IN CAT V1 WITH A REVERSE-HEBB MODIFICATION RULE. R. E. Soodak, The Rockefeller University, New York, NY 10021.

In a previous report (*Visual Neurosci.*, **6**) I showed that a reverse-Hebb synaptic modification rule led to experience-dependent enhancement of orientation tuning in simulations of simple cells in cat V1. In this presentation I will show that the model can be extended to account for experience-dependent enhancement of spatial frequency tuning as well. All structural parameters of the model, i.e. positions of ganglion cells in the retinal mosaic, cortical magnification factor and spread of geniculate afferents tangential to the cortical surface, were constrained by published anatomical and physiological data. In the general form of the synaptic modification rule, fractional change in synaptic weight, dW/W, is defined as a time weighted average, by a function H(t'), of the input-output cross-correlation, $X_{io}(t')$, where t' is the time interval between input and output. For the case of sinusoidal stimuli, as employed in these simulations, fractional change in synaptic weight is determined by the amplitudes of the pre- and post-synaptic activities, and a cosine term containing the phases of the pre- and post-synaptic activities and a phase variable defined by the Fourier transform of H(t') at the input frequency. The reverse-Hebb condition results when this phase variable has a value of 180 deg. In simulated development, the increase in slope of the low spatial frequency cutoff is more pronounced than that of the high frequency cutoff, in agreement with the data of Derrington and Fuchs (*J. Physiol.*, **316**, 1-10).

(Supported by NIH Grant EY-04613)

585.6

NERVE GROWTH FACTOR-RECEPTOR-IMMUNO-REACTIVITY IN CENTRAL VISUAL PATHWAYS OF THE DEVELOPING RABBIT BRAIN. Dennis Rickman, Nicholas Brecha and David Dawbarn*. Depts of Anatomy & Cell Biology and Medicine, Jules Stein Eye Institute and CURE, UCLA School of Medicine and VAMC-Wadsworth, Los Angeles, CA; University of Bristol, U.K.

Nerve growth factor (NGF) is a target-derived molecule which is crucial for the survival of, and neurite outgrowth from, sympathetic and primary sensory neurons. Recently it has been suggested that NGF plays a role in the development and maintenance of neurons in the central nervous system as well. In the developing rabbit retina, the low- affinity NGF-receptor (NGF-R) appears to be expressed transiently in Muller cells during the perinatal period. Here, using a mouse monoclonal antibody (ME 20.4) to the human low-affinity NGF-R, we examined the expression of NGF-R-immunoreactivity (NGF-R-IR) in central visual pathways of the developing rabbit brain. At embryonic day 25 and postnatal days 5, 7 and 10, NGF-R-IR was observed in the optic tract, the lateral geniculate nucleus of the thalamus, the nucleus of the optic tract, and the superficial (retinorecipient) layers of the superior colliculus. In addition, NGF-R-IR was observed in the medial and dorsal terminal nuclei of the accessory optic tract. No NGF-R-IR was observed in these central visual nuclei in the adult rabbit brain. These results suggest that NGF-R is expressed transiently during the perinatal period and may play a role in the innervation of central visual targets.

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585.7

NGF PREVENTS THE EFFECTS OF MONOCULAR DEPRIVATION. N. Berardi, G. Carmignoto, L. Domenici, V. Parisi*, T. Pizzorusso* and L. Maffei Neurofisiologia (CNR) and Scuola Normale Superiore, Pisa, 56100, Italy.

Monocular deprivation (MD) during the critical period of mammalian visual cortex development shifts the ocular dominance (OD) distribution of visual cortical neurons towards the non deprived eye. This effect is ascribed to competition between the thalamic afferents. We tested the hypotesis that this competition is for a neurotrophic factor such as NGF by assessing whether an exogenous supply of NGF could prevent MD effects. 20 hooded rats were monocularly deprived for one month from eye opening. 7 rats were left untreated, 9 rats were treated with ßNGF (intraventricular injections, 2 µl, 1-1.6 µg/µl), 4 rats were treated with cytochrome c. We found that in NGF treated rats the OD distribution in area 17 is similar to that in normal adults. By contrast MD shifts the OD distribution both in untreated and in cytochrome c treated rats. NGF treatment does not affect such spontaneous discharge and selectivity for stimulus parameters of cortical cells. We conclude that NGF prevents the effects of MD in the rat visual cortex.

585.8

EVIDENCE FOR A ROLE OF NGF IN NEURONAL PLASTICITY OF THE CAT VISUAL SYSTEM. G. Carmignoto, R. Canella*, P. Candeo* and M.C. Comelli* Fidia Research Laboratories, 35031 Abano Terme (PD), Italy.

Monocular deprivation in kittens causes the almost total loss of excitability of visual cortical neurons by stimulation of the deprived eye. We report that intraventricular administration of NGF through a cannula-mini-pump system in kittens subjected to monocular deprivation for 15 days prevents, virtually completely, the shift of ocular dominance of area 17 neurons toward the open eye. Conventional single unit recordings show that in NGF-treated animals (n=3; 208 cells) 57.1±12.1% of recorded neurons in area 17 were binocularly responsive compared to 9.6±0.6% and 6.3±1.7% in kittens treated with cytochrome c (n=3; 172 cells) or untreated (n=3; 148 cells), respectively. Receptive field properties of neurons in NGF-treated kittens did not show any abnormalities. Autoradiographic experiments utilizing ^{125}I-NGF are in progress to detect the possible presence of NGF binding sites in visual system-related nuclei. A specific role of NGF on visual cortical plasticity is proposed.

585.9

ORIENTATION AND DIRECTION SENSITIVE CELLS IN THE LGND AND STRIATE CORTEX OF DARK REARED CATS. Audie G. Leventhal, Kirk G. Thompson, Yifeng Zhou, Steven J. Ault Anat. Dept., Univ. of Utah Sch. of Med., Salt Lake City, UT 84132

LGNd and striate cortical (area 17) cells of cats reared in darkness from birth were studied quantitatively using gratings, bars, and spots generated under computer control. Statistical tests designed specifically to analyze orientation and direction were employed. These procedures revealed a higher percentage of orientation and direction selective cortical cells in dark reared cats than reported previously and provide evidence that dark rearing does not affect these properties of LGNd cells.

In dark reared animals, the orientation and direction sensitivity of LGNd relay cells and area 17 cells did not differ quantitatively or qualitatively. Most LGNd cells and cortical cells were orientation sensitive, 79% and 71% respectively, and many were also direction sensitive, 36% and 46% respectively. Dark reared area 17 cells exhibited mean orientation and direction biases of .17 and .11, respectively compared with mean values of .19 and .10, respectively for LGNd cells. Also, in both the LGNd and in area 17, cells were more selective when the test stimuli were gratings rather than bars and, in both areas cells exhibited clearer direction and orientation sensitivity when tested with relatively low and high spatial frequency gratings, respectively. The orientation and direction sensitive responses of LGNd cells were two times stronger and three times less variable than those of cortical cells.

Our results indicate that innate, subcortical orientation and direction sensitivity coupled with early visual experience contribute to the high degree of selectivity found normally in visual cortex.

585.10

RECOVERY FROM THE EFFECTS OF EARLY BINOCULAR DEPRIVATION IN THE CAT STRIATE CORTEX. S. V. Szapiel and T. N. Wiesel, Laboratory of Neurobiology, The Rockefeller University, N.Y., N. Y. 10021.

The effects of 3 months of binocular visual deprivation at birth by eyelid suture followed by opening of one eye and 3 months of recovery, were studied in the striate cortex of 6 cats by extracellular, single-unit recordings and in one animal also by optical recording of intrinsic signals. A total of 253 cells were recorded in anesthetized and paralyzed animals and their receptive field properties were analyzed using oriented stimuli. The majority of cells (65%) were driven solely by the opened eye and only 5% solely by the closed eye. This indicates a significant shift in eye preference associated with the recovery period, since as previously shown (Hubel and Wiesel, 1965) at 3 months after binocular deprivation without recovery, there is an equal number of monocular cells dominated by either eye. Analysis of orientation tuning in the current study revealed that 38% of all cells (96/253) exhibited normal orientation tuning, similar to a finding of 40% in the Hubel and Wiesel study. Of the remaining cells in their study, 27% were non-drivable and 32% were unoriented whereas, we found few cells which could not be driven, and a large number of poorly oriented or unoriented cells. This suggests that the high proportion of non-drivable cells found before recovery now seem to be drivable but show poor or no orientation specificity. These results may indicate that at least partial recovery of visual function is possible after the critical period. (Supported by NIH grants EY00305 and EY05253).

585.11

ACTIVITY-DEPENDENT PRUNING OF LONG-RANGE TANGENTIAL CONNECTIONS IN AREA 17 OF STRABISMIC CATS. Siegrid Löwel and Wolf Singer. Max-Planck-Institut für Hirnforschung, Deutschordenstr. 46, D-6000 Frankfurt 71, F.R.G.

A prominent feature of visual cortical circuitry is the system of long-range horizontal connections. These develop postnatally and acquire their selectivity through a pruning process. We examined whether this pruning is influenced by visual experience and in particular whether it is affected by the decorrelation of neuronal responses as it occurs with strabismus. In 4 cats, divergent strabismus was induced surgically at the age of 2-3 weeks. At age 2-3 months, intrinsic cortical connections were labelled with fluorescent tracers and ocular dominance (OD) columns were visualized using 2-DG autoradiography. Regions of increased 2-DG uptake extended in a columnar fashion through all cortical layers and formed a pattern similar to the OD-system seen in layer IV after intraocular injections of [^{3}H]proline. Clusters of retrogradely labelled cells extended up to 5mm from the injection site. Comparison of the two patterns in flat-mount sections revealed that labelled cells were predominantly confined to regions of the same OD as the respective injection sites, indicating that in strabismic cats the tangential fibers interconnect preferentially columns with the same OD. In normally reared cats, tangential connections were not confined to 2-DG territories labelled after monocular stimulation. These data suggest that 1) pruning of tangential connections is influenced by visual experience and 2) selection criterion for the stabilization of pathways is the correlation of activity in interconnected elements.

585.12

REMAPPING OF THE PROJECTION OF THE VISUAL FIELD ON THE SUPERIOR COLLICULUS AFTER NEONATAL ROTATIONAL SQUINT IN THE WALLABY (*MACROPUS EUGENII*). R.F. Mark, L.R. Marotte* and X.-M. Sheng. *
Centre for Visual Science, Developmental Neurobiology Group, Research School of Biological Sciences,Australian National University, Canberra ACT 2601, Australia.

For a short period after the eyes open different parts of the mammalian visual system are variably dependent upon visual experience for their normal development. Abnormal experience such as neonatal squint (misalignment of the eyes) produces changes in the receptive field properties of neurones of the cerebral cortex. The superior colliculus is not so susceptible.

In 7 wallaby pouch young one eye was surgically rotated about the visual axis just prior to eye opening. The animals were grown to maturity and maps made by electrophysiology of the projection of the visual field of each eye upon the contralateral superior coiliculus. In 3 additional mature animals a similar rotation was performed after anaesthesia immediately prior to mapping. In this latter group the projection of the visual field was as would be expected from the degree of rotation. In all of the early rotation group the projection of the visual field was distorted towards a complicated map in which elevation was in accord with eye rotation but azimuth was represented in the original orientation on the colliculus. This may indicate some developmental compensation in the colliculus towards restoring spatially correct vision from the misaligned eye.

NEUROBIOLOGY OF AFFECTIVE ILLNESS AND RELATED DISORDERS

586.1

REDUCED TYROSINE HYDROXYLASE IMMUNO-REACTIVITY IN LOCUS COERULEUS OF SUICIDE VICTIMS A. Biegon , Lawrence Berkeley Laboratory, Univ. of California, Berkeley, CA 94720

As part of a search for presynaptic noradrenergic markers in suicide and depression, we have investigated the fate of noradrenaline synthetising enzymes in suicide victims. Antibodies against tyrosine hydroxylase (TH), the rate limiting enzyme in norepinephrine synthesis, and dopaminre beta hydroxylase (DBH), the last enzyme in the synthesis, were used for immunohistochemical stainimg of human brain locus coeruleus sections obtained postmortem. Brains of 6 suicide victims and 6 age- and sex matched controls were obtained from the medical examiners' office. All subjects were drug- and neuropathology free at time of death and had no psychiatric history (controls) or none besides depression (suicide victims). Sections (40µ) were cut on a whole body cryostat, thaw mounted onto glass slides and postfixed in 10%formalin prior to staining. Mean cell number, cell density and immunreactivity (stain density) over individual cells (~100cells/subject) were quantified by a computerized image analysis system. Mean TH stain density, in optical density x 100 units, was significantly lower in the suicides (controls: 28.8±6.8, suicides: 19.9±5.8, $p<0.025$, Student's t-test for paired values). There was no significant difference between suicides and controls in DBH immunoreactivity , mean cell size or mean cell density. The results suggest that a presynaptic noradrenergic deficit does indeed exist in suicides, expressed as reduced levels of TH in the locus coeruleus.

586.2

BINDING TO SEROTONIN UPTAKE SITES IN PREFRONTAL AND TEMPORAL CORTEX OF SUICIDE VICTIMS. R.A. Henteleff, V. Arango and J.J. Mann. Laboratories of Neuropharmacology, University of Pittsburgh, Pittsburgh, PA 15213.

We have previously reported increased postsynaptic 5-HT$_2$ binding in the prefrontal cortex of suicide victims (*Arch. Gen. Psychiatry, 43:954-9, 1986; Arch. Gen. Psychiatry, 47:1038-47, 1990).* Conflicting reports exist in the literature as to whether binding to the serotonin transporter on serotonin nerve terminals is reduced in brain cortical regions of suicide victims. Using the selective radioligand ^{3}H-paroxetine, we sought to determine whether binding is altered in membrane homogenates from the prefrontal cortex (Brodmann area 9, PFC) and the temporal pole (Brodmann area 38, TC) of suicide victims compared to controls. All cases had negative toxicological screens. Subjects were matched for postmortem delay, age, gender, and where possible race (PFC N = 11 pairs; TC N = 7 pairs). P$_2$ pellets (5 mg/tube) were preincubated (30 min, 23°C) in 50 mM Tris-HCl buffer, pH 7.4 and then incubated (60 min, 23°C) in same buffer containing 120 mM NaCl, 5 mM KCl and 6 concentrations of ^{3}H-paroxetine (0.1-3.2 nM). Nonspecific binding was determined by 10 µM sertraline. In the suicide group binding was significantly reduced in the PFC (93.1 ± 12.3 *vs.* 150.8 ± 33.3 fmol/mg protein, $p = 0.026$) but not in the TC (127.2 ± 11.5 *vs.* 153.3 ± 23.7 fmol/mg protein, $p > 0.05$). K_D did not differ between groups in either area. Binding in both groups was positively correlated with age in TC ($r = 0.69$, $p = 0.007$, N = 14). A negative correlation with postmortem delay (range 7 to 22 hours) was found in TC of both groups ($r = -0.53$, $p = 0.05$, N = 14). We conclude that 5-HT uptake sites are reduced in the PFC but not in the TC of suicide victims, consistent with the hypothesis that there is a serotonergic deficiency associated with suicide. (Supported by PHS grants MH40210 and MH46745.)

586.3

QUANTITATIVE AUTORADIOGRAPHY OF 5-HT$_{1A}$ BINDING IN SUICIDE. V. Arango, W.E. Miller, M.L. Miller, M.D. Underwood, R.W. Smith and J.J. Mann. Laboratories of Neuropharmacology, University of Pittsburgh, Pittsburgh, PA 15213.

We have previously reported increased 5-HT$_2$ binding in the prefrontal cortex of suicide victims. Several subtypes of 5-HT$_1$ receptors have now been identified in human brain including the 5-HT$_{1A}$ site which appears to be involved in the actions of certain psychotropic agents. Using quantitative autoradiography we sought to determine whether 5-HT$_{1A}$ binding is altered in the brain of suicide victims by studying five Brodmann areas (11, 24, 8, 9 and 46) in the frontal cortex of suicide victims and controls, with negative toxicological screens. Subjects were matched for postmortem delay, age and gender (N = 10 pairs). Slide-mounted coronal sections (20µm) from the right hemicerebrum were incubated with 2nM ^{3}H-8-OH-DPAT and nonspecific binding determined by 1µM 5-HT. Sertraline blocked binding to 5-HT uptake sites. Tissue sections were exposed to Ultrofilm for 8 weeks and analyzed using a PC-based image analysis system (Imaging Research, Inc). 5-HT$_{1A}$ receptor distribution across cortical layers was similar in both groups forming five isodensity bands corresponding to layer I, layer II, outer layer III, layers III-IV, and layers V-VI. The gyrus had a higher level of binding than the sulcus. Layer II had the highest level of binding, approximately sixfold that of layers III-IV, which had the lowest. The suicide group differed from the control group in only one of the five brain areas. In the sulcus of area 46, layer II had 29% more 5-HT$_{1A}$ binding in the suicide group (Suicide: 31.1 ± 4.1 fmol/mg tissue; Control: 26.6 ± 4.2 fmol/mg tissue; $p < 0.05$). The binding was positively correlated with age ($r = 0.44$ to 0.66; $p = 0.003$ to 0.05) but not with postmortem delay. Females had greater 5-HT$_{1A}$ binding than males. We conclude that 5-HT$_{1A}$ binding is increased in some brain regions of suicide victims. Further studies should investigate other brain areas and other 5-HT$_1$ receptor subtypes and control for the effects of age and gender. (Supported by PHS grants MH40210 and MH46745.)

586.4

REDUCED PLATELET [^{3}H]-PAROXETINE AND [^{3}H]-IMIPRAMINE BINDING IN MAJOR DEPRESSION. C.B. Nemeroff, D.L. Knight, and K.R.R. Krishnan. Depts. Psychiat. & Pharmacol., Duke Univ. Med. Ctr. Durham, NC 27710

Considerable evidence has accumulated to implicate serotonergic neuronal dysfunction in the pathophysiology of major depression. One such line of evidence is the reduction in the density of binding sites for [^{3}H]-imipramine ([^{3}H]-IMI), a marker of the presynaptic 5HT uptake site, in postmortem brain tissue and platelets of patients with major depression. However, not all investigators have confirmed the reduction in [^{3}H]-IMI binding site density in drug-free depressed patients. This may be at least partly due to the fact that [^{3}H]-IMI is not the ideal ligand to label the presynaptic 5HT transporter as it binds to a variety of other neurotransmitter receptors. For all of these reasons we have used a very selective 5HT uptake inhibitor, [^{3}H]-paroxetine ([^{3}H]-PAR), to label the 5HT transporter to study the platelet binding kinetics of the 5HT transporter in drug-free depressed patients and age- and sex-matched controls. In the same platelet samples, we also measured [^{3}H]-IMI binding. Seven concentrations of [^{3}H]-IMI and [^{3}H]-PAR were used in these experiments and Scatchard analysis of these data were performed. The diagnostic groups studied were: (1) young normal controls, n=14, (<50 yrs); (2) elderly normal controls, n=14 (>60 yrs); (3) young major depression, n=14 (<50 yrs); (4) elderly major depression, n=14 (<60 yrs.) The number (Bmax) of high affinity binding sites for both [^{3}H]-PAR and [^{3}H]-IMI was significantly reduced in the depressed patients when compared to their age-matched controls. No change in Kd was observed. In addition, there was a significant correlation between the Bmax for [^{3}H]-PAR and [^{3}H]-IMI ($p < 0.005$). These findings represent the first study in which both [^{3}H]-PAR and [^{3}H]-IMI binding was measured in identical platelet samples. The results support a role for 5HT systems in the pathophysiology of depression. (Supported by NIMH MH-40159).

586.5

THE FOUR-ARM MAZE: AN IMPROVED MODEL FOR OBSESSIVE-COMPULSIVE DISORDER? E. Yadin and C. Thompson*. Department of Psychiatry, The Medical College of Pennsylvania, Philadelphia, PA 19129, USA.

The sensitivity of the traditional spontaneous alternation paradigm to serotonergic manipulation has been previously demonstrated. The present study used a modification of the spontaneous alternation task, the four-arm maze, as an improved model for the repetitive nature and indecisiveness seen in obsessive-compulsive disorder (OCD) in humans. Food-deprived rats were run in a black Plexiglas maze with 4 identical arms, equipped with white end-panels. All arms were baited with a small amount of chocolate milk. Each rat was placed in the center and allowed to explore the maze until 12 arm entries were completed (or a maximum time of 10 min). Chocolate milk was replenished after each choice. The percent of repeated arm entry patterns was recorded as well as the latency to complete 12 entries. After a stable baseline of arm entries was achieved the effects of manipulating the serotonergic system were tested.

Both the nonselective 5-HT agonist 5-MeODMT (1.25 mg/kg) and the more selective 5-HT_{1A} agonist 8-OH-DPAT (2 mg/kg) disrupted alternation patterns in the 4-arm maze, a change that was reversed by the nonselective antagonist methiothepin (0.1 mg/kg). A course of chronic treatment (2x5 mg/kg for 21 days) with the selective 5-HT uptake blocking agent fluoxetine had a protective effect on the 5-MeODMT-induced disruption of alternation behavior, whereas chronic treatment with the benzodiazepine chlordiazepoxide did not.

586.6

THE PHOSPHOLIPID THEORY OF DEPRESSION: IMPLICATIONS FOR TRH FUNCTION IN CNS. J. R. Hibbeln* and A. Sattin. DVA Sepulveda-UCLA Medical Center, Sepulveda, CA 91343-2099.

Many confirmed biological findings in depressed patients not explained by amine theories are compatible with increased activities of phospholipase A_2 (PLA_2; Hibbeln *et al, Biol. Psychiat.*, 25: 945, 1989). By altering membrane phospholipid composition and fluidity diverse enzymes and regulatory proteins may be affected. The theory explains depression-related altered activity of Na-K-ATPase, β_2- and α_2-adrenergic receptors, MAO, uptake of NE and 5-HT, and imipramine binding. PLA_2 reduction of tyrosine and tryptophan hydroxylase activity is consistent with amine theories. The theory explains the antidepressant effects of tricyclic antidepressants, Li^+, electroconvulsive shock (ECS) and some other antimanic agents. PLA_2 activation may reduce the binding of TRH and cortisol to their respective ligands, explaining the reduced responses to TRH and dexamethasone seen in many depressed patients. ECS activates phospholipase C→ arachidonic acid→ PGE_2 (prolonged elevation), an anticonvulsant which inhibits PLA_2. Reduced PLA_2 activity might increase TRH receptor efficacy in CNS (as in pituitary) wherein TRH synthesis and release are also augmented, in hippocampus, after ECS (Knoblach *et al, Abstr. Soc. Neurosci.*, 16: 1098, 1990). This apparent concerted augmentation of TRH function might be opposed by receptor down-regulation but the functional resultant remains unknown. Both ECS and TRH are anticonvulsant. These interactions are believed to contribute to the uniquely antidepressant properties of Electroconvulsive Treatment which is also anticonvulsant in humans.[suppt: DVA Res. Svc.]

586.7

ELEVATED CEREBRAL CORTEX G_s-ALPHA IMMUNOREACTIVITY IN BIPOLAR AFFECTIVE DISORDER. L.T. Young, P.P. Li, S.J. Kish, K.P. Siu* and J.J. Warsh. Clarke Institute of Psychiatry, Toronto, Canada, M5T 1R8.

Evidence implicating postreceptor disturbances in affective disorders, including bipolar affective disorder (BAD), together with the recent observations that lithium acts on the coupling of neuroreceptors to effector responses at the guanine nucleotide binding (G)-protein level suggest that abnormalities in G-protein function may occur in BAD. To test the hypothesis that G-protein levels are altered in brain of BAD patients we estimated the relative abundance of G-protein subunits in postmortem brain obtained from patients (N=7) who had been diagnosed with BAD (confirmed by chart review) and age and sex-matched controls (N=7). G-protein subunit immunoreactivities were determined in membranes prepared from postmortem prefrontal cortex (Brodman's area 10) using SDS-PAGE and immunoblotting with specific polyclonal antisera against specific G-protein subunits: $G_{s\alpha}$, $G_{i(1\&2)\alpha}$, $G_{o\alpha}$ and G_{β}. Immunoreactive bands were detected by enhanced chemiluminescence and quantitated by laser densitometry. Brain samples from BAD and matched control subjects were processed in a yoked design on the same blots to control for intrablot assay variation. Prefrontal cortical $G_{s\alpha}$ subunit (52 kDa species) immunoreactivity was significantly elevated (34%, p=.01) in BAD subjects compared with age and sex-matched controls. In contrast, no significant differences were found in the other G-protein subunits measured. Significantly increased (>80%, p<.05) mean $G_{s\alpha}$ immunoreactivity was also found in occipital cortex but not in cerebellum (22 %) obtained from the same subjects. On the basis that increased $G_{s\alpha}$ immunoreactivity reflects increased $G_{s\alpha}$ subunit concentration, and that increased levels may enhance functional responses to receptor activation, the present findings suggest that disturbances in G_s-mediated signal transduction may be involved in the pathophysiology of BAD.

586.8

GABA BLOCKADE IN THE REGION OF THE DORSOMEDIAL HYPOTHALAMUS (DMH) OF RATS: A POSSIBLE MODEL FOR PANIC ATTACKS. A. Shekhar, Dept. of Psychiatry, Indiana Univ. Med. Ctr. Indianapolis, IN 46202.

Blocking GABA neurotransmission in the region of the DMH of rats elicits a constellation of responses which include increases in heart rate (HR), respiratory rate (RR), blood pressure (BP), locomotor agitation, aversive responses and "anxiety" similar to severe anxiety states like panic attacks in humans. The present study was aimed at testing if this response can be blocked by treatment with standard human anti-panic drugs like imipramine. Rats were implanted with bilateral microinjection cannulae in the DMH and arterial and venous catheters. Rats were then randomized to either daily placebo injections or imipramine (15 mg/kg) injections intraperitoneally for 7 days. All treatments were double-blind and the physiological responses to BMI injection (25 ng/250 nl) into the DMH were recorded at baseline and on days 2, 7 and 10. The BMI response was almost completely blocked by systemic imipramine while the placebo treatment had no significant effect. These results support the hypothesis that GABA blockade in the DMH may be a model for severe anxiety states. (Supported by R29 MH45362-02).

586.9

PYRITHIAMINE-INDUCED THIAMINE DEFICIENCY RESULTS IN DECREASED Ca^{2+}-DEPENDENT RELEASE OF GLUTAMATE FROM RAT HIPPOCAMPAL SLICES. O. Le*, M. Héroux and R.F. Butterworth, Lab. of Neurochemistry, André-Viallet Clin. Res. Center, Hôpital St-Luc (Univ. of Montreal), Montreal, Quebec, Canada H2X 3J4.

Pyrithiamine-induced thiamine deficiency encephalopathy results in decreased brain glutamate content (Butterworth and Héroux, J. Neurochem. 52, 1079, 1989). In order to evaluate the effects of thiamine deficiency on the release of neurotransmitter glutamate, electrically-stimulated release of glutamate was studied in superfused hippocampal slices from symptomatic pyrithiamine-treated rats as well as in pair-fed controls. Stimulation-evoked release of glutamate was reduced by 42% ($p<0.02$) in the presence of 1.2 mM Ca^{2+}. These changes were accompanied by parallel decreases of activity of the thiamine-dependent enzyme α-ketoglutarate dehydrogenase (αKGDH) in hippocampi from symptomatic animals. These findings suggest an effect of thiamine deficiency on the glutamate content of nerve terminals in the CNS. Such decreases could result from decreased synthesis of neurotransmitter glutamate as a consequence of diminished activities of αKGDH. Glutamatergic dysfunction could play an important role in the pathogenesis of thiamine-deficiency encephalopathy (Supported by grants from MRC Canada and ABMRF).

587.1

DOES DOPAMINE ACTIVATE POTASSIUM CHANNELS OF MULLER CELLS? F.A.M. de-Azeredo and M.F.Ribeiro*,Laboratorio de Biociencias, Depto de Neurobiologia, Universidade Federal Fluminense, Caixa Postal 100229, Niteroi, RJ, 24000, Brazil.

In the retina, during spreading depression (SD), large spatial buffering K+ currents have been predicted to occur where the Muller cells are well suited to transfer K+ from the retina to the vitreous humor and the extracellular space. Recently, Karwoski,Lu and Newman (Science,244:578,1989) have reported that Ba2+ block K+ channels of Muller cells reducing the release of this ion to the vitreous humor during light stimulation. We then tested the action of Ba2+ on the velocity of propagation (VP) of SD and its influence on the the effect of a dopamine D1 agonist, SKF 38393. SD can be mechanically elicited at the isolated eye cup preparation of the chick retina and the wave followed with a stereomicroscope. VP is very sensitive to changes in the composition of the superfusate and it can measured based on the time elapsed between two parallel lines located at the ocular which correspond to 2 mm at the preparation. When SKF 38393 (10 uM) is added to the superfusate there is a significant increase in VP which is inhibited by SCH 23390,a D1 antagonist. VP also returns to control levels when the eye cup is superfused with normal physiological solution (PS). However, if the preparation is superfused with SKF 38393 after being initially bathed with Ba2+ (50 uM) no significant increase in VP is observed. Ba2+ when added at the bathing fluid at the begginning of the superfusion does not change the VP and if does the effect seems to be very slow. Returning to superfusate the retina with PS no residual effect of Ba2+ is observed. We suggest that Muller cell has a D1 dopamine receptor which regulates K+ channels responsible for the spatial buffering of this ion in the retina.

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587.2

SUBSTANCE P, SOMATOSTATIN AND MET-ENKEPHALIN REGULATE THE SAME K- CHANNEL IN CULTURED NORADRENERGIC NEURONS FROM THE LOCUS COERULEUS. *B.Velimirovic, K.Koyano, S.Nakajima and Y.Nakajima. Dept. of Anat. and Cell Biol. and Dept. of Pharmacol., Univ. of Illinois, College of Medicine at Chicago, Chicago, IL 60612.*

We reported that substance P (SP) excites noradrenergic neurons of the locus coeruleus by suppressing a K-conductance (Koyano et al.,Biophysical J. 59:391A,1991), whereas somatostatin (SOM) or methionine enkephalin (MENK) inhibits them by enhancing a K-conductance (Inoue et al.,J. Physiol. 407:177,1988; North et al.,Proc.Natl.Acad.Sci.USA 84:5487,1987). All these K-conductances showed inwardly rectifying properties. The SP response and SOM (or MENK) response seem to be mediated through different G proteins. We performed experiments to determine whether the same K-channel molecule is modulated by SP and by SOM (or MENK). The whole-cell patch clamp technique was used on cultured rat locus coeruleus neurons. Application of SP during a SOM (or MENK) response produced a larger conductance reduction than when SP was applied alone. In some neurons SP alone did not cause an obvious response, yet, SP was able to abolish the K-conductance that was induced by SOM or MENK. When SOM (or MENK) was applied soon after SP application, SOM (or MENK) induced a smaller conductance increase than when it was applied alone. Similar results were obtained in neurons preloaded with GTPγS. These results indicate that it is the same inwardly rectifying K-channel molecule which is responding (closing or opening) to SP and to SOM (or MENK). The SP action that closes the K-channel seems to be stronger than the SOM (or MENK) action that opens it. Supported by PHS grants AG06093 and DA05701.

587.3

Hormonal Regulation of K^+ Channels in Human T Lymphocytes. L.C. Schlichter*, P.A. Pahapill* and P.S. Pennefather, Department of Physiology, University of Toronto, Ontario, Canada, M5S 1A8.

In cell-attached patch-clamp recordings of intact human T cells we found voltage-activated K^+ channels in about 60% of patches. The I-V relation was inwardly rectifying and had a slope conductance of 9.4 pS to 23.8 pS. From the reversal potentials under various ionic gradients the cell resting potential was -51 $\pm$ 1 mV in normal NaCl saline and about 0 mV when the bath contained 150 mM KCl saline. Ensemble currents constructed from single channel recordings showed sigmoid kinetics of current activation and a monoexponential decay phase. These kinetics were well fitted by a Hodgkin-Huxley type n^4j kinetic model with time constants very similar to the whole-cell current of disrupted cells. K^+ channel activity at the resting potential increased about 10 fold when the temperature was raised from 22° to 30°C and the single-channel conductance increased. This voltage-dependent K^+ channel was inhibited by a rise in internal Ca^{2+} concentration produced by mitogens or ionomycin in the presence of external Ca^{2+}. Several agents that raise cyclic AMP levels in T lymphocytes caused an increase in K^+ channel activity in cell-attached patches (8BrcAMP, dbcAMP, cpt cAMP, forskolin, isoproterenol, isobutyl methyl xanthine, histamine and prostaglandin E_2). The cAMP-analogues had no effect on intracellular Ca^{2+}, as measured by Fluo-3 fluorescence. Supported by grants from MRC and NCIC (Canada).

587.4

STIMULATION OF ATP-SENSITIVE K^+ CHANNEL ACTIVITY BY ADP IN EXCISED PATCHES FROM CULTURED MOUSE PANCREATIC B-CELLS. W.F. Hopkins and D.L. Cook*, Depts. of Physiology/Biophysics and Medicine, Univ. of Washington and VA Medical Center, Seattle, WA 98108.

ATP and ADP are known to compete in regulating ATP-sensitive potassium channels (K(ATP)) in insulin-secreting cells, yet little is known about the receptor mechanisms for this effect. We have obtained concentration-response data for ADP on K(ATP) channel activity in excised, inside-out patches from cultured adult mouse B-cells. At millimolar concentrations, ADP inhibited K(ATP) channel activity, as has been described (Cook and Hales, 1984). Furthermore, we found that 10 mM ADP inhibited channel activity by 94 $\pm$ 3% (sem, N=7 patches) suggesting that it is a full rather than partial agonist for inhibition of K(ATP) channel activity. At micromolar concentrations, however, ADP stimulated K(ATP) channel activity in the absence of ATP. ADP (100 μM) enhanced channel activity by 221 $\pm$ 82% (N=15), while 10 μM ADP stimulated channel activity by 19 $\pm$ 7% (N=6). The stimulatory effect of ADP did not run-down with repeated applications, and was not mimicked by the non-hydrolyzable ADP analogue, ADP-ß-S (100 μM). In 20% of patches in which K(ATP) channels were blockable by 10 mM ADP, the excitatory effect of 100 μM ADP was not seen. These results suggest that ADP competes with ATP by binding to an ADP-site which opens the channel while ATP (and high concentrations of ADP) binds to a separate site which closes the channel. This competition may be the basis for channel regulation by ADP levels in the 10-100 μM range thought to exist in cells.

587.5

GLYBURIDE-SENSITIVE K^+ CHANNELS IN CULTURED RAT HIPPOCAMPAL NEURONS: ACTIVATION BY CROMAKALIM AND ENERGY DEPLETING CONDITIONS. D.M. Politi and M.A. Rogawski. Neuronal Excitability Section, Medical Neurology Branch, NINDS, NIH, Bethesda, MD 20892.

Previous studies in our laboratory have shown that cromakalim activates a tetraethylammonium-sensitive K^+ current in cultured embryonic rat hippocampal neurons. This phenomenon was further characterized using whole-cell voltage-clamp and single channel recording techniques. Glyburide (1-25 μM), an antagonist of ATP-dependent K^+ channels, produced a depression of the cromakalim-activated current, but did not affect resting or voltage-activated K^+ current in the absence of cromakalim. Exposure of the cells to energy depleting conditions [oligomycin (0.24 μg/ml) and 2-deoxy-D-glucose (10 mM)] also activated an outward current. Single channel recordings in the cell-attached configuration showed that cromakalim (100 μM) stimulated the opening of flickery single channels having a unitary conductance of ~26 pS and a prolonged burst duration (mean open time ~131 ms); similar channel openings were observed in patches from cells exposed to energy-depleting conditions. Preliminary studies with inside-out patches showed currents with a conductance of 26 pS in physiological solutions and 50 pS in symmetrical K^+ solutions; these channels are being further characterized for ATP sensitivity. Nevertheless, the observation that cromakalim and energy depleting conditions activate the same glyburide-sensitive channel supports the concept that the K^+ channels whose opening is stimulated by cromakalim are of the ATP-sensitive type.

587.6

Ca^{2+}-dependence of I_D in hippocampal pyramidal neurons. K.Krnjević,[1] V. Crépel* and Y. Ben-Ari. INSERM U29, Hôpital Port-Royal, 75014 , Paris, France and [1]Anaesthesia Research Dept., McGill University, Montréal, H3G 1Y6, Canada.

A fast-activating, slowly inactivating K current, which is generated by depolarizing pulses from a relatively negative membrane potential (< -60mV), was first identified (and named I_D) by Storm (1988, Nature 336:379). As originally described, I_D was insensitive to TEA, but was readily blocked by low concentrations of 4-AP (30 μM). Its persistence in the presence of 200 μM Cd^{2+} led to the conclusion that I_D is not Ca^{2+}-activated.

In experiments on CA3 pyramidal cells, in slices (from Wistar rats) kept submerged at 33° and in the presence of tetrodotoxin, we have repeatedly recorded a current having the characteristics of I_D: a very slowly decaying (time constant > 1s) outward current, evoked either by depolarizing pulses from V_H ~ -80mV, or more often as an off-current following large hyperpolarizing pulses from V_H ~ -50mV. Also in agreement with Storm (1988), this current was largely abolished by 30-40 μM 4-AP, but not by 1-10 mM TEA.

However, when the superfusing medium was changed to a low Ca^{2+} (0.2-0.5 mM) solution - with 0.2-0.5 mM Cd^{2+} or 4 mM Mn^{2+} added - we consistently observed a major suppression of I_D, comparable to the effect produced by 30 μM 4-AP. Neither 4-AP nor low Ca^{2+} solutions produced any major depression of the early transient outward current (I_A).

The sensitivity of I_D to Ca-deficient solutions (with Cd^{2+} or Mn^{2+} added) suggests that Ca^{2+} does play a role in the generation of I_D.

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587.7

THE DIVALENT CATION BINDING SITE ON POTASSIUM CHANNELS. T. Begenisich and S. Spires. Department of Physiology, University of Rochester, Rochester, NY 14642.

External divalent cations produce significant alteration in the kinetics of delayed rectifier potassium channels of squid (*Loligo pealei*) axons. The activation of the K channel current is slowed and the rate of deactivation is increased. These effects are produced by the physiologically important cation, Ca^{++}, but the kinetic alterations are more pronounced when Zn ions are used as probes. The modifications produced by low concentrations (2 to 5 mM) of Zn ions are qualitatively similar to but much larger than those produced by much larger concentrations of Ca ions. At normal pH values (7.5), these Zn concentrations produce a 3- to 4-fold slowing of the activation time constant: much too large to be consistent with any reasonable voltage shift produced by interaction with surface charges. The pK for hydrogen ion inhibition of the Zn effects is near 7.4. Treatment of the cells with histidine- or sulfhydryl-modifying reagents does not alter the Zn effects, however, Zn ions have almost no effect on the K channel kinetics after treatment with the amino-specific reagent trinitrobenzenesulfonicacid (TNBS). The external divalent cation binding site on the squid delayed rectifier K channel probably contains one or more important amino residues: lysine or a terminal amine.

To see if these properties of the divalent cation binding site are characteristics general to K channels we performed experiments on a K channel with a known sequence: the A-type K channel from Shaker *Drosophila*. The baculovirus vector expression system was used to insert A-type K channels from Shaker into the cell membranes of caterpillar cells (*Spodoptera frugiperda*). The caterpillar cells have no endogenous ion channels. Electrophysiologic studies were performed using standard voltage-clamp techniques. The interaction of Zn with the divalent cation binding site of the Shaker A-type channel produces some effects similar to those seen in squid: the activation and inactivation kinetics are slowed by Zn; and hydrogen ions compete with Zn ions for the binding site. However, there is a major difference: treatment of the cell with TNBS does not render the Zn inactive.

587.8

IMMATURE RAT MYOMETRIAL CELLS AND HUMAN UTERINE FIBROID CELLS IN CULTURE PREDOMINANTLY EXPRESS TRANSIENT OUTWARD K^+ CURRENTS

S.D. Erulkar, J. Ludmir*, B. Ger* and R. DePino*. Depts. of Pharmacol. and Obstet. and Gynecol., Univ. of Penn. Med. School, Philadelphia, PA 19104.

Whole cell patch clamp techniques were used to record K^+ currents from myometrial cells isolated from immature (23-25 days postpartum) rat uterus and human myomatous (fibroid) tissue. IRB approval has been given.

Immature rat myometrial cells responded to depolarizing voltage steps from holding potentials of -80mV to +30mV with fast outward currents that decayed with two time constants (6.87±0.98 (sem) msec and 75.77±15.92 msec (n=18)). When the animals are injected prior to cell isolation with 17β-estradiol, the probability of expression of these currents is reduced from 78.6% (n=42) in control cells to 29.8% (n=47) in cells from estrogen-injected animals. Cells from progesterone-injected animals showed little difference from control.

Fast transient outward currents have also been recorded predominantly in human myomatous cells (56.5%, n=23 in myomatous cells compared to 5.9%, n=34 in normal cells). Human myomatous cells, therefore, may reflect dedifferentiated myometrial cells.

587.9

ANALYSIS OF CURRENT-VOLTAGE RELATIONS OF THE DELAYED RECTIFIER CHANNEL (I_K) BY THE SINGLE VACANCY MODEL OF ION TRANSLOCATION. J.R. Clay. NIH, Bethesda, MD 20892

The I_K channel in squid axons is known to be multiply occupied by 2 or 3 K ions, based on tracer flux measurements (Begenisich and DeWeer, 1980; JGP **76**:83). The other measure of ion permeation is the net flux, i.e., the current-voltage (IV) relation, which for I_K is well described by the Goldman-Hodgkin-Katz flux equation (Clay, 1984; Biophys J **45**:481). Inferences about multiple occupancy cannot be drawn from the net flux. Nevertheless, the IV and tracer flux results taken together are suggestive of a paradox inasmuch as the GHK equation is based on independent movement of ions across the membrane. The paradox can be resolved by the single vacancy model of ion translocation in which the channel is either fully saturated or has, at most, a single vacancy (Kohler and Heckmann, 1979; JTB **79**:381; Schumaker and MacKinnon, 1990; Biophys J **58**:975). A 3 binding site version of the model in the low ionic strength (physiological) regime gives IV relations for I_K which are essentially equivalent to GHK for $-200 < V < 200$ mV. The model is also consistent with the tracer flux results. In other words, the resolution to the paradox is that the I_K channel may indeed obey the independence principle for ion vacancies rather than ion occupancies.

587.10

Evidence for the presence of a slowly inactivating potassium current in prefrontal pyramidal cells.

C. Hammond and F. Crepel, laboratoire de Neurobiologie, université Paris XI, URA CNRS 1121, Orsay, France.

Potassium currents of the I_A family, such as I_D, have been described in a variety of preparations and particularly in hippocampus (Storm '88). Their characteristics make them good candidates for participating in delayed integration of synaptic currents. We present here data showing the presence of a I_D-like current in pyramidal cells of the rat prefrontal cingular cortex, using an in vitro slice preparation and single electrode voltage clamp as well as current clamp recordings. Pyramidal cells were identified as regular firing or bursting cells and their morphology was studied following intracellular injection of Lucifer yellow. In a fraction of these cells, a depolarizing pulse to -40mV from a holding potential of -95mV evoked the fast outward I_A current followed by a slower outward current which declined during the 3s pulse. This slower outward current was inactivated at a holding potential of -60 mV. The voltage and time dependences of its activation and inactivation kinetics together with its sensitivity to low concentrations (40 μM) of 4-AP, suggest that this slowly inactivating outward current is similar to I_D.

Storm J.F. Nature 336 (1988) 379-81.

587.11

Characterization of the biosynthesis, processing and assembly of the drk1 K^+ channel α-peptide expressed in *Xenopus* oocytes and mammalian cells. J. S. Trimmer and A. Kleinklaus Dept. of Biochemistry & Cell Biology, SUNY Stony Brook, Stony Brook, NY 11794-5215.

The biochemical properties of the drk1 K^+ channel α-peptide expressed from the cloned cDNA (Frech et al., Nature 340:642, 1989) in transient expression systems have been studied. Using two distinct anti-drk1 sera, we have identified the drk1 α-peptide in *Xenopus* oocytes microinjected with drk1 cRNA that express large, voltage-sensitive K^+ currents identical to those previously reported. This drk1 α-peptide is a broad band centered at an M_r of 118 kD; the endogenous drk1 α-peptide in adult rat brain has an M_r of 130 kD. The drk1 cDNA sequence predicts a core peptide of 95.3 kD, thus the drk1 α-peptide in both *Xenopus* oocytes and rat brain has undergone extensive postranslational modification. To investigate these processes in mammalian cells, the drk1 α-peptide was expressed in a human embryonic kidney cell line and was found to have an M_r=113 kD. Pulse chase studies in these cells show a conversion of the initial M_r = 95 kD product to the final M_r = 113 kD product. These results show that post-translational processing makes a significant contribution to the final structure of the drk1 α-peptide in rat brain, *Xenopus* oocytes and cell lines.

587.12

XENOPUS MYOCYTES DIFFERENTIATING IN VITRO EXPRESS SEVERAL CLASSES OF TRANSIENT POTASSIUM CHANNELS. U. Ernsberger and N.C. Spitzer. Department of Biology, UCSD, La Jolla, CA 92093.

Currents through voltage-dependent potassium channels affect the amplitude and duration of action potentials and consequently regulate events which are secondary to membrane depolarization. Thus, developmental changes of potassium currents play an essential role in the differentiation of excitable cells. We are investigating the development of transient, A-like potassium channels in *Xenopus* myocytes differentiating in vitro. Single channel recordings are performed in the cell-attached and inside-out configuration of the patch clamp technique with myocytes grown in the presence of other differentiating cell types, in particular neurons.

Channels were identified by their steady-state inactivation, which was complete at -60 mV in the inside-out configuration. Upon depolarization from more negative holding potentials, the channels were activated, showing faster activation with more depolarized potentials. Inactivation during depolarizing voltage steps was indicated by the decay of ensemble average currents. In some patches the current decay could be best fitted with 2 exponentials, in others with one. Time constants of current decay were variable, but clustered around 10 and 200 ms in the inside-out as well as the cell-attached configuration. However, in excised patches, channels giving rise to rapidly decaying currents were observed less often than in cell-attached patches. These results indicate the presence of several classes of transient potassium channels.

The density of channels in cell-attached patches of myocyte surface membrane increased during the first day in vitro. However, activation times and decays of ensemble average currents were similar for channels at both developmental stages. The lack of change of kinetic properties suggests that an increase in the density of potassium channels rather than the expression of new classes of channels contributes to the developmental increase in density of transient potassium currents.

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588.1

ACTIVATION OF DIFFERENT PROTEIN KINASE C ISOENZYMES IN IMMORTALIZED LHRH NEURONS. W.C.Wetsel, H. Rivera*, and I. Merchenthaler. Lab. of Molecular and Integrative Neuroscience, NIEHS, Res. Tri. PK., NC 27709.

An immortalized, hypothalamic, neuronal cell line (GT1-7) has recently been developed which biosynthesizes and secretes LHRH (Mellon et al., Neuron 5:1, 1990). In vitro, LHRH nerve terminals in the median eminence are highly responsive to phorbol 12,13-dibutyrate (PDBu). The purpose of our study was to determine which protein kinase C (PKC) subspecies were present in the GT1-7 cells, whether these cells were responsive to PDBu, and which of the PKC subtypes were most susceptible to down-regulation following continuous stimulation with this agent. In order to determine the presence of the different PKC isoenzymes, polyclonal antisera specific for each of the 8 PKC subtypes were developed. Immunocytochemical and Western Blot analyses revealed that pKCα,-βI,-βII,-γ,-δ,-ε,-ε' and -ζ were present in the GT1-7 cells in different amounts. When PDBu (50 to 400 nM) was added to the media for 30 min, LHRH secretion was stimulated in a dose-response manner. Activation of secretion for 2 hours with 200 nM PDBu stimulated LHRH secretion 7.7-fold over that of an unstimulated control. At the same time, PKCα, -β_I, and -β_{II} were found to translocate from the cytosol to the membrane. Translocation of the other 5 PKC isoenzymes during the 2 hr period of stimulation was not readily apparent. These data indicate that LHRH secretion is rapidly stimulated by phorbol esters. In addition, these immortalized LHRH neurons contain at least 8 different isoforms of PKC and some of these species appear to be differentially reactive to PDBu. Our findings suggest that these 8 PKC isoenzymes may differentially participate in a variety of functions in LHRH neurons that may be critical for reproduction.

588.2

FURTHER STUDIES OF MODELS OF VASOPRESSIN SYNTHESIS. M.D. Fitzsimmons, M.M. Roberts, and A.G. Robinson. Dept of Endocrinology Univ. of Pittsburgh PA 15261.

Previously (*Neurosci Abs*, 20: 1143), we discussed the merits of mathematical models of vasopressin (AVP) synthesis based exclusively on one of three mechanisms (transcription, translation, or mRNA decay), concluding that a strictly transcriptional model best fit the available data with respect to the dynamics of pituitary AVP content and hypothalamic AVP mRNA levels. In order to prevent net changes in pituitary content, these models presume hormone synthesis is always just sufficient to replace released AVP. Subsequently, we have observed that young adult male rats synthesize considerably more than they release, with the difference reflected in a straight line, age-dependent pituitary AVP accumulation (elsewhere in this volume). This excess synthesis proceeds in spite of weight restriction but can be prevented by inducing hyponatremia.

By incorporating constant, age-dependent AVP synthesis into the transcriptional model (mRNA half-life=1.9 days), we have successfully accounted for three quantitative shortcomings of the original model. This modification accelerates the rate of pituitary AVP content recovery after chronic stimulation and explains why content recovers beyond baseline levels; both predictions are observed experimentally. This modification also explains the unexpectedly slow pituitary AVP recovery under hyponatremic conditions (which blocks the age-dependent synthesis). Finally, assuming that transcriptional mechanisms regulate both stimulated and age-dependent AVP synthesis, the modified transcriptional model predicts significantly lower peak mRNA levels (relative to baseline) resulting from chronic stimulation. These new predictions correspond much more closely to mRNA levels observed by us and others. The results from the modified transcriptional model suggest that AVP synthesis is controlled in response both to stimulation for release and to other, as yet uncharacterized age-dependent influences.

The transcriptional model has proved to be a valuable tool for designing experiments investigating AVP synthesis. In turn, these results have led to more realistic and quantitatively accurate models of neurohypophyseal regulation.

588.3

NEURAL PATHWAYS INVOLVED IN OSMOTICALLY-STIMULATED THIRST AND VASOPRESSIN SECRETION - A STUDY UTILISING C-FOS IMMUNOREACTIVITY AND NEURONAL TRACERS B.J. Oldfield, +R.J. Bicknell, D.K. Hards* and M.J. McKinley*. Howard Florey Institute, University of Melbourne, Parkville, Australia, 3052. +Institute of Animal Physiology and Genetic Research, Babraham, Cambridge, U.K.

In order to help delineate the neural circuitry subserving osmotically-stimulated thirst and vasopressin (AVP) secretion we have utilised elevated levels of C fos protein to identify neurons activated following iv infusion of hypertonic solutions. These studies have been combined with the retrograde labelling of neurons projecting to the supraoptic nucleus - a site responsible for the production of AVP. Twenty three Sprague Dawley rats were used and on day 1 all had femoral veins cannulated. Eight of these also had stereotaxically-placed, bilateral injections of 30nl of rhodamine-labelled microspheres into the supraoptic nuclei. Three days latter, 1.5 ml of either hypertonic (1.5 M) or isotonic (0.15 M) saline was infused iv over 10 minutes. After a further 90 minutes, all rats were perfused intracardially with 4% paraformaldehyde. Elevated levels of fos protein in the brain were detected immunocytochemically. In animals receiving the hypertonic stimulus, fos immunoreactivity was found in neurons of the lamina terminalis i.e., the subfornical organ (SFO), median preoptic nucleus (MnPO) and organum vasculosum lamina terminalis (OVLT) as well as in magnocellular neurons in the paraventricular and supraoptic nuclei. These areas were essentially free of immunoreactive neurons in control rats. In double labelling experiments, at least 20% of fos immunoreactive neurons in the SFO, MnPO, and OVLT projected to the supraoptic nuclei. These results taken together highlight the population of neurons in the forebrain which are responsive to osmotic challenges and the trajectory of some of their projections.

588.4

CHRONIC HYPONATREMIA PREVENTS SPROUTING OF OXYTOCINERGIC AXONS IN LONG-TERM PARAVENTRICULAR NUCLEUS (PVN) LESIONED RATS. J. Dohanics, G. E. Hoffman, and J. G. Verbalis, Depts. of Medicine and Physiology, University of Pittsburgh, Pittsburgh, PA 15261

Destruction of the PVN causes acute changes in hypothalamic morphology. Of these the most striking is the virtual disappearance of CRF-41 axons from the external zone of the median eminence (EZ-ME), accompanied by decreases of vasopressinergic (AVP) and oxytocinergic (OT) fibers in the internal zone of the median eminence (IZ-ME). However, 6 wks following PVN lesions, strong sprouting of OT, but not CRF-41 or AVP, fibers occurs in the EZ-ME. We investigated if similar sprouting also occurs in chronically hyponatremic rats following PVN lesions, since AVP and OT synthesis and secretion is inhibited in such rats. Hyponatremia was induced in adult male rats fed a liquid diet as their only food source and infused with DDAVP (5 ng/h) via subcutaneous osmotic minipumps. Immediately after implantation of the minipumps, PVN lesions were made using a triangle-shaped rotating knife. One wk or 6 wks after surgery rats were perfused and hypothalamic sections stained for AVP-neurophysin (AVP-NP) and OT-neurophysin (OT-NP) immunoreactivities. In normonatremic/sham-operated rats strong AVP-NP staining was present both in the EZ-ME and IZ-ME, while OT-NP staining was present only in the IZ-ME. One wk after PVN lesions both AVP-NP and OT-NP staining was virtually absent from the EZ-ME and reduced in the IZ-ME. As expected, 6 wks after PVN lesions the EZ-ME in normonatremic rats exhibited strong OT-NP immunoreactivity, while AVP-NP staining remained unchanged. However, no such increase in OT-NP staining occurred following PVN lesions in rats who were maintained hyponatremic for 6 wks following the lesions. These results suggest that chronic hyponatremia inhibits not only synthesis and secretion of AVP and OT, but also causes a more generalized decreased synthetic activity of magnocellular neurons which impairs cellular processes involved in cytoskeleton remodeling in response to PVN lesions.

588.5

EFFECTS OF DOPAMINE RECEPTOR AGONISTS UPON OXYTOCIN AND VASOPRESSIN SECRETION IN MONKEYS. J.A. Amico, S.M. Pomerantz, L.M. Layden*, and J.L. Cameron, Depts. of Medicine, Physiology, and Psychiatry, Univ. of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

Administration of the dopamine receptor agonist apomorphine (Apo) causes a dose-dependent increase in plasma oxytocin (OT) concentrations and dose-specific behavioral changes in monkeys (Soc Neurosci Abstr 20:471.8, 1142, 1990). To further explore the pharmacological mechanisms of Apo effects upon neurohypophysial hormone secretion, we administered graded doses of the respective D_1 and D_2 receptor agonists, CY 208-243 and LY 163502, to monkeys and monitored plasma concentrations of OT and vasopressin (AVP). Five female rhesus, 2 male rhesus and 2 male cynomolgus monkeys were implanted with chronic indwelling venous catheters and outfitted with standard jacket/tether/swivel systems to allow remote blood sampling. Blood samples were collected at 15 and 1 min prior to and at intervals 1 to 120 min after injection. Apo (50-400 μg/kg) and LY 163502 (10-100 μg/kg) elicited a dose-dependent stimulation of OT and AVP secretion. In contrast CY 208-243 (100-400 μg/kg) significantly increased OT and AVP secretion only at the 400 μg/kg dose. Low doses of Apo (50-100 μg/kg) and LY 163502 (10 μg/kg) elicited yawning and high doses of Apo (200-400 μg/kg) and LY 163502 (50-100 μg/kg) elicited stereotypy. No behavioral effects of Cy 208-243 (100-400 μg/kg) were observed. The magnitude of the secretory responses varied among animals, was similar in male and female monkeys, and was greater for AVP vs. OT. We conclude that Apo and LY 163502 elicit dose-related stimulation of OT and AVP secretion coupled with dose-specific behavioral changes in male and female monkeys. Apomorphine's effects upon OT and AVP secretion in primates may be mediated primarily by D_2 receptors.

588.6

RESPONSE OF THE MAGNOCELLULAR SYSTEM (MCS) TO HYPOVOLEMIA AND CHOLECYSTOKININ (CCK) DURING PREGNANCY AND LACTATION. E.Koehler*, G.McLemore*, J.Summy-Long, Dept. of Pharmacol., Hershey Med. Ctr., Penn.State Univ., Hershey, PA 17033

Oxytocin(OT) and vasopressin(VP) systems show a decreased sensitivity to osmotic stimulation during lactation(L). We examined further the responsiveness of the MCS to hypovolemia & CCK. Virgin(V),pregnant(d 20;P) & L(d 6) albino rats were injected s.c.(35ml/kg) with polyethylene glycol (PEG MW 20,000; 70-1200 mg/ml),4 hrs before killing to induce hypovolemia. In other studies, V, P & L rats were injected i.p.(4ml/kg) with CCK(100 ug/kg) 5 min before killing. Controls received isotonic saline (SAL),or no needle stick(NS). OT and VP were measured by RIA. During hypovolemia, the exponential relationship(linearized by log transformation) between volume depletion & plasma [OT] was different ($p<0.03$;partial F-tests) such that P>V>L whereas that for VP was unchanged(V=P=L). CCK increased plasma[OT] (pg/ml;NS vs SAL vs CCK)in V(9±1 & 9±1<35±4),P(10±1 & 12±1<26±3)& L(6±1 & 5±1<17±3). The OT response to CCK, however, was reduced in lactation(L<V & P, $p<0.01$ 2-way AOV).Plasma VP increased slightly after CCK (NS & SAL<CCK,$p<0.05$ 2-way AOV) but was not different among V,P & L rats. Thus,during lactation,the OT response to CCK, osmotic and hypovolemic stimuli is reduced, while late in pregnancy the OT system shows an enhanced response to hypovolemia.(Supported by grant # HD 25498).

588.7

OROPHARYNGEAL REGULATION OF WATER BALANCE IN POLYDIPSIC HYPONATREMIC SCHIZOPHRENICS.

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Objective: To assess oropharyngeal regulation of water balance in normals (Ns), and polydipsic schizophrenics with (PHSs) and without (PNSs) episodic hyponatremia.

Methods: Ns (n = 12) received two 2 hr 3% NaCl infusions (0.1 ml/Kg/min). 35" post-infusion each received either 10 ml/Kg water or nothing. Plasma vasopressin (pAVP), desire for water (DFW), and relevant stimuli were measured at 15,30,40,45,50,55,60,65 min. post-infusion. Matched PHSs (9) and PNSs (6) only ingested water post-infusion.

Results: pAVP dropped immediately in Ns following water (30"=4.8±.9 pg/ml, 40"=2.6±.7) vs nothing (30"=5.1±1.8, 40"= 5.1±2.3) prior to changes in stimuli. pAVP also fell immediately in both PHSs (30"=1.2±.8, 40"=.7±.3) and PNS (30"=3.1 ±1.9, 40"=1.6±1.2), and their responses resembled Ns. In contrast, desire for water dropped acutely in Ns (30"=1.9 +2.6 cups, 40"=1.3±2.2) and remained level; while PNSs had high basal DFW (5.6±1.7) which fell acutely (40"=1.8±2.1) but rapidly rebounded (55" 3.4 ±2.9). PHSs (30"1.8±2.6) showed no change over time.

Conclusions: Defects in oropharyngeal regulation of water balance are unlikely to account for our previously described defects in AVP regulation in PHSs; but may contribute to the possibly different etiologies of polydipsia in PHNs and PHSs. Supported by Scottish Rite Foundation(MBG), R29 MH43618 (MBG), GCRC M O1RR00055.

588.8

ENDOTOXIN REGULATION OF TRH AND CRH GENE EXPRESSION IN THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS (PVN) IS MEDIATED BY INTERLEUKIN-I.

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Lipopolysacharide (LPS), a bacterial endotoxin, elevates the plasma concentration of ACTH and suppresses TSH release. Although the effect of LPS on ACTH secretion is thought to be mediated primarily by interleukin-I (IL-I) on hypothalamic CRH secretion, little is known about the mechanism by which TSH is suppressed. To determine whether IL-I could have a central action on TRH production and thereby mediate the effect of LPS on TSH, we studied male S.D. rats after either intraventricular (icv) injection of recombinant human IL-Iß (1-100 ng) or saline or systemic treatment with LPS (E coli 055:35, 25 µg/kg, ip) 30 minutes after an icv injection of saline or the interleukin-I receptor antagonist (IL-Ira, 250 µg). Brains were fixed six hours later by perfusion with 4% paraformaldehyde and coronal sections were processed for *in situ* hybridization histochemistry using ^{35}S-labelled antisense RNA probes for CRH and TRH mRNA. Autoradiographs of the PVN were analyzed under darkfield microscopy by computer image analysis. Intraventricular IL-I induced a marked increase in CRH mRNA by 294% ($p<0.05$) but significantly decreased TRH mRNA by 52% ($p<0.05$) at a dose of 1 ng. LPS also increased CRH mRNA by 216% ($p<0.05$) and reduced TRH mRNA by 58% ($p<0.05$) compared to saline controls. This response could be inhibited (CRH) or prevented (TRH) by pretreatment with IL-Ira whereas the inhibitor alone had no significant effect. These studies indicate that IL-I is capable of exerting a central action on both CRH and TRH gene expression in the PVN and may mediate the effects of LPS on anterior pituitary secretion by acting through central IL-I receptors.

588.9

EFFECTS OF DIETARY PROTEIN INTAKE AND AMINO ACIDS ON TSH SECRETION.

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In previous studies we have investigated the effects of meals and precursor amino acids on prolactin and cortisol secretion. In this study, we analyzed the effects of dietary protein and amino acids on basal and stimulated TSH secretion in normal volunteers. TSH levels were determined in seven different experimental situations: administration of placebo, tyrsoine (5 and 10 g orally), tryptophan (5 g orally), prazosin (1 mg orally), prazosin and 10 g tyrosine combined and administration of an amino acid mixture (15 g, with 5.7 g of large neutral amino acids competing with tyrosine for blood brain barrier transport). TSH secretion was determined 90 minutes following nutriant/drug intake and following TRH stimulation (200µg). Basal levels of TSH were significantly elevated following the combined administration of l-tyrosine and the amino acid mixture (2.66 +/- 1.75 vs. 1.09 +/- 0.82 µE/ml). The TRH-stimulated TSH secretion was again most dramatically increased following l-tyrsoine plus amino acid mixture (14.05 vs. 7.40 µE/ml). Tyrsoine alone also significantly augmented TSH release following TRH stimulation. Prazosin administration did not alter TSH secretion. These data indicate that dietary protein intake enhances TSH secretion, most likely through tyrosine or its derivatives stimulating TRH release. In addition, other constituents of protein, such as phenylalanine, appear to contribute.

588.10

REGULATION OF DBI PEPTIDE AND MITOCHONDRIAL BENZODIAZEPINE RECEPTOR (MBR) EXPRESSION BY ADRENOCORTICOTROPIN (ACTH) IN RAT ADRENAL GLAND

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MBR and DBI are highly enriched in steroidogenic cells such as adrenocortical, Leydig, and glial cells. In these cells DBI acts on MBR and stimulates steroidogenesis (Endocrino.: 123: 2075, 1988; Papadopoulos V., Neurosc. Abst., 1991). We have investigated whether ACTH regulates MBR and DBI expression in rat adrenal gland, testes, cerebellum and C6-2B glioma cells. Seven days after hypophysectomy the amount of DBI-like immunoreactivity (DBI-LI) and DBI mRNA in rat adrenal glands decreased by almost 80%. Similarly binding of the specific MBR ligand, [^{3}H]4'Cl-diazepam, to crude adrenal homogenates, and MBR mRNA, decreased by 60-70%. The administration of ACTH, (15U/Kg, twice daily) reduced the decrease in adrenal DBI-LI, DBI mRNA, MBR binding sites and MBR mRNA caused by hypophysectomy. The regulation of DBI and MBR expression by ACTH specifically occurred in adrenal gland and was absent in testes and cerebellum. Addition of 10^{-7}M ACTH to 6C-2B glioma cells cultured in serum-free medium failed to change the content of DBI-LI and DBI mRNA.

588.11

THE ENDOGENOUS POLYPEPTIDE DBI AND OTHER DRUG LIGANDS OF MITOCHONDRIAL BENZODIAZEPINE RECEPTORS (MBR) STIMULATE STEROIDOGENESIS BY C6-2B GLIOMA CELL MITOCHONDRIA.

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Mitochondrial preparations of C6-2B cells convert 22(R)-hydroxycholesterol to pregnenolone by a mechanism blocked by aminoglutethimide. Immunoblotting confirmed the presence in C6-2B cell extracts and intact mitochondria of relatively high levels of cytochrome P-450 cholesterol side-chain cleavage enzyme. Furthermore, MBR and diazepam binding inhibitor (DBI), that are operative in mitochondrial steroidogenesis regulation are abundant in C6-2B cells. Pregnenolone synthesis in C6-2B mitochondria is stimulated by nanomolar concentrations of Ro5-4864, PK11195, or DBI as well as by triacontadecaneuropeptide (TTN; DBI_{17-50}) a naturally occurring processing product of DBI. In contrast, clonazepam and the octadecaneuropeptide (ODN; DBI_{33-50}) which exhibit a low affinity for MBR were ineffective in stimulating pregnenolone synthesis. Thus C6-2B cells exhibit a significant steroidogenic activity that appears to involve MBR and DBI as endogenous regulators.

588.12

PURIFICATION AND CHARACTERIZATION OF DIAZEPAM BINDING INHIBITOR (DBI) PROCESSING PRODUCTS.

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DBI is a protein with multiple biological actions that is located in selected neurons, glia, steroidogenic cells, and in pancreatic islets. DBI tryptic digestion yields several fragments including an 18 amino acid residue, ODN (DBI_{33-50}). Synthetic ODN a) elicits a flumazenil sensitive proconflict response in rats; b) inhibits glucose induced insulin secretion from pancreatic islets; c) displaces [^{3}H] flumazenil but not [^{3}H] 4'chlorodiazepam from brain crude synaptic membrane prepara-tions. Reverse phase HPLC of rat brain extract reveals several immunoreactive peaks with ODN antibodies (ODN-IR). The most abundant of them, TTN (DBI_{17-50}) was purified (Slobodyansky, J. Neurochem. 53:1276, 1989). It elicits a set of DBI responses different from those of ODN. a) It displaces [^{3}H] 4' chlorodiazepam but not [^{3}H] flumazenil; b) it elicits mitochondrial steroidogenesis; c) in rats it produces a flumazenil resistant proconflict response. Another ODN-IR peak emerges with the same HPLC retention time as ODN. This peptide was purified by reverse phase HPLC and by a Sepharose-ODN antibody column. The sequence of the first 15 amino acids is identical to that of ODN but the carboxy terminus sequence is not completed.

589.1

SHAGGY-ZESTE WHITE 3 AND THE DEVELOPMENT OF ECTOPIC SENSILLA IN THE WING OF *DROSOPHILA*.
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Mitotic recombination coupled with enhancer-trap marking techniques were used to follow the development of ectopic sensilla induced by the mutation *shaggy/zeste white 3* during wing development in *Drosophila*. This gene codes for a serine-threonine kinase thought to be involved in the transduction of positional signaling during the specification of sensilla in the developing imaginal discs. The mutation acted to induce the development of ectopic sensillar precursors during the period of normal sensillar development, as marked using sensillar specific enhancer trap lines. The timing of the appearance of these ectopic precursors depended upon the position of the clone, being markedly delayed in the posterior of the wing. Neurons were formed by ectopic precursors and almost always extended axons in the normal direction, despite their abnormal positions. Localized expression of *achaete-scute* genes are thought to play a primary role in the positioning of sensilla. Interestingly, ectopic sensilla were formed outside the regions of normal *achaete-scute* expression. Using marked clones, it is apparent that the mutation can induce ectopic anti-*achaete* immunoreactivity. Thus, *shaggy/zeste white 3* may play a role in the localization of *achaete-scute* expression in the developing wing.

589.2

FUNCTION OF THE *DROSOPHILA SINGLE-MINDED* GENE AND DEVELOPMENT OF THE CNS MIDLINE.
J. R. Nambu, J. O. Lewis*, and S. T. Crews. Dept. of Biology, Molecular Biology Institute, UCLA, Los Angeles, CA 90024.

The midline nerve cells of the *Drosophila* embryo possess unique anatomical, developmental, and morphological properties; they play an important role both in the formation and function of the CNS. We are characterizing the development and function of these cells via molecular, genetic, and cell biological approaches. The *single-minded* (*sim*) gene is specifically expressed in the midline cells and we have shown via genetic analyses that *sim* is required for proper midline cell differentiation and activation of "downstream" midline genes. It has been determined that *sim* is a member of the basic-helix-loop-helix family of transcription factors, strongly suggesting that *sim* acts to directly regulate midline gene transcription. We investigated the effect of ectopic *sim* expression on midline development and gene expression by generating transformant flies in which the expression of *sim* protein is driven by the hsp70 promoter. This heat-shock/*sim* construct allows us to induce ectopic *sim* expression during different developmental stages. Early induction of *sim* protein results in defective nervous system formation as well as ectopic expression of midline genes. The data indicate that *sim* is capable of directing other cell types to exhibit midline-like properties and may function as a master regulator of the midline lineage.

589.3

THREE CLASSES OF NEURON IN THE MEDIAN NEUROBLAST LINEAGE OF THE GRASSHOPPER.
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Our concern is the manner in which neuronal diversity is generated within a family of lineally related neurons. Each segmental ganglion of the grasshopper Schistocerca contains a midline group of neurons that are bilaterally symmetrical in their branching and that innervate bilaterally symmetrical targets. The neurons are unpaired (by contrast with motor neurons, for example) and arise during embryogenesis from segmentally iterated unpaired median neuroblasts (MNBs). The midline group of the third thoracic ganglion contains about 100 neurons. We show that the group is divided into three distinct classes of neuron [Thompson & Siegler (1991) J. Comp. Neurol. 305:659]. Based on differences in the shape and position among neurons of the different classes, we predict that the classes are produced sequentially during embryogenesis, in the following order: 1) octopamine-containing efferent modulatory neurons; 2) GABA-immunoreactive spiking local interneurons with an auditory function; 3) GABA-immunoreactive spiking intersegmental interneurons with a role in flight. In ongoing studies we are determining the relative numbers of the different neuronal types within the population and the rate at which the MNB progeny are produced. This will allow us to predict when, given a sequential model, each of the neuronal classes arises during embryogenesis.

589.4

TEMPORAL CHANGES OF PRECURSOR CELL PATTERNS IN DORSAL ROOT GANGLIA (DRG) AND SYMPATHETIC GANGLIA (SG) OF THE QUAIL EMBRYO.
R. S. Duff*, C. J. Langtimm, M. K. Richardson and M. Sieber-Blum. Department of Cellular Biology and Anatomy, Medical College of Wisconsin, Milwaukee, Wisconsin 53226.

We have performed *in vitro* clonal analyses to elucidate changes in the temporal and spatial patterns of pluripotent and apparently committed precursor cells in DRG and SG. Previous *in vitro* clonal analysis of quail neural crest differentiation indicated that all sympathoadrenal cells and some sensory neurons originate from pluripotent neural crest cells (M. Sieber-Blum, Science **243**, 1608, 1989). Our present data suggest the following. 1) All four classes of pluripotent and apparently committed cells found in the migrating neural crest are also present in young DRG and SG. 2) The proportion of pluripotent cells decreases with progressing age of the embryo. 3) Both types of ganglia contain an at least bipotent, possibly "neurogenic", precursor that persists at least half-way through the incubation period, albeit at a 10-fold lower concentration in the SG than in the corresponding DRG. 4) There is a subset of early-differentiating sensory neurons in the DRG, explaining and confirming previous studies by us and others. These data support the notion that the life-span of pluripotent cells within the neural crest as well as in DRG and SG correlates well with the timing of proven and putative location-specific environmental cues influencing the ultimate phenotype generated by these pluripotent cells. Supported by USPHS grant HD21423 and a Research Grant from the Familial Dysautonomia Foundation (M. S-B.) and NIH Training Grant GMO 7892-09 (R.S.D.).

589.5

PARTIAL RESTRICTION IN THE DEVELOPMENTAL POTENTIAL OF LATE EMIGRATING AVIAN NEURAL CREST CELLS.
Kristin B. Artinger* and Marianne Bronner-Fraser. Developmental Biology Center, University of California, Irvine, Ca. 92717

Trunk neural crest cells migrate along two major pathways: (1) a ventral pathway whose cells form neurons, glia and chromaffin cells and (2) a dorsolateral pathway whose cells form melanocytes. Neural crest cells fill their derivatives in a ventral to dorsal order, with the last emigrating cells moving dorsolaterally (Serbedzija *et al.*, 1989, Develop. 106: 806). Here, we test whether late emigrating neural crest cells are more restricted in developmental potential than early migrating cells. In culture, early migrating ("young") neural crest cells differentiate into pigmented and catecholamine-containing cells, among other derivatives, by 7 days. "Older" neural crest cells were prepared from stage 20 - 21 embryos, when the last neural crest cells are emigrating from the neural tube. When "older" neural crest cells were cultured for 10 - 14 days, pigment cells were detected but no catecholamine-containing cells were found. Because the culture system may lack important differentiation factors, "older" neural crest cells were injected into young chick embryos during the early stages of neural crest migration (stages 16 - 18). Cells were labelled with the vital dye, DiI, prior to injection to aid in their visualization. Injected embryos were allowed to develop for 2 - 3 days. Embryos that were injected with "young" neural crest cells contained DiI-labelled catecholamine-positive cells. In contrast, no DiI-labelled catecholamine-positive cells were observed in embryos injected with "older" neural crest cells. These results suggest that late emigrating neural crest cells have a more restricted developmental potential than early migrating neural crest cells. (supported by USPHS HD-25138)

589.6

CLONAL PATTERNS OF NEURON PROLIFERATION AND DISPERSAL IN THE BRAIN STEM OF THE CHICKEN EMBRYO.
Sharon G. Hemond and Joel C. Glover, Inst. of Physiology, Univ. of Oslo, 0317 Oslo, Norway.

The purpose of this study was to determine the importance of lineage in the determination of neuronal phenotype in the brain stem of the chicken embryo. We used the recombinant retrovirus LZ10 to introduce the marker gene lacZ into single progenitor cells in the brainstem proliferative zone. The virus was pressure-injected into the fourth ventricle on embryonic day (E)2. On E4-5 or E9 the embryos were sacrificed, and the brain stems were fixed and reacted in wholemount to detect the lacZ gene product, β-galactosidase.

On E4-5, clusters of labelled cells were observed in wholemount, and their positions noted. They were then examined in transverse sections. On the basis of an adopted set of criteria, 83 such clusters were defined as clones. These were distributed to all rostrocaudal levels and mediolateral locations and contained from 1 to 29 cells (mean = 5). 79/83 clones extended radially from the ventricular zone (77 had ventricular attachment = 93%). 4/83 were non-radial but contained only a few (1 - 4) cells. 21 of the 79 radial clones included outlier cells. These clones were located predominantly in the lateral region.

On E9, 10 clusters that satisfied our criteria for classification as clones were observed after sectioning. They contained from 3 to 14 cells (mean = 8). 6/9 had cells or attachments in the ventricular zone. 2/9 were radial, 3/9 had a radial component with outliers, and 4/9 were non-radial. The degree of cell dispersal, as measured by mediolateral extent in cell diameters, was greater at E9 than at E4-5. Mapping cell positions shows that most of the E9 clones contribute to several different brainstem nuclei.

Neural progenitors appear to differ in their mitotic rates at these stages. Clonally-related cells are spatially restricted during early migration but disperse to contribute to different functional neuron groups later. This suggests that lineage relationships at these stages are not of primary importance in the determination of neuronal phenotype in the developing brain stem.

589.7

MULTIPLE RETROVIRAL VECTORS ARE USED TO STUDY CLONAL DISTRIBUTION IN THE CHICK RETINA. D. M. Fekete, J. Perez-Miguelsanz*, and C.L. Cepko. Department of Genetics, Harvard Medical School, Boston, MA 02115

Lineage analysis of mouse and Xenopus retina has shown that clonally-related cells form radially-arrayed, cohesive columns. We now report results from lineage analysis in the chick showing a different spatial distribution of clonally-related cells generated at early times in development. Three different replication-defective avian retroviruses are used in paired combinations to co-infect chick retinas between embryonic day 3 (E3) and E7. Retinas are harvested between E14-E19 and processed for the presence of the retrovirally-encoded protein products: ß-galactosidase or alkaline phosphatase are detected by enzyme histochemistry while viral gag protein is detected immunohistochemically. Clones derived from infections at E3 are arrayed as clusters of 5-50 radial columns that are separated by unlabelled cells. Each radial column is composed of 10-50 cells that are often flanked by individual early-born cells (horizontal, photoreceptor or ganglion cells). Clones in the central retina have fewer radial columns than clones in the periphery. The dispersed pattern formed by each clone is evident using all three retroviral vectors. Such a pattern is consistent with an initial period of tangential mixing (or migration) of progenitor cells followed by radial expansion of clones to form separate cohesive columns.

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589.8

ARE GANGLION CELLS THE DEFAULT PHENOTYPE FOR RETINAL NEUROEPITHELIAL CELLS IN THE RAT? T. A. Reh and I. J. Kljavin†. University of Washington, Dept. of Biological Structure, Seattle WA, 98195 and †University of Calgary, Neurosciences Research Group, Calgary, Alta. Canada.

To determine the mechanisms that control the determination of cell phenotype in the developing central nervous system, we are using dissociated cell cultures of rodent retina. In this experiment, we asked whether the environment of a retinal neuroepithelial (NE) cell can influence its phenotype, by culturing rat retinal or cerebral cortical cells from different embryonic and postnatal ages at high density ($10^6/cm^2$) and plating 10-100 mouse retinal cells onto the rat monolayers. After three or four additional days of culture, the cells were fixed and the mouse neurons and glial were identified with monoclonal antibodies M2 and M6, respectively. We found that 82 % of the E11 or E12 mouse retinal cells, plated onto cerebral cortical monolayers, differentiated into ganglion cells, and none of these cells differentiated into rods. By contrast, when the same aged mouse cells were plated onto rat retinal cells only 27 % differentiated into ganglion cells and 18% differentiated into rods. These data indicate that the microenvironment can influence the commitment of the multipotent NE cells, and in the absence of these retinally derived microenvironmental cues, the NE cells differentiate into ganglion cells. NIH RO1 NS28308.

589.9

NONCOMPARTMENTAL CELL LINEAGE IN RAT NEOCORTEX: A STUDY USING A RETROVIRAL LIBRARY/PCR RESCUE TECHNIQUE. C. Walsh and C.L. Cepko, Dept. of Genetics, Harvard Med. Sch., Boston, MA.

Whereas cell lineage compartments correspond to patterned gene expression in the developing vertebrate hindbrain, developmental compartments in the forebrain have been elusive. We have addressed this question using a new technique which allows definition of clonal relationships regardless of the migration patterns of sibling cells.

A library of 95 retroviral vectors was made, all carrying the histochemical marker gene, lacZ. Each vector contained a unique, 30-600 bp fragment of genomic DNA, as a genetic "tag". This viral mixture was injected into the forebrain of fetal rats, to label cortical progenitor cells and their progeny. The brains of adult animals were then sectioned, reacted for ß-galactosidase, and the labeled cells plotted on drawings. Labeled cells were then removed from the tissue sections and processed using the polymerase chain reaction (PCR) to analyze the genetic tags. Sibling cells share the same tag, while cells in different clones essentially always contain distinct tags.

Clonally related cortical neurons varied widely in their degree of separation from one another. About 50% of multicellular clones formed clusters of 2-5 cells from 50-800µm across. These clusters, similar to the pattern previously described (Walsh and Cepko, 1988, Science 241:1342), cross functional subdivisions of the cortex such as "barrels" of the somatosensory cortex. Surprisingly, the other 50% of multicellular clones spread for 1 millimeter or more, with several clones crossing major cytoarchitectonic subdivisions of the neocortex. The most widespread clones covered more than half of the antero-posterior or medial-lateral dimensions of the neocortex. Thus, the neocortex shows no simple compartmental or segmental development. Furthermore, the control of some aspects of cell number and phenotype may be independent of later specification of the neocortex into cytoarchitectonic zones. Supported by the Dana Foundation, the Howard Hughes Medical Institute, and the NIH.

589.10

VENTRICULAR ZONE HETEROGENEITY IN THE DEVELOPING RAT FOREBRAIN. S. E. Acklin and D. van der Kooy. Univ. Toronto, Toronto, Canada.

In the developing mammalian telencephalon there is evidence to suggest that some aspects of the fates of forebrain cells are predetermined while they are still proliferating in the ventricular zone (Krushel et al. 89, Soc. Neurosci. Abstr. 241.4; Fishell et al. 90, Devel. Biol.141,70-83; Barbe&Levitt, 91, J. Neurosci. **11**, 519-533). We hypothesized that such early predetermination might manifest itself in heterogeneous proliferation kinetics among individual cell lineages within the ventricular zone. In order to investigate cell cycle kinetics within individual cell lineages we devised a novel double labeling technique which combines retroviral identification of clonal populations within the ventricular zone with simultaneous radioactive labeling of all dividing cells. The retrovirus used (Psi 2 Bag alpha) introduces a histochemical marker gene directly into the genome of an infected progenitor cell; this marker is inherited undiluted by all the daughter cells and therefore serves to identify individual clones. The radioactive thymidine label is also taken up by all dividing cells, but then is successively diluted by half with every further division. By counting the number of silver grains (3H-thy) in all retrovirally labeled cells of a given clone it is possible to draw conclusions concerning (1) cell cycle kinetics (2) mode of proliferation (exponential vs steady state), and (3) cell death, for each clone analyzed. In our experiments the retroviral label was injected directly into the lateral ventricles of E 17 rat embryos in utero, and simultaneously the radioactive thymidine was administered systematically to the mother. After a survival time of 48 hours, serial sections through the embryonic rat forebrains were analyzed. Within the developing cerebral cortex, the results revealed a segregation into four spatially distinct proliferating cell populations. The individual populations differed in cell cycle kinetics, mode of proliferation as well as in the amount of cell death that occurred. The proliferative zone of the developing striatum shows similar differences in cell cycle characteristics among individual cell lineages, however the spatial segregation of the these different populations was not as prominent as in the cortex. We speculate that some aspects of the adult mammalian cortex might already be predetermined within the various proliferating cell populations in the embryonic ventricular zone.

589.11

CLUSTERS OF COUPLED CELLS IN THE VENTRICULAR ZONE OF NEOCORTEX J. L. Lo Turco and A. R. Kriegstein. Dept. of Neurology and Neurological Sciences, Stanford Univ. Sch. of Med., Stanford, CA 94305.

Cell to cell interactions may participate in determining the fates of cells in the central nervous system. We have used whole-cell patch clamp recordings to characterize physiological interactions between cells in the fetal rat neocortex. Cells in the ventricular zone had much lower input resistances and generated much larger currents in response to GABA than did neurons in the cortical plate. When Lucifer Yellow was injected into ventricular zone cells we found that large numbers of cells (15-90) were dye-coupled into radially oriented columns, whereas neurons in the cortical plate were not dye-coupled. To determine the nature of this coupling, both HRP and Lucifer Yellow were simulataneously injected into cells. While Lucifer Yellow filled a large number of cells, HRP staining was confined to single cells. In addition, manipulations that uncouple gap junctions (halothane, acidification, and arachidonic acid) increased the apparent resistance of ventricular zone cells. These experiments indicate that cells in the ventricular zone are coupled together by gap junction channels.

The number of cells in clusters decreases as neurogenesis proceeds in rat cortex (E15=60±20, E17=32±12, E19=7±4 cells), and mitotic figures at the ventricular surface are not coupled to cells in clusters. This suggests that during proliferation cells are first coupled to clusters and then uncouple before they enter M-phase. We are currently exploring with Brdu when during development cells decide to uncouple from clusters. We have also begun to characterize how cell clusters respond to neurotransmitters that may be released into developing cortex. Cells in clusters depolarize upon the application of GABA, glutamate and norepinephrine. We conclude that proliferating cells in the ventricular zone interact directly with each other through gap junction channels and can respond to transmitters well before the formation of synapses.

589.12

GABAERGIC CORTICAL NEURONS EXPRESS THEIR TRANSMITTER PHENOTYPE DURING MIGRATION AND EARLY CORTICAL PLATE FORMATION IN DEVELOPING MONKEY. D.L. Meinecke, M.L. Schwartz, and P. Rakic. Section Neurobiology, Yale Sch. Med. New Haven, CT 06510.

Recent evidence suggests that the restriction of cell phenotype in the cerebrum may occur early in development before neurons attain their final destination. For example, the commitment of fetal neurons to projection or local circuit subclasses may occur prior to the completion of their migratory phase, since it has been shown that some of these cells have callosal axons (Schwartz et al. '91, PNAS: 88). Additionally, patch clamp analysis shows that some neurotransmitter receptors also emerge before postmitotic cells settle in their laminar positions (Lo Turco et al.'91, Science). Here we report the early expression of GABA in cells of the developing monkey telencephalon. GABA antisera were used to immunolabel frontal and occipital lobes at stages when cortical neurons are generated (embryonic ages E41-E90; birth is at E165). GABA positive cells were present in the cortical plate in both frontal and occipital lobes at all ages examined, including E41 when the cortical plate is as yet only 2-3 cell layers wide. GABA-containing neurons were also present in the ventricular and subplate zones, and some of these cells had ultrastructural features typical of migrating neurons. Since GABA is commonly associated with inhibitory local circuit neurons, the present results indicate that their phenotype is determined early in neurogenesis independent of prospective synaptic interactions, and before they reach their final positions. Supported by NIH grants.

590.1

INTRAMUSCULAR INJECTION OF WGA YIELDS SYSTEMIC DISTRIBUTION ADEQUATE FOR IMAGING OF AXONAL TRANSPORT IN INTACT ANIMALS. A. G. Filler, H. R. Winn*, L. E. Westrum, P. Sirrotta*, K. Krohn*, and T. W. Deacon§. Dept. Neurol. Surg. and Dept. Nuc. Med., Univ. of Wash., Seattle, WA 98195, and §Dept. Anthro., Harvard University, Cambridge, MA 02138.

To explore the possibility of PET or MR imaging of axonal transport in humans, a series of preliminary biodistribution studies were undertaken in rats to learn whether tracer concentrations in nerve and among other tissues could achieve imageable relative distributions. The underlying principle is an extension of techniques in which retrograde transport from small intramuscular injections of wheat germ agglutinin (WGA) are used to selectively label and distinguish spinal cord neurons innervating closely juxtaposed muscles.

Concentrated [^{125}I]-WGA injected percutaneously into rat forearm or hindlimb muscle in various microliter quantities using methacrylate to seal the puncture site was allowed three to ten days to distribute after which animals were sacrificed, perfused, and 25 different tissues including blood, lymph nodes, liver, lung, muscle, ipsilateral and contralateral peripheral nerve, spinal root/dorsal ganglia, and various spinal cord segments were collected, weighed, and assayed in a gamma counter.

Excluding the injection site (as could be done in a clinical image) WGA concentrations (as counts per min./mg) in ipsilateral nerve and ganglia were up to five to ten times greater than that in any other tissue in the same animal when injection technique and dose were optimized. Spinal cord segment concentrations were greater than in any non-neural tissue except liver.

These results clearly show that biodistributions of axonal tracers can be achieved which may permit clinical imaging of phenomena involving axonal transport in humans if MR or PET dectectable labels can be delivered in adequate quantities. Direct application of axon transport imaging in the evaluation of nerve compression, spinal cord injury, and neuropathy as well as other reapplications of a variety of transport related basic neuroscience techniques into the clinical realm are possible.

590.2

IMPLEMENTATION OF AN ULTRAVIOLET (UV) LASER AS LIGHT SOURCE FOR FLUORESCENCE CONFOCAL SCANNING MICROSCOPY. Brun Ulfhake (1), Ulf Arvidsson (1), Kjell Carlsson (2) and Karin Mossberg (2). Department of Anatomy, Karolinska Institutet ,S-104 01 Stockholm (1), and Physics IV, The Royal Institute of Technology S-100 44 Stockholm (2), (Sweden)

We here describe the use of an UV laser as light source for confocal microscopy. We used a *Spectra-Physics 171-18* laser providing three lines in UV, namely *334, 351* and *364* nm . These lines can be used simultaneously or individually. With this instrument it is possible to use fluorophores not previously available for fluorescence confocal imaging as e.g. *Fluoro-Gold* and *AMCA* (7-amino-4-methyl coumarine-3-acetic acid). In this study we show confocal laser microscopical imaging of fluorescent motoneurons labeled by retrograde transport of Fluoro-Gold and AMCA- fluorescent axon terminals labeled with antiserum against various immunogenes. These two fluorophores may be recorded *simultaneously, or separately* by using filters that suppresses the emission of one of the fluorophores.

Measurements performed in reflected and fluorescent light indicated that the *resolution* along the optical axis *improved* by about *25%* when UV light (351nm) is used instead of visible light (514 nm). This figure is close to that expected on theoretical basis. There are, however, also serious problems related to the use of UV-light. Firstly, the objectives must be selected based on their *UV transmission properties*. Secondly, *chromatic aberration* may cause a substantial *focal shift* between illuminating and emitted light. Remedies for these technical problems are presented and discussed.

590.3

DYNAMIC INTRACELLULAR CALCIUM COMPARTMENTS: THREE DIMENSIONAL CONFOCAL MICROSCOPY OF FLO-3 IN PC12 CELLS. L.R. Mills and J.K.Stevens, Volume Investigation Lab. Playfair Neuroscience Unit,The Toronto Hospital, 399 Bathurst St. Toronto. ON Canada M5T 2S8

A confocal microscope (Biorad MRC-600) and an ICAR 80.8 Volume Investigation workstation were used to study the three dimensional distribution of the calcium indicator dye flo-3 in NGF activated PC12 cells. In addition to its expected cytoplasmic distribution flo-3 was also found to label the nucleus and a variety of subcellular organelles. Labelling with rhodamine 123 identified one class of organelle labelled by flo-3 as mitochondria, other organelles are as yet unidentified. In cells loaded with flo-3 in the presence of serum at 37 degrees, rest calcium levels were significantly higher in the mitochondria than in the cytoplasm: on average calcium levels in the cytoplasm were 74+8 nM (n=21 cells), compared to 129+11 nM (n=20 cells), in the mitochondria. Experimental perturbation of intracellular calcium levels using the calcium ionophore A23187 or removal of extracellular calcium indicate that flo-3 can act as a calcium indicator in all compartments examined. Furthermore, calcium dynamics appears to be different in different subcellular compartments.

590.4

IN-VIVO IMAGING OF INTRACELLULAR ION CONCENTRATIONS IN RAT BRAIN NEOCORTEX: A FEASIBILITY STUDY. U.Dirnagl, A. Villringer, A.Them*, G.Sixt*, K.M.Einhäupl*. Dept.of Neurology, University of Munich, 8000 Munich 70, F.R.G.

We tested the feasibility of in-vivo loading and imaging of brain cells in situ with ion-sensitive dyes. Anesthetized and ventilated Wistar rats were equipped with a closed cranial window (dura mater removed), and the brain surface was superfused with the pH-sensitive dyes BCECF-AM ($\lambda_{ex\text{-}max}$=488nm) and/or the Ca^{2+}-sensitive dye Calcium Crimson-AM ($\lambda_{ex\text{-}max}$=590nm; both dyes from Molecular Probes, 10 μM in artificial CSF). Loading and washout time was 30 minutes each, with a flow rate of 4ml/h. Images were generated using a confocal laser scanning microscope (CLSM, Biorad MRC-600) with an Ar/Kr-laser (488/568/ 647 nm) as a light source. Image formation was triggered to respiration. Leakage of dyes was assessed through the loss of intracellular signal over time and with spectro-fluorimetrical determination of dye concentrations in the outwash CSF. At the end of the experiment, calibration of intracellular pH (pH_i) was attempted by clamping pH_i to extracellular pH (pH_e) with iso-osmotic buffers titrated to pH 6 and 8 respectively (nigericin/high potassium method). Autofluorescence of the brain was low. On optical sections dye related signal was retrieved up to 200 μm beneath the cortical surface, single dye loaded cells could be visualized down to 50 μm. Double labelling allowed simultaneous imaging of pH_i and Ca^{2+} related signal. The rate of photobleaching was low, and leakage of the dyes was less than 15 % per hour. Motion artifacts and changes in brain geometry with experimental manipulations (f.e. clamping) impeded the accuracy of the measurements. In these experiments we have shown that 1) loading with AM-dye-esters, 2) CLSM imaging, and 3) pH_i/pH_e-clamping of brain cells in situ is feasible.

590.5

INTRINSIC OPTICAL IMAGING OF THE CAT DORSAL HIPPOCAMPUS FOLLOWING INTRAVENOUS COCAINE ADMINISTRATION R. M. Harper, D. M. Rector*, G. R. Poe*. Department of Anatomy and Cell Biology and the Interdepartmental Neuroscience Program, UCLA School of Medicine, Los Angeles, CA, 90024-1763.

Cocaine induces marked changes in neuronal discharge of limbic structures, including pronounced dimunition or cessation of firing in amygdala regions, an effect maintained for periods of 60-150 min. We wished to relate electrophysiological activity with local metabolic actions of cocaine, and thus assessed the time course of local metabolism in the dorsal hippocampus following intravenous cocaine delivery using a coherent-image technique. Electrodes were placed to record EEG, eye movement and diaphragmatic activity in three cats under surgical anesthesia, and a 1.6 mm diameter image probe was placed over the dorsal hippocampus. After recovery, a 2.5 mg/kg intravenous cocaine dose was administered following a baseline recording, and the time course of successive frames of reflected 700 nm optical activity was assessed over a 60 min recording period. Frames were collected together with digitized cortical and hippocampal EEG. Images were averaged over 1 min epochs, and a pre-cocaine epoch was subtracted from all subsequent epochs to eliminate background activity. A multiplication also was performed to amplify the differences, and ANOVA procedures for each individual pixel were used to determine the significance of the comparisons using an alpha of .05. Cocaine induced a profound overall diminution in reflected light from the dorsal hippocampus; this decline began as early as 1 min, with the maximal decline varying, depending on the preadministration state of the animal; typically maximal declines occurred at 2 min post administration, with recovery to baseline levels requiring approximately 15 minutes.

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590.6

DYNAMIC SPATIAL PATTERNS OF OPTICAL ACTIVITY FROM THE CAT DORSAL HIPPOCAMPUS FOLLOWING INTRAVENOUS COCAINE ADMINISTRATION D. M. Rector*, G. R. Poe*, R. K. Harper, and R. M. Harper. Department of Anatomy and Cell Biology, Interdepartmental Neuroscience Program, UCLA School of Medicine, Los Angeles, CA, 90024.

We observed intrinsic optical properties of tissue from the dorsal hippocampus of the cat using an optical probe and reflected 700 nm light. The probe consists of a 1.6 mm diameter coherent image fiber bundle coupled to a 128 by 128 pixel CCD, cooled with liquid nitrogen to reduce thermal noise. In three cats under surgical anesthesia, electrodes were placed to record cortical EEG, and the image probe was lowered to the surface of the dorsal hippocampus. Diaphragmatic leads were placed to record respiratory and ECG activity. After surgical recovery, recordings of cortical and hippocampal EEG were digitized simultaneously with the hippocampal optical activity. Images were digitzed at the peak of the ECG R-wave to eliminate cardiac artifact. Each fiber in the probe was 25 um in diameter, which allowed the simultaneous visualization of optical activity in 10,000 individual regions, each covering an area of 450 um^2. One fiber was approximately represented as a pixel on the video image. Previous studies indicate that optical activity at 700 nm correlated with regional blood flow and local metabolism. Images were collected 10 min prior to, and 30 min after 2.5 mg/kg intravenous cocaine adminstration. Animations were created by displaying successive images which illustrated changes in optical activity for the area of tissue under each fiber. Individual pixels of the image alternated between light and dark levels at a steady rate over time before administration of cocaine. As early as one minute after cocaine, the light and dark alternation of individual pixels accelerated, with an average level that was darker than precocaine conditions. The change in pattern created banding patterns on the image and an overall diminution of light reflected from the surface of the dorsal hippocampus.

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590.7

COMPUTER ANALYSIS AND VIDEO-DEMONSTRATION OF THREE-DIMENSIONAL MORPHOLOGY OF NEURONS OF APLYSIA . H. KOIKE, Y. UMITSU*, M. IMANISHI* & H. SHIBUTANI*. Dept. Neurophysiol., Tokyo Metropolitan Institute for Neurosciences, Fuchu, Tokyo 183, JAPAN.

A computer constructed realistic three-dimensional image of neurons will be presented on a video-screen. The neuron in the abdominal ganglion of Aplysia juliana was stained by an intra-cellular injection of cobaltic-lysine in combination with the silver-intensification procedure. The ganglion was serially sectioned (2-3μm) for the light-microscopy using an adhesive tape method (Umitsu, 1990). The video microscopic images of the serial sections (around 200) were sequentially acquired to a computer (Hamamatsu Photonics C2000). The acquired images were processed as 16-bit gradient natural images without any human handlings such as tracings or binary slicings. These images were reconstructed by our computer programs to produce hypothetical pictures of the stained neuron viewed from any angles other than actually observed. By presenting these calculated pictures of different view angles sequentially on the video screen, full rotation of the three-dimensional natural-looking image of the neuron was produced satisfactorily. We also precisely determined the volumes, surface areas and lengths of the cell body, axons and neurites of the stained neurons three-dimensionally.

590.8

AUTOMATIC GRAIN COUNTING. J. Nissanov, S. Mani*, and O.J. Tretiak*. Imaging and Computer Vision Center, Drexel University, Philadelphia, PA 19104.

While exposed silver grains are often dark objects on a lighter background, automatic grain counting techniques which segment the image on the basis of gray value alone are inaccurate. We report on an algorithm with improved performance which exploits the size as well as the gray value discrepancy between the grains and the background objects.

This algorithm convolves the original image with a low-pass filter followed by subtraction of the filtered image from the original. This high-pass result is then automatically thresholded to separate the grains from the background.

The performance of variants of this algorithm was measured on 42 images taken through a 100X objective. We compared the computer and visual grain count for 4 different low-pass filters, average, median, high, and F75, each at 5 different kernel sizes. The best results (r^2= 0.870) was achieved with a 7 X 7 median filter. (Supported by NIH grant # 5 P41 RR01638).

REGENERATION

591.1

THE SPECIFICITY OF MOTOR AXON REGENERATION DECREASES WITH AGE. T.M.Brushart. Depts. of Neurology and Orthopaedics, Johns Hopkins, and Curtis Hand Center, Baltimore, MD 21218.

The superior results of peripheral nerve repair in children have been attributed to CNS plasticity (Almquist '83), allowing better use of inappropriate peripheral connections. However, the specificity of peripheral axon regeneration itself may also be age dependent. These experiments examine the effects of age on Preferential Motor Reinnervation, the tendency for regenerating motor axons to reinnervate a distal motor branch in in preference to a sensory one. Both femoral nerves of juvenile (3 wks) and aged (1 yr) rats were sharply transected and sutured with proper alignment (40 nerves), 90° rotation of the distal stump (40 nerves), or enclosure of a 1/2 mm interstump gap within silicone tubing (40 nerves). After 12 wks of regeneration, HRP was used to identify motoneurons regenerating correctly down the motor branch (20 nerves/group) or incorrectly down the sensory branch (20 nerves/group). Significantly more motoneurons were labeled from the motor branch ($p < .01$) except in the aged animals with rotated repairs ($p = .07$). The difference between mean motoneuron counts from the sensory and motor branches, an indication of specificity, was significantly greater in the juvenile animals ($p < .0001$) regardless of repair type. The specificity of motor axon regeneration, and especially the ability to overcome repair malalignment, thus decreases with advancing age in the rat.

591.2

THE DEVELOPMENTAL HISTORY OF SUPRASPINAL PROJECTIONS TO THE LUMBOSACRAL CORD IN THE NORTH AMERICAN OPOSSUM, DIDELPHIS VIRGINIANA. POSSIBLE RELEVANCE TO DEVELOPMENTAL PLASTICITY. George F. Martin and Xiao Ming Xu. Dept. of Cell Biology, Neurobiology and Anatomy, The Ohio State Univ., Columbus, OH 43210.

In order to study the origins of supraspinal projections to the lumbosacral cord in developing opossums, we have used the retrograde transport of Fast Blue (FB). When injections were made into the caudal cord at postnatal day (PD)1, 12-13 days after conception, labeled neurons were present within the brainstem where they were most numerous within the reticular formation of the pons and the coeruleus complex. A few neurons were also labeled in the bulbar reticular formation and raphe, and in some cases, within the vestibular complex. By PD3, labeled neurons were much more numerous and present in most of the brainstem areas labeled by comparable injections in adult animals. A notable exception was the red nucleus which could not be labeled with certainty until approximately PD13. We have shown that rubral axons are able to grow around a lesion of their spinal pathway during development and it is our hypothesis that all brainstem-spinal axons are capable of comparable plasticity. If the potential for plasticity is based on maturation at the time of injury, we predict from the above results that the critical period for reticulospinal and vestibulospinal plasticity, if it occurs, ends earlier than that for the rubrospinal axons. (Supported by NS-25095 and NS-10165).

591.3

A SUBSTANTIAL FRACTION OF RETINAL GANGLION CELLS SURVIVE THE TRANSECTION OF THE RABBIT'S OPTIC NERVE. Jutta Schnitzer and Leo Peichl. MPI für Hirnforschung, Deutschordenstr. 46, W-6000 Frankfurt/Main, Germany

Cutting the optic nerve of adult mammals leads to degeneration of retinal ganglion cells (GC). Our study shows, however, that even 1-2 years after cutting the optic nerve of rabbits, a substantial fraction of the GC (about 10%) survive. We confirmed completeness of the cut by the failure of retrograde transport of HRP to the surviving GC, or by the absence of GC axons in the optic nerve anterograde to the cut. The surviving GC bodies and axons in the retina were detected by neurofilament-labeling and by reduced-silver stain. Lucifer Yellow injections revealed some alpha, bi-stratified and nonclassified GC.

Ultrastructurally the surviving axons appeared healthy; most were myelinated in the medullary rays (MR). Some oligodendrocyte cell bodies in the 'lesioned' retina were covered with myelin. Thus, after degeneration of about 90% of the axons, oligodendroglia myelinate 'aberrant' cellular elements. The proportion of myelinated vs. unmyelinated axons in the MR was higher in the 'lesioned' than in the control retina. Whether the enhanced proportion of myelinated axons is caused by 'aberrant' myelination, or whether myelinated axons survive to a higher extent, is still open. It remains to be shown why some GC are capable of surviving and whether they could regenerate their axon in a 'suitable' environment.

591.4

RETINAL GANGLION CELL AXON REGENERATION FAILURE IN GOLDFISH. A.D. Springer Department of Cell Biology and Anatomy, New York Medical College, Valhalla, NY 10595.

Retinal ganglion cell (RGC) axon regeneration occurs in goldfish after the optic nerve is crushed. This ability in nonmammalian vertebrates, and its absence in mammals, can be interpreted as reflecting species differences in regenerative capacities. However, failures of axon regeneration in mammals have been shown recently to be due, at least in part, to inhibitory factors present at the site of nerve damage.

Microenvironmental factors in the goldfish visual system also can interfere with RGC axon regeneration. RGC axons were transected intraretinally. At various post-surgical times, the eye was removed and cobaltous-lysine was placed on the optic nerve to backfill the RGCs. RGCs having undamaged axons were filled throughout the retina. However, there were very few labeled RGCs in the retina peripheral to the slit. The possibility that the sectioned axons had regenerated into the optic nerve, but that the corresponding cells could not be filled readily, was ruled out. In a control study, RGCs were easily filled several weeks after damaging their axons by crushing the optic nerve.

To determine the origin of the inhibition, small pieces of retina were explanted into poly-lysine coated dishes. Neurite outgrowth was quantified 8 days following explantation. A supernatant, prepared from the contents of the eye, inhibited neurite outgrowth completely when added to the media at the time of explantation. Thus, following severe retinal, but not optic nerve trauma, an inhibitory factor enters the microenvironment of the damaged RGC axons.

591.5

REGENERATION OF THE AMPHIBIAN OPTIC NERVE THROUGH AN AUTOLOGOUS PERIPHERAL NERVE GRAFT. F. Scalia and S. Roca*
Dept. Anat./Cell. Biol., SUNY-HSCB, Brooklyn, NY 11203.

The nerve-graft method of Aguayo and coworkers was used to conduct regenerating optic nerve axons to their normal targets in R. pipiens, by joining the ocular stump of the left optic nerve to the right optic tract with a length of peroneal nerve. The optic nerve was severed close behind the eye. One end of the nerve graft was passed through the optic foramen toward the eye, and secured in apposition to the optic nerve stump. The other end was inserted into the contralateral optic tract close to the optic chiasma. By 17 wks p.o., the frogs were actively locating and capturing meal worms presented to either eye. Prior to sacrifice, the affected eye was injected with 3H-labeled amino acids to trace the central projections of the regenerated nerve. At sacrifice, segments of the autologous nerve bridge were also excised and post-fixed in osmium tetroxide. The radiolabeled optic axons were observed to enter the optic tract from the graft and to terminate in the normal targets of the optic projection. The projection to the contralateral tectum was organized into its normal pattern of lamination. In the nerve graft, the radiolabel was confined within its perineurial compartments, where the EM material showed the optic fibers to be associated with a profusion of Schwann cells either individually, as myelinated axons, or as bundles of unmyelinated axons. Supported by PHS grant No. EY05284.

591.6

COMBINED NGF INFUSIONS AND HIPPOCAMPAL GRAFTS INDUCE FUNCTIONAL RECOVERY IN THE LESIONED SEPTOHIPPOCAMPAL PROJECTION.

M. H. Tuszynski and F. H. Gage. Department of Neurosciences, University of California-San Diego, La Jolla, CA. 92093

Septohippocampal (S-HC) lesions induce degeneration of septal cholinergic neurons and impair spatial memory in rats. Previous studies have shown that fetal neuronal grafts that span a fimbria-fornix lesion cavity (bridging grafts) induce partial reinnervation of the host hippocampus by promoting fiber regrowth from the host septum. We sought to determine whether NGF infusions combined with fetal hippocampal bridging grafts to the bilaterally lesioned S-HC projection of the Fischer 344 rat could restore host circuitry and induce *functional* recovery. Animals underwent bilateral fimbria-fornix lesions, placement of E18 fetal hippocampal bridging grafts in the lesion cavity, and 9 week intraventricular infusions of NGF (total NGF dose 60 ug). Control animals received either lesions alone, lesions plus NGF infusions, or lesions plus fetal hippocampal bridging grafts (total n=38). After a total eight month survival period, functional performance was assessed in a Morris water maze and a habituation task. Animals receiving combined NGF infusions and grafts showed significant, partial recovery on the habituation task and in the water maze. Morphologically, all animals receiving transient 9 week NGF infusions had long-term recovery of 80% of septal cholinergic neurons ($p<0.001$), independent of the presence of a bridging graft. Only animals with grafts spanning the lesion cavity showed hippocampal reinnervation. Thus, long-term but transient NGF infusions combined with bridging grafts promote partial anatomical and functional recovery in the adult brain. More complete restoration of the septohippocampal pathway is needed for complete functional recovery.

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591.7

FUNCTIONAL EFFECTS OF LOCALLY APPLIED EXTRACELLULAR MATRIX WITH AND WITHOUT NGF AND bFGF FOLLOWING SPINAL CORD TRANSECTION. B.B.Walters, J.R.Encarnacion*, T.E.Melin*, and S.N. Schwirck*. Div. of Neurosurgery, Univ. of North Carolina at Chapel Hill, NC 27599-7060.

67 Sprague-Dawley rats underwent an open midthoracic procedure under methoxyflurane anesthesia within 36 hours of birth. 12 had laminectomies alone (LAMI), 17 had intradural transection and one segment myelectomy (TRANS), 17 had myelectomies with implantation of approx. 7 µl of Matrigel (ECM), 14 had implants containing approx. 3.5 µg of 2.5S NGF, and 7 had implants containing approx. 15 µg of bFGF. The animals were evaluated by the combined behavioral score of Wrathall (Exp. Neurol. 88: 123, 1985) 3 times weekly for 15 weeks in blinded fashion. The LAMI animals performed significantly better ($p<10^{-10}$, ANOVA) than all other groups on weeks 3-15. ECM outperformed TRANS during weeks 5, 6, and 8 ($p<0.05$ to <0.001) while bFGF outperformed TRANS during weeks 5-13 ($p<0.01$ to <0.001 except week 11). bFGF also outperformed ECM during weeks 5, 7, 9, 12 and 13 ($p<0.05$ to <0.01). In contrast, NGF performed the same as TRANS through week 6 and then performed more poorly ($p<0.05$ to <0.001). NGF performed more poorly than ECM after week 4 ($p<0.05$ to <0.001) and more poorly than bFGF after week 2 ($p<0.01$ to <0.001). Quantitative histological correlates of these results will be presented. Supported by the American Paralysis Association.

591.8

SURVIVAL AND AXONAL GROWTH OF RETINAL GANGLION CELLS IN ADULT RATS IS ENHANCED BY THE USE OF 'CONDITIONED' SCIATIC NERVE GRAFT. M. Bähr , G.W. Eschweiler* and H. Wolburg+. Max-Planck Institut für Entwicklungsbiologie and Neurologische Universitätsklinik Tübingen, +Pathologisches Institut der Universität Tübingen, Spemannstr. 35/1, W-7400 Tübingen, Germany

We have examined the influence of normal and precrushed ('conditioned') sciatic nerve grafts on the survival and axonal growth of retinal ganglion cells (RGCs) in adult rats. Normal sciatic nerves (group A) or sciatic nerves which had been crushed one week before transplantation (group B, 'conditioned' grafts) were used as grafts. The nerves were removed and sutured to the proximal stump of intraorbitally axotomized optic nerves. Neuronal survival and axon growth were determined by counting the numbers of surviving DiI prelabeled RGCs, cresylviolet stained RGCs and the numbers of axons which had grown into the grafts three and six months after transplantation. Counting of axons was performed by combined use of light- and electronmicroscopy.

We observed that the use of conditioned grafts (group B) significantly enhanced RGC survival and axonal regrowth as compared to normal grafts at 3 months after transplantation. Six months after grafting, RGC survival (as determined in DiI stained retinae) and axonal growth were not significantly different in both groups. When cresylviolet stained retinae were analysed, significantly more RGCs survived in group B compared to animals with normal grafts. These results suggest that the functional status of a peripheral nerve used for grafting in the CNS influences neuronal viability and axonal reelongation especially during the first 3 months after grafting. Long-term RGC survival, however, may be determined by functional reconnection of regenerating RGC axons rather than by the graft itself.

G.W.E. is a fellow of the Boehringer Ingelheim Fonds.

591.9

RAT PERIPHERAL NERVE REGENERATION ACROSS 18-mm GAPS AND SCHWANN CELL MIGRATION: INFLUENCE OF FIBRONECTIN. S.B. Bailey[1], M.E. Eichler[2], A. Villadiego*[2], K.M. Rich[2,3], Departments of [1]Otolaryngology, [2]Neurosurgery, and [3]Neurobiology, Washington University School of Medicine, St. Louis MO 63110

Silicone chambers, filled with sterile saline solutions of fibronectin (FB, 500 µg/ml), laminin (LN, 500 µg/ml), the combination of fibronectin-laminin (FB/LN, 500 µg/ml each), cytochrome-C (1 mg/ml cyto-C, control), or nerve growth factor (NGF, 1mg/ml), were sutured between the severed ends of sciatic nerves to form gaps of 18 mm, a distance chosen to stress the rat regenerative capacity. After 4 months, the various experimental groups were examined to determine the success of regeneration. Although NGF slightly increased cable formation -- the initial step for regeneration, all the groups had at least a 60% incidence of cables across the 18-mm gaps. The number of myelinated axons in the sural nerve distal to the repair was increased with either FB or FB/LN. HRP-labeling demonstrated that significantly more sensory and motor neurons in the FB or FB/LN groups had extended axons through the cables.

A possible reaction that may improve peripheral nerve regeneration is the augmentation of Schwann cell migration. As above, sterile saline solutions of FB, LN, FB/LN, NGF, or cyto-C were added to silicone chambers forming 5-mm gaps to determine the immediate effect, within the first 10 days, on Schwann cell invasion of the cables. Quantification revealed a greater influx of Schwann cells into the regenerate segment of the FB/LN group than of control.

Thus, although NGF does improve short-term regeneration in rat sciatic nerve, fibronectin provided a greater, over-all effect in the stressed system and affected the early influence of Schwann cells.

591.10

FLUNARIZINE IMPROVES LONG-TERM REGENERATION AFTER SCIATIC NERVE INJURY K.M. RICH, M.E. EICHLER, J.P. HOLLOWELL
Depts. of Neurosurg. & Neurobiol., Wash. Univ. Sch. Med., St. Louis, MO

The diphenylpiperazine, flunarizine, improves long-term regeneration in newborn rats after sciatic nerve crush. Experiments were done to determine whether prolonged neuronal protection with flunarizine during the period of axonal regeneration would be beneficial and result in long-term improvement in regeneration. Two groups of newborn rats were studied in parallel; the first group (experimental) received systemic administration of flunarizine, while the second group (control) received injections with vehicle only. Newborn rats (PND-1) were injected subcutaneously with flunarizine (25 mg/kg) every 12 hours. A unilateral sciatic nerve crush at the midthigh was performed under hypothermia anesthesia on PND-2 with continuation of flunarizine injections. On PND-3, 24 hours after nerve injury, the dose was increased to 50 mg/kg every 12 hours and continued for 28 days. The rats were then allowed to survive for an additional 3 months after the last flunarizine injection (4 months after injury). Total myelinated axonal counts of the sural nerves showed a 50% decrease on the lesioned side in the control group (947 ± 45 vs. 473 ± 48) compared with only a 26% decrease in the flunarizine-treated group (942 ± 72 vs. 700 ± 72). At the time the sural nerves were removed, the rats underwent injection with HRP in the sciatic nerve distal to the crush site and retrograde labeling studies were done to determine the number of neurons that survived and regenerated distal to the crush lesion. The control and flunarizine-treated groups respectively showed 1406 ± 346 vs. 4716 ± 811 in lumbar DRG and 194 ± 35 vs. 599 ± 132 in the ventral horn of the lumbar spinal cord. Differences between corresponding control and experimental lesioned groups were statistically significant, $P<0.05$, Student's t-Test, independent variables. Flunarizine treatment after sciatic nerve crush provides significant neuronal protection allowing enhancement of peripheral nerve regeneration.

591.11

INTERLEUKIN-1 RECEPTOR ANTOGONIST RELEASED FROM A POLYMERIC GUIDANCE CHANNEL IMPEDES PERIPHERAL NERVE REGENERATION. P. Aebischer[1], V. Guénard[1], C.A. Dinarello[2]*. [1]Brown University, Providence, RI and [2]Tufts University and New England Medical Center Hospital, Boston, MA.

Schwann cell-mediated nerve growth factor (NGF) effects play a key role in regenerative processes in the PNS. Upon injury, interleukin-1 (IL-1), a cytokine released by macrophages, triggers NGF synthesis by Schwann cells. Activated macrophages also release IL-1 receptor antagonist (IL-1ra), a true antagonist which binds to IL-1 receptors without inducing any IL-1 effects. Ethylene vinyl acetate (EVA) copolymer tubes releasing controlled amounts of either IL-1ra or deactivated IL-1ra (dIL-1ra) combined with bovine serum albumin (BSA) were tested in the mouse transected sciatic nerve model across a 4 mm nerve gap. Channels releasing BSA only were used as controls. Four weeks postimplantation, cables bridged the nerve stumps in all the tubes. Regardless of the channel type, at any point along the tubes, regenerated cables were morphologically similar. The regenerated tissue consisted of an epineurial-like sheath enclosing blood vessels and nerve microfascicles containing unmyelinated and myelinated axons with their associated Schwan cells. However, cables extending through BSA-IL-1ra-releasing channels contained significantly less unmyelinated and myelinated axons as compared to cables regenerated through BSA- and BSA-dIL-1ra-releasing channels. This study demonstrates that a true antogonist to IL-1 receptors impedes peripheral nerve regeneration and suggests that macrophages modulate peripheral nerve regeneration by releasing stimulatory and/or inhibitory molecules.

591.12

LOW ENERGY LASER IRRADIATION ALTERS IMMUNO-FLUORESCENCE OF CHOLINE ACETYLTRANSFERASE (ChAT) IN REGENERATING RAT FACIAL NEURONS. J.J.Anders, R.Borke and S.Woolery*. Dept. of Anatomy and Cell Biology, USUHS, Bethesda, MD 20814.

The authors have shown that low energy He-Ne laser irradiation (632nm, 8.5mW, 90 min) increases the rate of facial nerve regeneration after crush injury. ChAT decreases during the first week post-crush and returns to normal levels by 20 days in motoneurons (Curtis and Borke, Anat. Rec., 226:24A, 1990). The purpose of this study was to determine if irradiation could effect ChAT changes during nerve degeneration and regeneration. The facial nerve was crushed unilaterally in anesthetized rats for 90 s. The wound was irradiated transcutaneously, daily. Rats were fixed by intracardiac perfusion on days 5 and 11 and sections were processed for immunofluorescence. Fluorescence levels of ChAT were quantified using a Biorad Confocal Imaging System. A significant increase ($p<.05$) in ChAT fluorescence intensity/μm^2 was detected in motoneurons of irradiated versus nonirradiated injured rats at 5 and 11 days. Whether this increase in ChAT correlates with a decrease in the initial degenerative events or signifies an earlier return of acetylcholine systhesis is being determined.

AUDITORY SYSTEM: CENTRAL PATHWAYS V

592.1

MIDBRAIN AUDITORY PHYSIOLOGY IN TWO SPECIES OF MORMYRIDS. J.D. Crawford. Parmly Hearing Institute, Loyola U., Chicago, IL 60626.

All of the African mormyrid fishes have specialized ears consisting of a gas-filled bladder within each labyrinth, and the direct coupling of an otolithic organ (sacculus) to this bladder. Even though several mormyrid species are now known to use acoustic signals in communication, little is known about the processing of sounds by the ear and brain in these animals. As a part of an ongoing study of the processing of temporally modulated communication signals, I have conducted a single-unit survey of the midbrain auditory nucleus in two species of mormyrids: *Pollimyrus isidori*, a species known to use sounds in communication, and *Brienomyrus niger*, a related fish but one which is not known to produce sounds.

Microelectrodes were used to record from single neurons while fish were submerged in a calibrated, water-filled, tank. Recording sites were marked with 15 µm diameter deposits of metal from the recording electrode. Response areas (RAs) were constructed by presenting an array of 20 tone bursts (105 to 1500 Hz) over a 60 dB range. From the RA, each neuron was classified on the basis of characteristic frequency (CF), best sensitivity (BS), and degree of frequency tuning (Q_{10dB}). Both fish were found to be highly responsive to low frequency tones, with the most common characteristic frequencies around 235 Hz. Other CF classes had single excitatory zones located in the vacinity of 150 Hz and 450 Hz respectively, and another class had broad RAs including two excitatory zones. The majority of neurons were phasic, producing large onset responses with much less sustained activity during a 200 ms tone burst (50 ms rise/fall). Tonic units were much less common, and typically had low BSs and were relatively sharply tuned. In *P. isiodri* some neurons had quite complex response properties in that they rarely responded to tones even at high levels, but gave robust responses to clicks. Preliminary experiments with click trains suggest that certain neurons in *P. isidori* may be selective for particular inter-click-intervals. (NIH DC00020-02 & DC00293-06).

592.2

"GLYCINE RECEPTOR" IMMUNOSTAINING SITES MARK CLASSES OF AUDITORY AND VESTIBULAR CELLS. J.C. ADAMS ENT Dept., Mass. Eye & Ear Infirmary Boston, MA 02114.

A monoclonal antibody(BM) raised against strychnine binding sites recognizes sites that do not bind strychnine nor contain much glycine. One example is that of inclusion bodies within the cytoplasm of auditory and vestibular neurons. These cells are in the MNTB, primary vestibular neurons, octopus cells, spherical cells in the VNLL, and spherical cells located caudal and ventral to the LSO. Three of these cell classes are immunoreactive for glycine. Octopus cells and Scarpa's ganglion cells are not. One trait shared by cells containing inclusion bodies is the sparsity of immunoreactive sites for the "glycine receptor" on their surfaces. This suggests that the inclusion bodies are probably not sites where the receptor is made. The role of the inclusion bodies is obscure, but their presence can be used for identifying cell classes. For example, with the inclusion body as the defining trait of octopus cells: humans have very many; cats have many; rodents have few. This is another example of a closer similarity of cat and human auditory systems as contrasted with rodents.
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592.3

Sound-localization deficits induced by lesions in the owl's auditory space map. H. Wagner. Max-Planck-Institut für biologische Kybernetik, Spemannstrasse 38, D-7400 Tübingen, FRG

Barn owls possess a map of auditory space. The map is synthesized in the external nucleus of the inferior colliculus (ICx) and then projected to the optic tectum (OT). Azimuth is mapped from antero-medial to postero-lateral, while elevation is mapped vertically.

To find out whether the map plays a role in the localization of acoustic stimuli, head movements of owls were recorded before and after lesioning ICx and/or OT. After focal electrolytic lesions, I observed increased response latencies, reduced turning amplitudes and an increased number of turns away from the sound source. These deficits occurred at azimuths expected from the physiological data obtained at the lesion site before passing current. The deficits extended over a range of some 20 degrees and were obvious from 3 to 20 days after the lesion. After this transient phase, mean response latency remained increased in 1 of 6 cases, and the number of turns with very short latencies was reduced in 2 cases. Thus, this is the first unambiguous demonstration, for sound localization, of a deficit covering a locally well-circumscribed area within a hemisphere. Since the major lesion deficits were transient, two lesions, one on each side of the brain, were made in each of the three animals. Since the behavioral deficit induced by the second lesion disappeared in the same way as did the deficit induced by the first lesion, the possibility is excluded that the animals learned to respond tactically. The deficits were also not due to motor impairment, because immediately after the lesion, the animal responded normally to stimulation from outside the well-circumscribed region of the lesion.

592.4

LATENT NEURONAL ACTIVITY IN HUMAN AUDITORY CORTEX Z.L. Lü*, S.J. Williamson and L. Kaufman. Neuromagnetism Lab., Depts. of Physics and Psychology and Center for Neural Science, N.Y.U., New York, NY 10003**.

The auditory event-related response detected magnetically about 100 msec following the onset of a tone burst with a long interstimulus interval (ISI) reveals a component of neuronal activity that overlaps in time with the response in primary auditory cortex (N100m). We call this activity the 'latent' component (L100m) since it requires a much longer ISI than N100m to reach a full response amplitude when stimuli are presented at a fixed rate. Amplitudes of L100m and N100m were measured at their separate magnetic field extrema with interstimulus intervals ranging from 0.8 to 16 sec. The resulting ISI-dependence for each could be well fit by an exponential curve using a least square criterion. Statistically significant fits showed that suppression of the response amplitude caused by the preceding stimuli decreased exponentially with ISI, with the time constant for L100m being about 1.5 sec longer than for N100m, the latter ranging from 1.5 to 3.5 sec across individuals. The locations of the neuronal activity that produce L100m and N100m were deduced from the field pattern by a least squares fit of 2 current dipoles. By comparison with MRI, both were found to lie on the supratemporal plane with the source of N100m lying in primary auditory cortex and the source of L100m lying about 1.5 cm more posterior and 1 cm more inferior than that of N100m. The second location was identified as the auditory association cortex.

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592.5

DISRUPTION OF INTERHEMISPHERIC INTEGRATION OF COMPLEX AUDITORY INFORMATION FOLLOWING FOCAL HEMORRHAGE INTO THE POSTERIOR BODY OF THE CORPUS CALLOSUM M.J. Tramo, F.E. Musiek, and M.S. Gazzaniga
Program in Cognitive Neuroscience and Division of Audiology, Dartmouth-Hitchcock Medical Center, Hanover, NH 03756

In subhuman primates, the posterior body of the corpus callosum contains interhemispheric projections of auditory cortical neurons in the supratemporal plane and posterior superior temporal cortex (Pandya & Seltzer 1986). To our knowledge, in only one human has the posterior body of the callosum been selectively sectioned (Dimond et al. 1977), but the extension of the surgical lesion (and possibly the pathological lesion) into the left hemisphere precludes a clear interpretation of the unusual auditory findings in this case. Evidence that a segment of the posterior callosum mediates interhemispheric auditory integration in humans has been derived from dichotic studies of patients with partial callosotomies extending from the splenium forward (Geffen 1980; Musiek et al. 1985) and of patients with left hemisphere lesions lying deep in the periventricular parietal or parieto-occipital white matter (Damasio & Damasio 1979).

We report a case of focal hemorrhage into the posterior body of the callosum caused by delayed post-traumatic rupture of a pericallosal artery aneurysm. Thin-section sagittal and coronal MR permitted the precise localization of callosal damage and documented the limited extent of intrahemispheric involvement. Pure tone thresholds were within normal limits in each ear. Consonant-vowel (CV) phonemes presented monaurally 50dB above sensation level were correctly repeated in 30/30 trials in each ear. When CV phoneme pairs were presented dichotically, 30/30 vs. 0/30 presented to the right ear vs. left ear, respectively, were reported. When monosyllabic homophone pairs were presented dichotically, 25/30 vs. 3/30 presented to the right vs. left ear were reported. In none of the 60 trials were both right and left ear stimuli reported. Other notable findings included: no extinction to double simultaneous visual or tactile stimulation; normal naming of line drawings presented tachistoscopically in the left visual field; normal tactile naming in the left hand; and mild left hand dyspraxia.

These findings indicate that: 1) the posterior body of the corpus callosum mediates the interhemispheric integration of phonemic information; and 2) the topography of callosal auditory projections is homologous in humans and nonhuman primates.

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592.6

HOMOTONIC SEGREGATION IN THE ORIGIN OF BILATERAL ASCENDING PROJECTIONS FROM THE FERRET LATERAL SUPERIOR OLIVARY NUCLEUS. C. K. Henkel and J. K. Brunso-Bechtold, Dept. of Neurobiology and Anatomy, Bowman Gray Sch. of Med., Winston-Salem, NC 27157-1010.

In this study of the adult and early postnatal ferret lateral superior olivary nuclei (LSO), it was observed that cells giving rise to the ipsilateral and contralateral ascending projections were segregated spatially within areas of similar frequency representation. This homotonic segregation was far more striking in the ferret LSO than the heterotonic variation that has been previously reported. The cellular region of the intermediate limb of the LSO was divided arbitrarily into eight parallel lanes from core to margin. Labeled projection cells on the ipsilateral and contralateral side with respect to tracer injections in the inferior colliculus were mapped and their position plotted as a function of the lanes they occupied. There was a clear tendency for ipsilateral projection cells to occupy marginal lanes and contralateral projection cells to occupy core lanes. Approximately two-thirds of the ipsilateral projection cells were found in the two inner and outer lanes compared to only one-third of the contralateral projection cells. The distribution curves varied from case to case and limb to limb, probably in part due to the irregular orientation of the limbs of the LSO with respect to standard anatomical planes. Some homotonic segregation in the LSO was evident at birth indicating that this rule of organization may be in place at least four weeks prior to the onset of hearing. Cells marked by other phenotypic traits reveal similar homotonic segregation. Glycine- and GABA-immunoreactive cells were most frequent in the marginal lanes. Similar immunoreactive cells ringed the nucleus in the developing ferret. No homotonic gradient of immunoreactive puncta in the LSO was apparent suggesting that the output but not necessarily the input to the LSO may be segregated. Further studies are underway to address these hypotheses. Supported in part by NIH grant DC00335.

592.7

AN ANIMAL BEHAVIORAL MODEL OF HALLUCINATIONS IN RATS. P.J. Jastreboff[1] and J.F. Brennan[2]. [1]Dept Surgery, Univ Maryland Sch Medicine, Baltimore, MD 21201; and [2]Dept Psychology, Univ of Massachusetts/Boston, MA 02125.

An animal model of phantom auditory perception has been tested on salicylate-induced auditory sensation in rats (Behavioral Neurosc. 102:811-822, 1988) and involves continuous exposure of rats to background noise or light with offset of the background serving as a conditioned stimulus (CS). The model has been expanded to accommodate visual and auditory hallucinations by introducing complex background of pulses of tone or light of random length presented randomly, with the offset of the background serving as CS. Pulses of light/tone of different traits superimposed on this background resulted in modality-specific changes in behavior consistent with our previous findings.

The additional use of scopolamine (0.5-, 0.75- and 1.0 mg/kg/day, s.c.), which has been reported in humans to induce visual but not auditory hallucinations, produced changes in extinction among groups trained with visual modality, but not with the auditory modality, in a dose-dependent manner. These results are consistent with the postulate that scopolamine induces visual hallucinations in rats, detected by our paradigm. This offers a possibility for creating a behavioral animal model for schizophrenia. (NIH NIDCD Grant DC00445).

592.8

IDLING 10-HZ RHYTHM IN THE HUMAN AUDITORY CORTEX? R. Hari. Low Temperature Laboratory, Helsinki University of Technology, 02150 Espoo, Finland.

The 10-Hz alpha rhythm in the visual cortex and the corresponding mu rhythm in the somatomotor area are considered 'idling' rhythms of the sensory systems; they are blocked by visual and tactile stimuli, respectively. We have recorded magnetic spontaneous activity with a 24-channel superconducting quantum-interference device (SQUID) gradiometer to find out whether the human auditory areas also display an idling rhythm [1,2].

Magnetic 8–10 Hz rhythm was observed over the temporal regions. It was damped by auditory stimuli but it did not decrease when the eyes were opened or when the fist was clenched. The field patterns over the auditory cortex could be satisfactorily explained by equivalent current dipoles, situated within 2 cm from the source of the 100-ms auditory evoked response, generated in the supratemporal auditory cortex. During light drowsiness, when the occipital electric alpha became discontinuous and 'spread' to more anterior scalp locations, the temporal magnetic 10-Hz rhythm accentuated and resembled the frontocentral electric activity. Since currents at the superior surface of the temporal lobe would produce the maximum potentials in the frontocentral midline, the 'spread' of electric 'alpha' during drowsiness might reflect activity of the temporal-lobe 10-Hz generators.

The results suggest that each sensory projection cortex has its own idling rhythm.

[1] Tiihonen J, Hari R, Kajola M, Karhu J, Ahlfors S and Tissari S. Submitted for publication. [2] Lu S-T, Kajola M, Joutsiniemi S-L, Knuutila J and Hari R. Spontaneous MEG-activity during sleep. In preparation.

592.9

TOPOGRAPHY OF INTENISTY ENCODING BY SINGLE NEURONS IN CAT PRIMARY AUDITORY CORTEX. M.L. Sutter and C.E. Schreiner. Coleman Laboratory, Dept of Otolaryngology, University of California, San Francisco, CA 94143-0732.

The topography of the sharpness of amplitude tuning (monotonicity) of single neurons was studied within the isofrequency domain of high frequency (CFs > 4 kHz) primary auditory cortex (AI). To pool data across animals, the multiple unit monotonicity (Schreiner et al. 1988: ARO abstracts 11: 36) and excitatory bandwidth maps were used as topographical frames of references (Sutter and Schreiner 1991: J. Neurophysiol. 65). The multiple unit monotonicity map consisted of two non-monotonic (sharply tuned for amplitude) areas along the isofrequency (roughly dorsal-ventral) domain of AI. Non-monotonic response profiles were more common in single unit (56%) than multiple unit (36%) recordings. In ventral AI, the spatial distribution of single neuron monotonicity ratios (response at highest tested intensity divided by response at best intensity) paralleled the shape of the multiple unit spatial distribution. In dorsal AI, pooled single neurons displayed no clear topographical order with respect to amplitude tuning. At the two non-monotonic regions, a sharp decrease in the scatter of intensity thresholds for single neurons was observed, implying that non-monotonic multiple unit areas are a result of tightly overlapping intensity receptive fields for single units. These data indicate that parcelling AI into dorsal (AId) and ventral (AIv) regions, as supported by the excitatory bandwidth topography, is justified; however, physiological properties of the ventral non-monotonic area lying at the AId/AIv border need to be considered in more detail.

The ventral multiple unit non-monotonic area roughly aligns with the area of sharply frequency tuned units described by Schreiner and Mendelson (1990: J. Neurophysiol. 64: 1142-1160). This region, with low intensity thresholds, narrow intensity tuning, and narrow frequency tuning might correspond to an intensity fovea for low intensity narrow-band stimuli, just above the background noise. (This work was supported by NINDS/NIH grant NS10414, NIH training grant GM08155, the Coleman Fund, and Hearing Research Inc.)

592.10

BRAINSTEM INFUSION OF KYNURENIC ACID REDUCES SPONGIFORM LESIONS IN GERBIL COCHLEAR NUCLEI, SUGGESTING AN EXCITOTOXIC ORIGIN. M.D.McGinn and B.T. Faddis*. Dept. of Otolaryngology, University of California, Davis CA 95616

We hypothesized that spongiform lesions in gerbil cochlear nuclei are the result of chronic excess of an excitatory amino acid neurotransmitter. For eight days we used osmotic minipumps to infuse a magnesium-free 50 mM solution of kynurenic acid, a non-specific glutamate antagonist, through brainstem cannulae whose tips were unilaterally placed near cochlear nuclei. Sequential methacrylate sections showed that lesions were significantly reduced in the cochlear nuclei ipsilateral to the cannulae when compared with the untreated side. The greatest reduction in spongioid lesions was located near the cannulae tips, apparently reflecting diffusion gradients. The results suggest that the reduction of lesions is due to blockade of amino acid receptors in the cochlear nuclei.

592.11

REORGANIZATION IN AUDITORY CORTEX OF ADULT CATS WITH UNILATERAL RESTRICTED COCHLEAR LESIONS. D. R. F. Irvine, R. Rajan, L. Z. Wise and P. Heil. Dept. of Psychology, Monash University, Clayton, Vic. 3168, Australia.

In a previous study (Robertson & Irvine, J. Comp. Neurol. 282:456-471, 1989), restricted cochlear lesions in adult guinea pigs were found to result in a reorganization of the representation of the lesioned cochlea in the contralateral auditory cortex. One question raised by this result concerns the effect of this reorganization on the representation of the normal (ipsilateral) cochlea in that cortex. This question was examined in adult cats in which restricted unilateral cochlear lesions were produced by direct mechanical trauma. Following a 2-6 month post-lesion recovery period, the animals were reanesthetized, the extent of the peripheral hearing loss in the lesioned ear was established by means of the compound action potential audiogram, and the frequency organization of primary auditory cortex (AI) contralateral to the lesioned ear was determined using conventional microelectrode mapping techniques. In the representation of the lesioned contralateral cochlea the region deprived of its normal input by the lesion was partially occupied by an expanded representation of the frequency at the edge of the lesion, but the ipsilateral frequency map in this region showed a normal frequency progression. Evidence on the thresholds at their new characteristic frequencies of neuronal responses in the enlarged contralateral representation indicated that the expanded representation reflected a reorganization of cortex rather than simply residual drive at frequencies unaffected by the lesion. The lack of change in the ipsilateral frequency map results in a loss of the normal registration between the two frequency maps, and has implications for the mechanisms underlying auditory cortical reorganization.

PRESYNAPTIC MECHANISMS: ION CHANNELS

593.1

5-HT INCREASES Ca^{++} INFLUX DURING ACTION POTENTIALS IN *APLYSIA* SENSORY NEURONS BY SPIKE BROADENING AND BY DIRECT CALCIUM CHANNEL MODULATION. H. Blumenfeld, L.S. Eliot, B.W. Edmonds, E.R. Kandel and S.A. Siegelbaum. Columbia Univ., H.H.M.I., N.Y., N.Y. 10032.

Aplysia sensory neurons contain "N-like" Ca^{++} channels which participate in transmitter release, and dihydropyridine-sensitive "L-like" Ca^{++} channels which do not contribute to release (Edmonds et al., 1990). 5-HT produces presynaptic facilitation of the *Aplysia* sensory-motor neuron synapse, and increases the sensory neuron $[Ca^{++}]_i$ transient during action potentials. The increase in the $[Ca^{++}]_i$ transient is due to two processes: 1) Indirect modulation whereby $S\text{-}K^+$ channel closure and resulting spike broadening increase influx through both N and L channels, and 2) A direct increase in L Ca^{++} current. Since only N Ca^{++} current contributes to presynaptic facilitation (Edmonds et al., 1990), we have used fura-2 $[Ca^{++}]_i$ measurements with or without nitrendipine to assess the relative contributions of N and L currents to the $[Ca^{++}]_i$ transient and its modulation by 5-HT.

5-HT caused a 77 ± 14% increase (mean ± SEM; $n = 10$; $p<0.001$, two tailed t-test) in the amplitude of $[Ca^{++}]_i$ transients in the cell body produced by action potentials. However, in the presence of 10 μM nitrendipine (which blocks the 5-HT-induced increase in L current by 87 ± 4%), 5-HT caused only a 28 ± 8% increase in $[Ca^{++}]_i$ transient amplitude ($n = 9$), significantly different both from control ($p<0.01$) and from 5-HT alone ($p<0.01$). This 28% increase presumably reflects increased Ca^{++} influx via the N channels due to spike broadening. In nitrendipine, the amount of spike broadening produced by 5-HT was roughly correlated with the increase in $[Ca^{++}]_i$ transient amplitude. As shown in the accompanying abstract (Eliot et al., 1991), a similar increase in Ca^{++} influx is seen in presynaptic sensory neuron processes in 5-HT + nitrendipine, supporting its role in presynaptic facilitation.

593.2

IMAGING $[Ca]_i$ TRANSIENTS AT *APLYSIA* SENSORIMOTOR SYNAPSES: CONTRIBUTIONS OF DIRECT AND INDIRECT MODULATION TO PRESYNAPTIC FACILITATION. L.S. Eliot, H. Blumenfeld, B.W. Edmonds, E.R. Kandel and S.A. Siegelbaum. Ctr. Neurobiol & Behav, HHMI, Columbia Univ., N.Y., N.Y. 10032.

The accompanying abstract (Blumenfeld et al., 1991), demonstrated that 5-HT increases Ca transients in *Aplysia* sensory neurons both by direct modulation of the L-type Ca current and by action potential broadening, which indirectly enhances influx through both L- and N-type Ca currents. Here, we examine the contributions of these two mechanisms to the increase in Ca influx in synaptic terminal regions.

Serotonin produced a 39 ± 9% ($N = 5$) increase in the $[Ca]_i$ transient in regions of the sensory neuron making contact with a postsynaptic motor neuron, and facilitated the EPSP by 47 ± 16%. Nitrendipine reduced this increase by 65%, but did not reduce facilitation of the EPSP. In separate cultures, nitrendipine by itself reduced the Ca transient to 80 ± 5% of control ($N = 5$). Subsequent 5-HT addition increased the Ca transient by 27 ± 6% and facilitated the EPSP by 109 ± 46% over nitrendipine alone. These results indicate that direct modulation of the L-type current contributes roughly 2/3 of the enhancement of Ca influx by 5-HT, but this does not contribute to facilitation. The remaining enhancement of Ca influx occurs through N-type Ca channels as an indirect consequence of spike broadening. There was a significant correlation between enhancement of the Ca transient in the presence of nitrendipine and facilitation of the EPSP ($R = .737$, $p < .05$, $N = 8$) fit by a 2.4 power relation, consistent with the view that enhanced Ca influx via the N-type channels contributes to presynaptic facilitation. These experiments also provide some evidence that the two classes of Ca channels are differentially localized.

593.3

SYNAPTIC PLASTICITY OF A DUAL-ACTION POTENTIAL MEDIATED BY METABOLITES OF ARACHIDONIC ACID IN *APLYSIA*. E. Shapiro, Howard Hughes Medical Institute, New York, NY 10032

In the abdominal ganglion of *Aplysia*, an identified cluster of interneurons called L32 cells makes a dual-action synaptic potential onto L14 cells, motor neurons controlling the defensive release of ink. The dual-action consists of a fast early depolarizing phase followed by a slower hyperpolarization. Previous work has suggested that both phases of the dual-action may be mediated by stimulation of arachidonic acid (AA) metabolism to produce the active 12-lipoxygenase products 12-hydroperoxy-5,8,10,14-icosatetraenoic acid (12-HPETE) and 8-hydroxy-11,12-epoxy-5,9,14 icosatrienoic acid (8-OH). I have begun to examine plasticity of this dual-action response in order to gain insight into the regulation of signalling by the AA cascade.

The L32-L14 dual-action synapse displays quite variable strength. Within the same L32 cluster different individual cells may produce almost pure excitation or almost pure inhibition on the same L14 follower cell. Similar to other dual-action responses, the L32-L14 dual-action is frequency-dependent. At low frequencies of stimulation (< 1Hz) the early excitatory phase predominates, while at higher frequencies of stimulation, the depolarizing phase undergoes synaptic depression, and the slow inhibitory phase predominates. When L32 cells are tetanized (e.g. 20Hz stimulation for 20 sec) the slow IPSP component of the synaptic response elicited by a short train of spikes (20 Hz for 1 sec) exhibits post-tetanic potentiation. In each ganglion up to seven L32 cells converge on L14. I am currently testing whether activity in one L32 cell may affect the synaptic response of L14 to other L32 cells.

593.4

ADENOSINE MEDIATES SYNAPTIC DEPRESSION IN *APLYSIA*. S.M. Fredman. Department of Physiology, Meharry Medical College, Nashville, TN 37208.

Low frequency stimulation of the A-B neuron synapse in the cerebral ganglion of *Aplysia* produces a long-lasting depression. Only 5 spikes presented at 3 min intervals results in a 49±14% ($n=10$) reduction in EPSP amplitude. I have obtained evidence that this depression may be mediated by the neuromodulator adenosine. Synaptic depression was assessed by measuring EPSP amplitudes in B neurons evoked by stimulating A neurons at 3 min intervals and by changes in potentiated EPSP amplitudes (Slow Developing Potentiation, SDP; Fredman, 1988) produced by 2 sec 20 Hz trains in the A neurons.

Adenosine agonists increased depression. Bath application of 100 μM adenosine (ADO) or 150 nM of the A_1 agonist, N^6-cyclohexyladenosine reduced SDP and increased synaptic depression. ADO antagonists decreased depression. Application of 100 μM DPSPX reduced depression and increased SDP. ADO antagonists with phosphodiesterase activity had two effects. At low concentrations, both 100-400 μM IBMX and 200 μM theophylline reduced or blocked synaptic depression. At high concentrations (1-2 mM) they increased it. The latter effect suggested that depression might be mediated by cyclic AMP. Both bath application of DB-cAMP and direct injection of cAMP into A neurons reduced SDP. Adenosine deaminase (1 mg/ml) which hydrolyses ADO significantly reduced depression, indicating that endogenous ADO may contribute to depression of the synapse. These results suggest that at the A-B neuron synapse in *Aplysia*, depression may be mediated by presynaptic adenosine autoreceptors acting via a cAMP dependent-mechanism. Thus, as in the mammalian CNS, adenosine may also modulate synapses in *Aplysia*.

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593.5

PRESYNAPTIC CALCIUM CONCENTRATION MEASURED BY THE EFFECTS OF CALCIUM BUFFERS ON CALCIUM-ACTIVATED POTASSIUM CHANNELS THAT COLOCALIZE WITH ACTIVE ZONES IN SACCULAR HAIR CELLS OF *RANA PIPIENS*. W.M. Roberts. Inst. of Neuroscience, Univ. of Oregon, Eugene, OR 97403.

We have proposed that the entry and action of Ca^{2+} in frog saccular hair cells is confined to the immediate vicinity of a 250 nm-diameter cluster of ion channels at the center of each synaptic active zone [Roberts, Jacobs & Hudspeth, *J. Neurosci.* (1991) **10**:3664]. Each active zone is purported to contain ~90 Ca^{2+} channels that can pass a 50 pA Ca^{2+} current, and ~40 K_{Ca} channels that open when the cell is depolarized and the local $[Ca^{2+}]_i$ exceeds ~10 μM. To test this model, I introduced Ca^{2+} buffers through whole-cell recording pipettes and examined how the concentration and association rate constant of the buffer affected the K_{Ca} current. At concentrations of 1 mM, EGTA had little effect, whereas BAPTA, which binds Ca^{2+} >10 times more rapidly, reduced K_{Ca} channel activation. This difference indicates that 1 mM-EGTA was too slow to intercept a significant fraction of the Ca^{2+} as it diffused the short distance between the clustered Ca^{2+}- and K_{Ca} channels, and implies a diffusion distance <300 nm. K_{Ca} channel activation was reduced by 500 μM BAPTA during small depolarizations that evoked submaximal Ca^{2+} currents, but the buffer saturated during larger Ca^{2+} currents. This saturation indicates that local $[Ca^{2+}]_i$ exceeded 500 μM during large depolarizations. Thus, diffusible buffers reduced the local steady-state $[Ca^{2+}]_i$ only when present at high concentration, and the buffer's binding rate was a crucial factor in its effectiveness close to the site of Ca^{2+} entry.

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593.6

CALCIUM DYNAMICS IN MOUSE MOTOR NERVE TERMINALS REVEALED BY FURA-2 RATIO IMAGING. Z.-P. Fang and N. Robbins. Dept. of Neurosciences, Case Western Reserve Univ., Sch. of Med., Cleveland, OH 44106.

Until now, the amplitude and time course of changes in free calcium concentration in mammalian nerve terminals has been estimated only indirectly by measuring MEPP frequency or EPP amplitude. Therefore, it was of interest to determine cytosolic calcium dynamics more directly; particularly those transients which occur during physiological pattern of neuronal activity. We studied cytosolic calcium dynamics in mouse motor nerve terminals by direct measurement with the calcium indicator fura-2. The nerve-muscle preparation was perfused in normal Krebs solution at room temperature. After the dye was injected into preterminal axons in soleus muscles, it rapidly diffused into the nerve terminals (Fang & Robbins, *Neurosci. Abstr.* **15**:484, 1989). Fluorescence signals at two excitation wavelengths, detected by a photomultiplier tube or a video camera, were ratioed to generate average terminal calcium concentration data. During a tetanus of 10-20 Hz (the natural firing rate in the soleus), $[Ca^{++}]_i$ increased from a resting level of 100-200 nM to a plateau level of 500-1000 nM in about 10 seconds. At the end of the tetanus, $[Ca^{++}]_i$ decayed exponentially to the resting level with a time constant of 10-20 seconds. When a higher stimulation frequency of 50-100 Hz (typical of fast twitch muscle) was used, $[Ca^{++}]_i$ reached plateau levels as high as 1500 nM or more in 3-5 seconds, and recovery could take more than 20 minutes. These data in soleus muscle will be compared to those in fast twitch muscles which have different firing patterns. Supported by NIA grant AG08886.

593.7

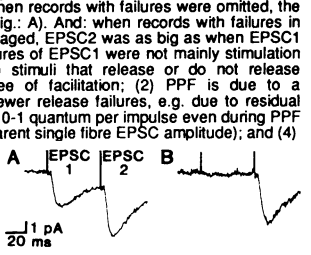
CALCIUM-EVOKED [^{3}H]ACETYLCHOLINE RELEASE FROM CALCIUM-NAIVE RAT HIPPOCAMPAL SYNAPTOSOMES: INFLUENCE OF MUSCARINIC CHOLINERGIC AUTORECEPTORS AND CATION CHANNELS. T.W. Vickroy, C.J. Schneider* and J.M. Hildreth*. Depts. Physiol. Sci. and Neuroscience, Univ. Florida, Gainesville, FL 32610.

Studies were carried out to delineate presynaptic mechanism(s) which regulate the ability of calcium (Ca^{2+}) to promote [^{3}H]acetylcholine ([^{3}H]ACh) release in a novel model system. Rat hippocampal synaptosomes were prepared, labelled with [^{3}H]choline and superfused under Ca^{2+}-free conditions (zero Ca^{2+} plus 50μM EGTA) at 37°C. Infusion of buffers containing Ca^{2+}, Sr^{2+} or Ba^{2+} produced a four to twelve-fold increase in the rate of [^{3}H]ACh efflux. Tetrodotoxin, an inhibitor of voltage-activated Na^+ channels, caused near-complete blockade of cation-stimulated [^{3}H]ACh release thereby indicating an integral role for voltage-activated Na^+ channels in this exocytotic event. Pharmacological studies with Ca^{2+} channel blockers demonstrated that the response to Ca^{2+} (and Ba^{2+}) was sensitive to selected agents, including (IC_{50}'s in parentheses) ω-conotoxin GVIA (0.9nM), $CdCl_2$ (1.6μM), $CoCo_3$ (300μM), $MgCl_2$ (<20mM) and $LaCl_3$ (<1mM). The dihydropyridine Ca^{2+} channel antagonist nifedipine (10μM) was without effect, consistent with the profile for N-type voltage-sensitive Ca^{2+} channels. Membrane depolarization with elevated extracellular potassium (up to 30mM) selectively enhanced the secretagogue action of Ca^{2+}. In addition, pretreatment with potassium channel blockers, including (EC_{50}'s in parentheses) charybdotoxin (3nM) 4-aminopyridine (10μM) and tetraethylammonium (800μM), nearly tripled the maximal response to Ca^{2+}. Pretreatment with muscarinic cholinergic agonists (carbachol or oxotremorine) significantly reduced Ca^{2+}-stimulated [^{3}H]ACh release in an atropine-sensitive manner. Together, these results demonstrate that Ca^{2+}-naive synaptosomes are a useful model for studies of presynaptic receptors and ion channels which regulate Ca^{2+}-dependent neurotransmitter release in CNS tissues. (supported by NS-28568).

593.8

A STUDY OF SYNAPTIC FACILITATION IN CA1 PYRAMIDAL CELLS USING WHOLE CELL RECORDING AND STIMULATION OF ONE OR A FEW PRESYNAPTIC FIBERS J.F. Storm & M. Raastad, Inst. of Neurophysiology, Pb 1104 Blindern, N-0317 Oslo, Norway.

Paired-pulse facilitation (PPF) is a widespread form of short-term synaptic plasticity, which may be due to residual Ca in the presynaptic terminal following the first impulse. We have used whole-cell recording to study PPF in CA1 cells in rat hippocampal slices (in 10 uM bicuculline). Two electrical stimuli (stim.1 and 2, 30-60 ms apart) were given to radiatum fibres once every 1-2 s, each eliciting a postsynaptic excitatory current (EPSC1 and 2). Strong stimuli from a glass pipette (30-50 um tip) elicited regular EPSCs (peak amplitude 10-50 pA; no failures). Each EPSC2 was larger than EPSC1, reflecting PPF of the summed EPSC from many synapses. In contrast, weak stimuli from a small pipette (2-6 um tip; to stimulate a single presynaptic fiber; Raastad, Storm, Andersen, Soc. Neurosci Abstr. 16, 674, 1990; Raastad, this meeting), elicited small EPSCs of highly variable amplitude (typically < 10 pA) intermixed with no detectable response (failures). Now, PPF was only seen as fewer failures; the mean amplitude of non-zero EPSCs did not change. When medium pipette sizes (5-20 um) or stimulation strenghts were used (to stimulate a few fibres), there were still some failures, but when records with failures were omitted, the mean EPSCs showed clear PPF (Fig.: A). And: when records with failures in EPSC1 but not in EPSC2 were averaged, EPSC2 was as big as when EPSC1 did not fail (Fig.: B). Hence, the failures of EPSC1 were not mainly stimulation failures. This suggests that: (1) stimuli that release or do not release transmitter cause the same degree of facilitation; (2) PPF is due to a presynaptic mechanism (probably fewer release failures, e.g. due to residual Ca); (3) each synapse releases only 0-1 quantum per impulse even during PPF (since PPF did not increase the apparent single fibre EPSC amplitude); and (4) if the residual Ca hypothesis is correct, the release failures are probably not due to variations in presynaptic Ca influx (since EPSC1 failures were not correlated with a smaller EPSC2).



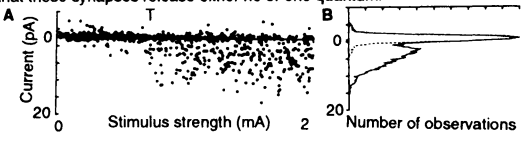
593.9

A METHOD FOR RELIABLE ACTIVATION OF A SINGLE EXCITATORY SYNAPSE IN CA1 PYRAMIDAL CELLS
M. Raastad, Inst. of Neurophysiology, Pb 1104, N-0317 Oslo, Norway.

A reliable single synaptic input is a useful tool for a detailed study of synaptic mechanisms. By stimulating with a small (2-4um) glass pipette filled with extracellular solution, positioned 100-200um off-centre in the dendritic tree, and using a random stimulation-strength pattern, it is possible to demonstrate regular activation of a single synaptic afferent to CA1 cells.

CA1 cells in submerged hippocampal slices from rats were patch-clamped deep in the tissue without cleaning. 200us long stimuli varying randomly between 0 and 2mA, were delivered at 1Hz. Above threshold, there was a good linear correlation between the stimulus strength and the amplitude of extracellular field potential. In contrast, there was no correlation between the size of the excitatory postsynaptic current (EPSC) and stimulus strength above threshold (T in fig.A). The mean amplitude and the variability remained constant. In conclusion, only one synapse was activated within this range of stimuli. Below the threshold the amplitudes were distributed normally around zero (failures, dotted in fig.B), whereas above threshold the amplitudes were distributed around two values, either around zero or around a given mean amplitude, with a considerable variability (EPSCs, continuous line in fig.B). The mean amplitude varied considerably between synapses.

Above threshold, paired pulses 30ms apart, reduced the number of failures (facilitation), but the mean amplitude of the EPSCs was not affected, confirming that only one synapse was regularly activated, and suggesting that these synapses release either no or one quantum.



593.10

PRIMARY CULTURES OF NEURONS FROM RAT HIPPOCAMPUS RELEASE CORTICOTROPIN RELEASING FACTOR (CRF)
D.L.Birkle. Dept. Pharmacology & Toxicology, West Virginia University, Morgantown, WV 26506

CRF-containing cells have been localized in a number of brain areas outside the hypothalamus, including the hippocampus and frontal cortex. Changes in CRF levels in these sites occur in response to psychoactive drugs, stress and anxiety. However, the factors that regulate CRF release from extrahypothalamic sites have not been identified. Primary cultures of hippocampal neurons were prepared from fetal rat brain (E17), and grown in poly-lysine coated 35 mm wells. Cells were treated at day 3 with ARA-C, and used on culture day 12. Cells were incubated 1 hr in physiological buffer (basal period), then re-incubated 1 hr in fresh buffer containing 50 mM K^+. CRF immunoreactivity (CRF-IR) in the incubation media was measured by radioimmunoassay. Basal levels of CRF-IR were 154 ± 3 pg/10^8 cells (n = 12). Depolarization with K^+ caused a 100% increase in CRF-IR to 300 ± 4 pg/10^8 cells. Cultures of neurons from specific brain regions will provide a useful model system for studying the regulation of CRF release. Supported by Pharmaceutical Manufacturers Association and the National Science Foundation.

593.11

PHORBOL ESTERS INCREASE THE FREQUENCY OF SPONTANEOUS EXCITATORY POSTSYNAPTIC CURRENTS IN THE HIPPOCAMPUS BY A PRESYNAPTIC, CALCIUM-DEPENDENT MECHANISM. K.D. Parfitt and D.V. Madison, *Dept. of Molecular & Cellular Physiology, Stanford Univ. Sch. of Med., Stanford CA 94305-5426.*

Phorbol esters, which are activators of protein kinase C, induce a synaptic potentiation in the hippocampus that may share many common mechanisms with LTP. Using whole-cell voltage clamp techniques to study synaptic transmission in area CA1 of 500 uM-thick hippocampal slices, we have found (Parfitt & Madison, Soc. Neurosci. Abstr. 1990) that phorbol esters are capable of potentiating stimulus-evoked synaptic transmission even when induction of LTP is blocked by postsynaptic dialysis, suggesting a presynaptic mechanism for the phorbol potentiation. Using the same techniques, we have recorded spontaneous excitatory postsynaptic currents (EPSCs) in pyramidal neurons in the presence of tetrodotoxin and picrotoxin. Thus, these events are the product of non-action potential-dependent excitatory transmitter release. We have observed an approximate 8-fold increase in the frequency of spontaneous EPSCs during bath application of phorbol diacetate (PDAc, 10 uM), with no change in the amplitude distribution of these events. This phorbol ester-induced increase in frequency of spontaneous EPSCs is significantly attenuated by voltage-dependent calcium channel (VDCC) blockers, such as cadmium, dihydropyridines, or omega conotoxin. These VDCC antagonists did not affect the basal frequency or amplitude of spontaneous EPSCs. A glutamate antagonist known to act postsynaptically, 6-cyano-nitroquinoxaline-2,3-dione (CNQX, 100 nM), shifted the amplitude distribution to the left without significantly diminishing the frequency of spontaneous events. Taken together, these results strengthen our hypothesis that phorbol esters potentiate synaptic transmission by enhancing the opening of presynaptic voltage-dependent calcium channels, thereby augmenting transmitter release.

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

593.12

DIFFERENTIAL MODULATION OF INHIBITORY TRANSMISSION IN THE HIPPOCAMPUS BY CARBACHOL, ADENOSINE, BACLOFEN, SEROTONIN AND NOREPINEPHRINE. G.A. Cohen, V.A. Doze and D.V. Madison, *Department of Molecular & Cellular Physiology, Stanford School of Medicine, Stanford, CA 94305.*

Carbachol (CARB), baclofen (BACLO), serotonin (5-HT), norepinephrine (NE), and 2-Cl-adenosine (ADEN), all reduce the polysynaptic evoked inhibitory postsynaptic potential (ipsp) in area CA1 of the rat hippocampus.

Intracellular recording and whole cell patch clamping were used to monitor evoked ipsps and miniature ipscs, respectively, in rat CA1 pyramidal cells. We studied the effects of the listed neurotransmitter agonists on polysynaptic-evoked ipsps, monosynaptic evoked ipsps (KMeSO4 and QX-314 in the intracellular electrode; CNQX and APV in the extracellular ACSF), and spontaneous miniature ipscs (KCl and QX-314 in the patch electrode; CNQX, APV and TTX in the ACSF).

CARB (10uM), or BACLO (10uM) inhibited the monosynaptic ipsp more than 60%. At 1uM, BACLO reduced the monosynaptic evoked ipsp 30%. 5-HT, NE, or ADEN (all 10uM) had no effect on the monosynaptic evoked ipsp. CARB (10uM) reduced the frequency of spontaneous miniature ipscs more than 50%, while the overall shape of their amplitude distribution was not changed. Baclo (10uM) caused a 10% reduction in frequency, and had no effect at 1uM. 5-HT, NE, or ADEN (10uM) had no effect on spontaneous miniature ipscs.

These results show that different neurotransmitters that reduce inhibitory transmission may exert this effect by acting at different sites in the synaptic circuitry. 5-HT, NE, and ADEN appear to inhibit interneurons at a somatic location or by inhibiting excitatory synaptic activation of interneurons. At low concentrations BACLO does not directly decrease spontaneous GABA release but may still act at the interneuron terminal (e.g., by decreasing calcium currents). CARB directly reduces GABA release from interneuronal synaptic terminals.

D.V.M. is a Lucille P. Markey Scholar and this work was supported by a grant from the Lucille P. Markey Charitable Trust. G.A.C. is a Howard Hughes Medical Institute Predoctoral Fellow. V.A.D. is a NIMH Predoctoral Fellow.

SEROTONIN III

594.1

GLIAL BUT NOT NEURONAL ACTIVATION OF Na^+/K^+-ATPase BY SEROTONIN IN THE RAT BRAIN. J. Hernández, R. Mercado* and G. Chagoya*. CINVESTAV-IPN. A.P. 14-740, México, D.F. 07000 México.

In-vitro and in-vivo experiments from our and other laboratories have shown a regulatory role of serotonin (5-HT) on the activity of Na^+/K^+-ATPase in rat brain. We further observed that the Vmax of the enzyme kinetics was significantly elevated by 5-HT, only in cerebral cortex homogenates, but not in synaptosomes or microsomes, where glial membranes are not predominant.

The possibility of a selective activation of the glial enzyme by 5-HT was tested in enriched glial or neuronal fractions isolated from rat brain and also in cultured astrocytes or neurons.

The results clearly indicate that 5-HT selectively activates the glial enzyme with significant response - curves from 10^{-7} to 10^{-3} M. No activation of the neuronal enzyme was observed at physiological 5-HT concentrations. Potassium activation curves and (^{3}H) Ouabain binding in glial enriched fractions and cultured astrocytes, suggest that 5-HT may act by exposing more active sites of the enzyme. Our results suggest that regulation of glial Na^+/K^+-ATPase by a neurotransmitter may play an important role in maintaining the cationic balance for neuronal function.

594.2

THE 5-HT_2 ANTAGONIST RP 62203 SELECTIVELY BLOCKS SEROTONINERGIC INHIBITION IN THE RAT PREFRONTAL CORTEX. R. Godbout, J. Mantz*, J. Glowinski* and A.M. Thierry*. Collège de France, INSERM U. 114, 75231 Paris cedex 05, France.

The electrical stimulation of the median raphé nucleus (MRN) inhibits the spontaneous firing of medial prefrontal cortical (PFC) neurons, and this effect is likely mediated by 5-HT_2 receptors (Mantz et al., *Brain Res.*, **524**: 22-30, 1990). Biochemical and autoradiographic studies have shown that RP 62203, a novel naphtosultam derivative, is a potent and selective antagonist of 5-HT_2 receptors. To further characterize the selective influence of RP 62203 on 5-HT neurotransmission in the PFC, we analysed the effect of i.p. administration of RP 62203 on: 1) the 5-HT inhibitory responses induced by MRN stimulation and 2) the dopaminergic (DA) inhibitory responses elicited by stimulation of the ventral tegmental area (VTA). A single dose was administered and 1 PFC cell was tested in each of 10 ketamine-anesthetized rats. In 8 of the 10 PFC cells inhibited by both MRN (dur = 87.0±5ms; lat = 10.4±2ms) and VTA (dur = 114.0±18ms; lat = 6.0±2ms), RP 62203 blocked MRN-induced inhibitory responses in a dose-dependent and reversible fashion; in contrast, it failed to affect the inhibition elicited by VTA stimulation. These results show that RP 62203 selectively blocks 5-HT but not DA neurotransmission in the rat PFC and further support the involvement of 5-HT_2 receptors in this effect.

594.3

INHIBITION OF PLATELET SEROTONIN UPTAKE BY PHORBOL ESTER: DOES PROTEIN KINASE C ACTIVATION DOWN-REGULATE THE SEROTONIN TRANSPORTER? **G.M. Anderson*, W.C. Horne*, L.M. Hall*, B.A. Shaywitz, D.J. Cohen*. Child Study Center, Yale University School of Medicine, New Haven, CT 06510.**

Treatment of human platelets with 4-beta-12-0-tetradecanoylphorbol-13-acetate (beta-TPA), an activator of protein kinase C (PKC), resulted in a mean 34% decrease (N=16, p=.0001) in the V_{max} of serotonin (5-HT) uptake. A smaller effect on K_m was also observed (mean 15% decrease, N=16, p <.0005). 4-Alpha-TPA was without effect on either the V_{max} or K_m of platelet 5-HT uptake. Treatment with the calcium ionophore A23187 produced a smaller, non-significant, decrease in V_{max}, while dibutyrylcyclic AMP treatment had no effect. Phorbol ester (beta-TPA) did not cause a decrease in [^{3}H]-imipramine binding in intact platelets. Experiments in which sodium/proton antiporter or the chloride/bicarbonate exchangers were inhibited suggest that about one-third of the TPA effect may be due to change in the transmembrane sodium gradient.

The data indicate that PKC activation may down-regulate the activity of the 5-HT transporter in platelets. Most of the effect of PKC activation appears to be independent of a reduction in the number of 5-HT transporters at the platelet surface or alterations in ion gradients. The apparent regulation of 5-HT uptake by PKC raises the possibility that the transporter may be receptor-regulated as has been demonstrated for several other transport systems.

594.4

COLOCALIZATION OF PROGESTIN RECEPTORS AND SEROTONIN IN RAPHE NEURONS OF MACAQUE. C. L. Bethea, Div. of Reproductive Biology and Behavior, Oregon Regional Primate Res. Ctr., Beaverton, OR 97006.

Serotonin (5HT) neurons play a significant role in the regulation of several endocrine systems. Dysfunction of 5HT neurons has also been implicated in various affective disorders, including depression. We have shown that progesterone (P) increases prolactin secretion in estrogen-primed monkeys, and that lactotropes do not contain progestin receptors (PR). Since stimulation of 5HT increases prolactin secretion, we questioned whether P could act on 5HT neurons to increase prolactin secretion. Pontine and medullary tissue blocks were obtained at autopsy of a female Japanese macaque. 5HT neurons were immunohistochemically identified in dorsal and ventral pontine raphe groups, as well as in a more caudal medullary group using an antibody to 5HT-BSA conjugate (Incstar) and an avidin-biotin alkaline phosphatase bridging technique (Vector Labs, ABC-AP Kit). Development in Substrate Kit III yielded blue 5HT positive (+) neurons. PR expressing neurons were identified with B39, a monoclonal antibody against PR and an avidin-biotin peroxidase bridge with development in DAB (Vector Labs, ABC-HP Kit). The blue AP reaction product for 5HT localizes in the cytoplasm of neurons while the brown DAB reaction product for PR localizes in the nucleus of neurons. All 5HT+ neurons in both raphe groups exhibited PR+ nuclei when the whole cell was visible in the section. Many PR+ nuclei were also observed in 5HT negative neurons in adjacent areas. These data indicate that the midbrain 5HT neurons are progestin target cells and suggest that P can modulate the function of 5HT neurons. Supported by HD17269, HD18185, RR00163.

594.5

INCREASED EXTRACELLULAR CONCENTRATION OF SEROTONIN IN THE RAPHE NUCLEI AFTER UPTAKE INHIBITION MEASURED WITH IN VIVO MICRODIALYSIS. SECONDARY EFFECTS ON 5-HT_{1A} AUTORECEPTORS F. Artigas*, A. Adell, R. Cortés, P. Celada*. Department of Neurochemistry, C.S.I.C. E-08034 Barcelona, Spain

Extracellular serotonin (5-HT) occurs in the raphe nuclei in concentrations similar to other brain areas rich in nerve terminals. We have examined the effects of several drugs and ions affecting 5-HT neuronal activity on this extracellular pool in the raphe using the technique of intracerebral microdialysis. Dialysate 5-HT was significantly increased (400%) by K^+ ions but it was insensitive to removal of Ca^{2+} ions from the dialysis fluid. The treatment with reserpine (5 mg/kg, 24 h) did not change dialysate 5-HT. However, it increased after a single injection of p-chloroamphetamine in both saline- and reserpine-treated animals (550% and 350%, respectively). 8-OH-DPAT, a 5-HT_{1A} agonist, locally applied through the dialysis cannula placed in the raphe did not change extracellular 5-HT in this area but it reduced it in the frontal cortex of animals with two cannulae. These results and the low density of 5-HT nerve endings in the raphe suggest a somatodendritic release of the amine. The treatment with 5-HT uptake inhibitors increased several-fold extracellular 5-HT in the raphe. Given the high density of 5-HT_{1A} receptors in the dorsal raphe nucleus, we have examined the effects of repeated treatment with two 5-HT uptake inhibitors used as antidepressants (fluoxetine and clomipramine, 10 and 20 mg/kg daily, respectively) on its density using autoradiography with ^{3}H-8-OH-DPAT as ligand. Preliminary results showed that two weeks of treatment with either drug did not alter 5-HT_{1A} receptor density.

594.6

ACUTE AND LONG TERM EFFECTS OF CERICLAMINE, A SELECTIVE 5HT UPTAKE INHIBITOR, ON THE FIRING OF DORSAL RAPHE NEURONS. T. Jolas*, J. Adrien*, L. Lanfumey, S. Hai-Dahmane* and M. Hamon*, (SPON: European Neuroscience Association)
INSERM U288, CHU Pitié-Salpêtrière, 75013 Paris, France.

Under acute treatment, serotonin (5HT) uptake inhibitors reduce the discharge frequency of 5HT neurons in the Dorsal Raphe nucleus (DRN) by triggering a negative feedback through somatodentritic 5HT_{1A} autoreceptors. Under chronic treatment, these drugs have been claimed to be less effective, due to a desensitization of the latter receptors. Further assessment of such changes was made using Cericlamine (JO 1017, Jouveinal Labs), a new highly selective 5HT uptake inhibitor with potent antidepressant properties.

Acute treatment: Extracellular recordings were performed in male Sprague-Dawley rats under chloral hydrate anaesthesia. Cericlamine (0.25-10mg/kg i.v.) induced a dose-dependent decrease in the firing frequency of DRN 5HT neurons (IC_{50}=0.45mg/kg), which could be reversed by the 5HT_{1A} antagonist Tertatolol (2mg/kg i.v.). In contrast, the same doses of Cericlamine had no effect on the discharge frequency of noradrenergic neurons in the Locus Cæruleus.

Chronic treatment: 24 h after completion of a 14 day-treatment with Cericlamine (16mg/kg i.p. twice daily), the potency of this drug to depress the firing rate of DRN 5HT neurons recorded *in vivo* was significantly less than in controls (IC_{50}=1.53mg/kg). Moreover, the inhibitory effect of the 5HT_{1A} agonist 8-OH-DPAT (10-50nM) on the firing rate of DRN 5HT neurons recorded *in vitro* (in brain stem slices) was significantly smaller in treated rats (IC_{50}=10nM) than in controls (IC_{50}=18nM).

These data support that the desensitization of somatodentritic 5HT_{1A} autoreceptors might be a common effect of 5HT uptake inhibitors when administred chronically for alleviating depression.

594.7

INFLUENCE OF 5,7-DHT HYPOTHALMIC LESION ON ANTEROGRADE TRANSPORT OF 5HT MEASURED BY IN SITU SYNTHESIZED α-METHYL SEROTONIN M. Diksic and A. Takada*. Department of Neurology and Neurosurgery, Montreal Neurological Institute, McGill University, Montreal, Quebec, H3A 2B4, CANADA

α-methyl serotonin (α-M5HT), a chemical and functional analogue of serotonin synthesized in situ, was used to measure anterograde transport of 5HT along the medial forebrain bundle (MFB) of 5,7-DHT stereotaxicly lesioned rats.

Lesions were produced in the brains of 250g Sprague-Dawley rats by injecting 3μg of 5,7-DHT (free base) in 250 nL of saline at the following coordinates: -3.14 (post); 1.0 (lat); 8.1 (vent) (Paxonos and Watson). In a control rat, 250 nL of saline alone was injected. Five days later, 500-800 μCi of [^{3}H] labelled α-MTrp was injected. In each animal, it was possible to compare the rate of transport in the lesioned and the contralateral MFB.

Destruction of the terminals by 5,7-DHT increased the rate of anterograde transport of 5HT by at least 30%. Destruction of the terminals also resulted in a tenfold increase in the amount of 5HT transported anterogradely. The amount transported was estimated from the area under the curve, obtained by plotting the radioactivity ratio in the MFB and that found in the hypothalamus on the non-lesioned side, as a function of the distance from the dorsal/medial raphe plane.

Our data support the notion that there is a presynaptic receptor/autoreceptor that controls the rate and the amount of anterogradely transported 5-HT. We also conclude that anterogradely transported 5HT has some physiological relevance, as there would otherwise be no need for presynaptic control of the rate and amount of 5HT transported from the cell bodies towards terminals.

The work described here was supported in part by a grant from the MRC (MA-10232).

594.8

HYPOINNERVATION OF SEROTONIN NEURONS IN THE FOREBRIAN OF ALCOHOL-PREFFERING (P) RATS. Li, T.-K., Lumeng, L., Pu, C.F., Farber, S., and Zhou, F.C.
Depts. of Anatomy and Medicine, Indiana University School of Medicine and VAMC Indianapolis, IN 46202 USA

We have previously found that the levels of serotonin (5-HT), and 5-hydroxyindole-acetic acid (5-HIAA) content are decreased in the hippocampus, accumbens, striatum, cortex, and thalamus of selectively bred alcohol-preferring P rats, compared with the alcohol-nonpreferring NP rats. This study shows that the number of 5-HT fibers and 5-HT neurons are low in P rats compared with NP rats. 5-HT immunofibers were lower in cingulate cortex, and medial nucleus accumbens, layer III-V of anterior frontal cortex, CA4 and fascicularis cinarium of dorsal hippocampus and dentate gyrus of ventral hippocampus, hypothalamus and striatum (unpaired Student's t-tests, n>4) of the P rats. 5-HT neurons were counted in serial sections through the entire ascending raphe nuclei. Overcounting in adjacent sections was corrected with use of Abercrombie's formula. There were significantly fewer 5-HT neurons in the median raphe in P (1542±76) than in NP (1975±81) rats (n=5 each), while no significant difference was observed in the B9 group (P=1359±132, NP=1489±151). This result indicates that the lower content of 5-HT and the decreased 5-HT fiber density seen in P rats may be partially due to decrease number of 5-HT neruons in the median raphe. (Support: PO1 AA 08553, P50AA07611)

594.9

Antipeptide antibodies against rat brain tryptophan hydroxylase Azmitia, E.C.[1,2], Yu, I.J.[2], Akbari, H.M.[1], Chen,Y.[1] and Marshak, D.R.[2]
[1]Dept. of Biology, New York University, Washington Square East, N.Y., N.Y. 10003 and [2]Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. 11724

N- and C-terminal segments from rat tryptophan hydroxylase were selected with no homology to tyrosine or phenylalanine hydroxylase. The peptides were coupled to KLH and injected into adult rabbits. Radio-immuno assay of the immune serum up to 1/8000 showed higher binding (5-18X) than the pre-immune serum. Immunoblotting against whole cell extracts (30-75 ng) from pineal and midbrain raphe tissue from adult rats were run on 12.5% SDS-PAGE and transferred to nitrocellulose. Both antibodies at 1/100-1/2000 revealed specific bands. A single band (approximate M.WT. 48,700) was seen for the rat pineal and midbrain raphe using the C-terminal peptide derived antibody (WOH-412). Staining with the N-terminal region antibody (WOH-66) showed 3 specific bands (approximate MW 52,500, 48,400, and 47,800) for the pineal and single band (MW 47,800) for the midbrain raphe. The specificity was further characterized by using immunohistochemical staining of rat brain sections. The staining with WOH-66 showed specific staining in all the classical raphe nuclei at dilutions as low as 1:7000 using glutaraldehyde, paraformaldehyde or picric acid as fixatives. In addition labelled cells were seen in the pineal gland and in the area postrema. The perikarya, dendrites and axons, but not the nuclei, were stained by WOH-66. The rare ependymal cell in the fourth ventricle was positively stained. No staining was seen in the locus coeruleus, substantia nigra or in the hypothalamus. No staining was seen with antibody WOH-412. The multiple bands seen with the pineal extract after immuno-blot with WOH-66 but not WOH-412 indicate post-translational modification or alternative splicing at the C-terminus. Support: NSF grant 88-12892 and Natl Down Syndrome Soc.

594.10

EVALUATION OF POTENTIAL NEUROTOXIC EFFECTS OF AMPHETAMINE-RELATED ANORECTIC AGENTS ON BRAIN SEROTONIN AND DOPAMINE IN THE RAT. M.S. Kleven, W.L. Woolverton, and L.S. Seiden. Department of Pharmacological and Physiological Sciences, The University of Chicago, Chicago, IL 60637.

Amphetamine, N-methylamphetamine, and several related analogs have neurotoxic effects on brain dopamine (DA) and/or serotonin (5-HT) neurons. In this study, possible long-lasting neurochemical effects of several structurally dissimilar anorectic agents currently available for clinical use were examined. Saline or phentermine (25, 50 or 100 mg/kg), phenmetrazine (31.3, 62.5, or 125 mg/kg), phendimetrazine (31.3, 62.5, or 125 mg/kg), or benzphetamine (75, 150, or 300 mg/kg) were administered s.c. to separate groups of rats (n=6-9/group) twice daily for four consecutive days and levels of DA and 5-HT were assayed after a two week survival period. Doses used were approximately 5, 10, and 20 time the anorectic ED50 for suppression of milk intake obtained in separate groups of rats presented sweetened condensed milk. The four day regimen of phentermine and benzphetamine produced significant, dose-related decreases in DA levels in striatum (to 73.6±8.3 and 66.5±3.1% of control, phentermine and benzphetamine, respectively). Depletions of 5-HT were also observed in hippocampus (to 50.5±4.9% and 71.8±6.1%) and cortex (to 70.7±13.0 and 76.3±8.9) following repeated phentermine and benzphetamine injection. In contrast, comparable monoamine depletions were in general, not observed following repeated administration of phenmetrazine or phendimetrazine, nor did any of the drugs cause substantial depletions of 5-HT in striatum or hypothalamus. The results indicate that, like amphetamine, the α,α-dimethyl homolog phentermine and the N-dialkyl homolog benzphetamine may be neurotoxic to DA and 5-HT neurons. In contrast, the phenylmorpholine derivatives, phenmetrazine and phendimetrazine probably lack such effects. (Supported by NIDA Grant DA-00085 and RSA MH-10562 [L.S.S.])

594.11

PROLONGED ADMINISTRATION OF D-FENFLURAMINE IS NOT ASSOCIATED WITH ANY LONG LASTING EFFECT ON NEOCORTICAL 5HT INNERVATION. M. Kalia Dept. Pharmcol, Jefferson Med Col., Thomas Jefferson Univ., Philadelphia. PA 19107.

Previous studies from this laboratory revealed that in the rat, orally administered D-Fenfluramine for short periods (up to 4 days) produced short-lived and reversible immunocytochemical changes in neocortical 5HT innervation. (*Kalia, M., Brain Res. 1991)* In this report additional questions have been addressed: How long does the depletion of 5HT from the neocortex last following cessation of treatment? Is this effect reversible?. We selected two oral doses, (8mg/kg/day and 16 mg/kg/day) which were administered for 21 & 30 days. The animals were examined at 3 different post-treatment time points: 18 hrs, 30 days and 60 days. Immunocytochemical methods, identical with those described previously were used to evaluate the brains postmortem. A predictable reduction in immunocytochemical staining of neocortical 5HT nerve networks was seen 18 hrs following cessation of treatment. The 5HT immunocytochemical staining 30 and 60 days post-treatment was indistinguishable from pair-fed controls. When the results of 18 hour post-treatment were compared with existing data from 4 day treated animals, we found qualitative differences. Chronic treatment with D Fenfluramine produced fewer "thick" immunoreactive 5HT fibers and the fine fibers were very distinct and finely beaded. These results confirm our hypothesis that the predictable reduction in neocortical 5HT immunoreactivity is a function of transient depletion of 5HT and is not associated with any long-term change in the 5HT innervation of this region.

CIRCUITRY AND PATTERN GENERATION II

595.1

MUTATIONS AFFECTING PERIODIC BEHAVIOR IN *C. ELEGANS*. Dennis WC. Liu and James Thomas*, Department of Genetics University of Washington, Seattle, WA 98195.

Defecation is a periodic behavior in *C. elegans*, occurring about every 45 seconds in well-fed, adult wild-type worms. The cycle periodicity (CP) may be under the control of a neuronal pattern generator. Wild-type worms will stop defecating in the absence of food and CP is correlated with the rate at which the pharynx pumps in food; pumping defective mutants defecate less often. Extensive screens for constipated (Con) mutants yielded only one mutation in one gene (*dec-1*) which caused lengthening of the defecation CP (Thomas, Genetics 124:855-872, 1990). We reasoned that Con screens might fail to reveal many mutants with long cycles and should miss mutants with shorter than normal CP.

To isolate short and long-cycle mutants we undertook a direct screen for animals with altered CP, after EMS mutagenesis. CP was timed in individual animals, and a mutant candidate was picked: 1) if it repeated the behavior twice or more in one minute, 2) if it repeated the behavior less often than once per minute.

The screen has led to the identification of three classes of mutations which affect CP by causing; 1) long cycles, 2)short cycles , and 3) variable CP. Most of the mutations we have isolated are of the first class. An example is the *sa73* mutation, which causes an average period of 100 seconds. We have isolated one mutation in the second class, *sa92*, which causes a CP of about 30 seconds. Finally we have isolated a mutation *(sa96)*, which causes animals to have a variable CP ranging from 10-150 seconds. Thus far, none of the mutations map to the same loci and we are carrying out epistasis analysis.

We are currently examing the effects of these mutations on other rhythmic behaviors such as egg-laying and locomotion. In order to identify the cells which are responsible for controlling periodic behavior, we are using a microsope-focused laser to kill neurons in wild-type worms.

595.2

STIMULATION OF THE SECOND THORACIC ROOTS AFFECTS THE SWIMMERET, THE WALKING AND THE ABDOMINAL POSITIONING SYSTEMS OF THE CRAYFISH. A. Chrachri and D.M. Neil. University of Glasgow, Department of Zoology, Glasgow G12 8QQ Scotland, U.K.

In crayfish, the rhythmical activity of the swimmeret system is strongly modified by stimulating the second thoracic roots (STR). This stimulation increases the frequency of the swimmeret rhythm and the frequency of spikes within bursts, it also increases the total amplitude of membrane potential oscillations of certain swimmeret neurons. Sometimes, an increase in the intensity of stimulation inhibits the swimmeret rhythm.

Recently, we have found that stimulation of STR affects not only the swimmeret system but also the abdominal positioning and the walking systems. The STR effects are most of the time comparable to that of spontaneous activity in these systems often obtained during the same experiment. In the present study we have also been able to record intracellularly from a number of abdominal interneurons. Most of these interneurons respond to the stimulation of the STR with a long-lasting depolarization, while others with only a short-lasting membrane depolarization and/or EPSPs. These properties may explain the variety of the STR effects.

In conclusion, axons in the STR seems to have access to a wide range of abdominal interneurons, some with specific effects (i.e. activation or inhibition of the swimmeret rhythm), while others seems to have a multiple motor output properties. At least some of these interneurons seems to induce effects that are established slowly and are of long duration suggesting that the stimulation of the STR might involve command neurons. Finally, intracellular recording from the same interneuron in two different ganglia suggests that they are descending interneurons.

This work has been supported by an SERC grant GR/E/86918.

595.3

MODULATION OF IDENTIFIED STOMATOGASTRIC NEURONS IN PRIMARY CELL CULTURE. G.G. Turrigiano and E. Marder, Dept. of Biology, Brandeis Univ., Waltham, MA 02254

We have begun to use current and voltage clamp techniques to study the properties of identified stomatogastric neurons in primary cell culture. Gastric mill and pyloric neurons from the stomatogastric ganglion of the spiny lobster *Panulirus interruptus* are identified electrophysiologically, ganglia are enzymatically treated, neurons are dissociated by aspiration into micropipettes, and plated onto uncoated Nunclon culture dishes in defined medium. Over the course of several days these neurons regenerate neurites, and regain the ability to produce action potentials, to oscillate in the presence of TEA, and to respond to neuromodulators. The DG neuron, a likely target of octopamine *in situ*, is modulated by octopamine in culture: octopamine acts in part by reducing the magnitude of an A-type current in this neuron. The PD neuron in culture is excited by the muscarinic agonist pilocarpine, inhibited by dopamine, and is not affected by serotonin; these responses are similar to those exhibited by PD *in situ*. These preliminary results suggest that pyloric and gastric mill neurons are expressing the same complement of receptors in culture as *in situ*, and are retaining some of their physiological properties. Supported by NS-08971 to G.G.T. and BNS-9009251 to E.M.

595.4

MECHANISMS SUBSERVING CONSTRUCTION OF A NOVEL MOTOR-PATTERN GENERATING NETWORK BY A MULTIACTION INTERNEURON. A. J. Simmers*, P. Meyrand* and M. Moulins. Lab. de Neurobiologie et Physiologie Comparées, Univ. de Bordeaux I, CNRS, Arcachon, 33120 FRANCE.

We have recently reported that in the stomatogastric nervous system (STNS) of lobsters (*Homarus gammarus*), the rhythmic discharge of an identified modulatory neuron (PS) is able to construct *de novo* the central pattern generator (CPG) for swallowing-like behavior from neurons belonging to other functional CPGs (Nature, 1991, in press). Thus, when PS is active, neurons otherwise operating independently as integral elements of different neuronal networks are reconfigured into an completely new functional circuit.

We report here on the cellular mechanisms underlying this network reconfiguration. A study of direct effects of PS on individual STNS neurons has revealed three major types of postsynaptic action. First, PS acts directly on many neurons via conventional short-term synapses that evoke discrete excitatory or inhibitory postsynaptic potentials (PSPs). Second, PS can exert long-term influences that far outlast the discharge of the interneuron itself. These modulatory influences involve the suppression of intrinsic bursting properties in certain STNS neurons, while in others, a sustained membrane depolarization is produced. Third, PS may act on the same target neuron via both short and long-term influences, giving rise to transient PSPs in combination with the modulation of bursting properties.

Our results show how a neuronal network can be dynamically assembled from neurons of disparate origin by the multiple actions of a single interneuron; conventional synapses serve to coordinate different individuals to the cell own firing pattern, while modulatory influences appear to dictate both functional membership in the new circuit and the duration of its existence.

595.5

LOCAL INTERACTIONS OF DESCENDING INPUTS WITH RHYTHMICALLY ACTIVE NEURAL NETWORKS. M.P. Nusbaum. Neurobiology Research Center and Dept. of Physiol. & Biophysics; Univ. of Alabama, Birmingham; Birmingham, AL 35294.

The pyloric and gastric mill networks in the crustacean stomatogastric ganglion (STG) are influenced by descending modulatory inputs from anterior ganglia. These inputs extend axons for long distances to arborize in the STG neuropil, but little is known about events occurring locally in these STG arbors. I am therefore recording intra-axonally, at the entrance to the STG, from descending Stomatogastric Nerve Axons (SNAXs) in the crab, *Cancer borealis*. These recordings are electrotonically close to the STG neuropil, enabling recordings of discrete PSPs to be made from a SNAX.

Individual SNAXs are identifiable in successive preparations, based on their interactions with STG targets. Each SNAX that influenced the STG networks was also found to receive local synaptic feed-back from these networks. This feed-back must occur locally, in the STG neuropil, since it persisted in the isolated STG. All recorded feed-back was via IPSPs and/or electrical coupling.

SNAX 1, for example, can initiate or enhance the pyloric and gastric mill rhythms. This SNAX received short latency electrotonic EPSPs and chemically-mediated IPSPs from gastric mill neuron LG. The pyloric network also affected SNAX 1. During pyloric or gastric mill rhythms, this feed-back imposed a rhythmic inhibition on SNAX 1. This rhythmic inhibition was sufficiently strong to suppress impulses in a tonically stimulated SNAX 1, thereby transforming its activity from tonic to rhythmic. These results suggest that endogenous, spontaneous descending activity may also be locally reconfigured.

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595.6

COMMAND OF FEEDING IN *APLYSIA*: IMPLICATION OF A NETWORK OF IDENTIFIED INTERGANGLIONIC INTERNEURONS IN THE BUCCAL AND CEREBRAL GANGLIA. S.C. Rosen, K.R. Weiss and I. Kupfermann. Cntr. for Neurobiol. and Behav., NYS Psychiatric Inst., New York, NY 10032; Dept. of Physiol. and Biophys., Mount Sinai Sch. of Med., New York, NY 10023.

Identified cerebral to buccal interneurons (CBIs 1-4) can initiate and regulate "feeding" motor programs. CBI-2, in particular, effectively drives a motor program that produces rhythmic, biting-like movements of the buccal mass. CBI-2 is a phasic, command-like cell that fires in periodic bursts when stimulated with constant, intracellular current. Its phasing is due to feedback inhibition and excitation generated by a buccal central pattern generator (CPG). CBI-2 acts both "permissively" and "instructively" since it: (a) produces a rhythmic, motor program even when phasic feedback is blocked and the cell fires tonically; and (b) drives salivary duct and lip (C11) motor neurons independent of CPG activity. In order to further elucidate CBI-2's functions, we examined two interganglionic buccal neurons that are antiphasically active in the program driven by CBI-2 and are monosynaptically connected to it. B17 is a postsynaptic cell that receives powerful, slow excitatory input from CBI-2. B17, in turn, produces direct PSPs in multiaction interneurons (B4,B5) and in buccal and cerebral motor neurons (B15,B16,C4,C5,C11,C12). B17 itself drives a motor program. 5(6)-Carboxyfluorescein dye fills indicate that B17 has bilateral axons in the buccal and cerebral ganglia and in buccal nerves 3. B19 is a presynaptic cell that evokes a complex, slow inhibitory and excitatory PSP in CBI-2. The PSP contributes to CBI-2 phasing across cycles of the program. B19 stimulation phase advances the CBI-2 driven program. B19 also directly excites cerebral and buccal motor neurons (many of which are reciprocally driven by B17). B19 has a single, ipsilateral axon that projects to the cerebral ganglion. The data indicate that command-like functions for feeding involve interganglionic CPG neurons that exhibit feedforward and feedback connections. Many of the dynamic properties of the neural circuitry can be simulated by a simple computer model.

595.7

TWO POPULATIONS OF RHYTHMICALLY FIRING TRIGEMINAL COMMISSURAL INTERNEURONS STUDIED DURING FICTIVE MASTICATION IN THE ANAESTHETIZED RABBIT. J.P. LUND & R. DONGA. Cent. rech. sci. neurol. Univ. de Montréal, Montréal, CANADA, H3C 3J7.

In rabbits anaesthetized with urethane, paralyzed with gallamine triethiodide and artificially ventilated, we have previously shown that trigeminal (V) commissural last-order interneurons in the left V sensory nuclei and adjacent cell groups can be antidromically identified by stimulating the right V motor nucleus (NVmot). When fictive mastication is induced by stimulation of the sensorimotor cortex (SMC) in this preparation, some of these interneurons fire rhythmically (Donga & Lund, J. Physiol. 423: 74P). We have now found that rhythmically firing interneurons (n=20) can be sub-divided into two distinct categories based on whether they received short-latency excitatory inputs (SLEIs) from the SMC (n=11) or not (n=9), using post stimulus time histogram analysis. Approximately equal numbers in both groups fired during the two main phases (jaw opening and jaw closing). 7 neurons in the SLEI category were only rhythmically active during stimulation of the contralateral SMC, 2 had ipsilateral SMC inputs only while the other 2 fired to stimulation of either SMC. The mean of the modal latencies to stimulation of the contralateral SMC was 8.3 ms (n=9) compared to 9.6 ms (n=4) for the ipsilateral SMC. It was concluded that rhythmical firing of most SLEI interneurons resulted from phasic inhibition of tonic SLEIs by other parts of the masticatory central pattern generator (CPG). It also appears that the rhythmical firing of neurons that did not receive SLEIs was due to phasic excitation from other CPG neurons. In some cases, phasic inhibition also occurred. Supported by the Canadian MRC.

595.8

A NEURALLY-INSPIRED MODEL FOR TEMPORAL AND SPATIAL PATTERN LEARNING. James L. Adams, Division of Neurobiology, Barrow Neurological Institute, 350 W. Thomas Road, Phoenix, AZ 85013.

In this model, pairs of adjacent excitatory synapses, receiving randomly-assigned input, function as local processing units. A hypothetical neuron is divided into multiple spatial/temporal levels with multiple synaptic pairs per level. During learning, a trigger event initiates a process which passes sequentially from the cell body to the tips of the dendrites. As the process passes each level, it records the ratios of the current input signal strengths at each synaptic pair on that level. After recording, whenever a given pair of synapses is presented with stimuli in the same ratio as during recording, that pair produces its maximum postsynaptic effect. Other ratios yield lesser effects. Thresholding is applied at each neuronal level to reduce noise.

Initial simulations tested the model with three neurons of 122 levels and 12 synaptic pairs per level recording three simple musical pieces of up to 73 notes in length. After recording and while each piece was input numerically note by note, the neuron which had recorded it responded continuously and vigorously and the other two neurons remained nearly inactive. Each neuron also responded to the playing of a fragment of its recorded piece or to the playing of the piece at a somewhat different rate.

The model is non-Hebbian in that the reinforcement of individual synapses does not depend upon, nor reflect, the amount of overall postsynaptic depolarization of the cell. It also does not require that the input be highly active. Once recording is triggered, the process simply records the activation ratios of each synaptic pair. Several physiological, biochemical, and molecular biological mechanisms are envisioned which could perform the operations of this model.

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595.9

A NEURAL NETWORK MODEL OF CHEMOTAXIS IN SIMPLE NERVOUS SYSTEMS. S. R. Lockery and T. J. Sejnowski. Salk Institute, La Jolla, CA 92037.

Some properties of neural circuits can be predicted by training a model network to reproduce the activity of input and output neurons in the biological network. However, in most systems, more is known about the animal's behavior than about the activity of individual neurons. Using chemotaxis as a model behavior, we trained neural networks in which the desired output was the movement of the animal. The gradient was a one-dimensional gaussian-shaped distribution of an attractant. The network had two sensory units and two motor units. The velocity and position of the animal were represented by two additional units. Activation of the sensory units was determined by the position of the animal. Sensory units were separated in space, allowing the animal to compute the local gradient. Sensory units excited motor units which determined the animal's velocity up or down the gradient. The position unit integrated the activity of the velocity unit. This network could be trained successfully using recurrent backpropagation to find and remain at the center of the gradient from arbitrary initial positions. The model can be made more realistic by including interneurons and known connections. Additional constraints on the neural circuit should allow the model to predict the contribution of real neurons to observed behaviors.

595.10

SEROTONIN ENHANCES POSTINHIBITORY REBOUND IN OSCILLATOR INTERNEURONS OF THE LEECH SWIM CIRCUIT. P.S. Mangan and W. Otto Friesen. Dept. of Biology, Univ. of Virginia, Charlottesville, VA 22901

Application of micromolar concentrations of serotonin (5-HT) elicits recurrent swim episodes in isolated leech ventral nerve cords (Willard, J. Neurosci. 1:936-944, 1981). Investigation has shown that endogenous 5-HT induces or enhances postinhibitory rebound (PIR) in swim motor neurons (Friesen et al., in preparation). Motor neuron PIR occurs in two forms. In inhibitor cells, PIR is induced by 5-HT, is prolonged, and is of low amplitude (< 5mV). In excitor cells, 5-HT enhances existing PIR which is higher amplitude (up to 10 mV) and occurs with a faster time course than inhibitor cell PIR. We report here that serotonin enhancement of PIR occurs also in oscillator interneurons 28, 208, and 115 of the leech swim circuit.

We examined interneurons exposed either to normal saline or saline with 50 μM 5-HT. Cells were injected with constant, hyperpolarizing current (1 s, -2.0 to -0.5 nA). PIR was monitored following release from inhibition. As in motor neurons, PIR was of two types. Cell 115 PIR occurred as a brief peak, analogous to excitor motor neurons, while PIR in cells 28 and 208 was more prolonged, as in inhibitor motor neurons. In either case, PIR amplitude was less than 3mV. Serotonin enhancement of time dependent, non-linear cellular properties, such as PIR, in oscillator cells could facilitate oscillatory activity in the reciprocally inhibitory interactions prevalent in the leech swim oscillator network. We conclude that, because changes in PIR of oscillator interneurons are small relative to PIR in swim motor neurons, the interactions and activity of motor neurons, which have reciprocally inhibitory connections with oscillator network cells, may be critical to the serotonin-enhanced expression of swimming in the isolated leech nerve cord. Supported by grants NS08781 (P.S.M.) and NS21778 (W.O.F.).

596.1

SYNCHRONIZATION OF INTERNEURONS IN THE HIPPOCAMPUS. H.B. Michelson and R.K.S. Wong. Dept. of Pharmacology, State Univ. of New York, Health Science Center, Brooklyn, N.Y. 11203.

Synchronized inhibitory post-synaptic potentials (IPSPs) can be recorded in guinea pig hippocampal pyramidal and granule cells in the absence of glutamate-mediated excitation. These synchronized IPSPs are recorded in the slice in 4-aminopyridine (4-AP, 75 μM), CNQX (10 μM) and CPP (10 μM). Direct recordings from inhibitory interneurons confirmed that a population of these cells were firing simultaneously to generate the synchronized IPSPs in principal cells.

Picrotoxin (50 μM) initially suppressed the rhythmic synchronized IPSP in principal cells. A second, different hyperpolarizing event subsequently emerged. This synchronized event had a slower time-to peak (303.3 ± 15.4 ms), was blocked by saclofen, and occurred at a lower frequency than the picrotoxin-sensitive event.

Picrotoxin (50 μM) blocked the burst events in a subpopulation of interneurons, indicating that excitation between this group of interneurons was mediated by GABA. Some interneurons continued to burst in the presence of picrotoxin. These bursts occurred simultaneously with the slower synchronized IPSP in principal cells. We conclude that GABAergic interneurons can become synchronized by at least two non-glutamate dependent processes: excitation mediated by the $GABA_A$ receptor, and a process independent of amino acid transmission. (Supported by N.I.H. and the American Epilepsy Society)

596.2

EPILEPTOGENESIS FOLLOWING CORTICAL INJURY. D. A. Prince and G.-F.Tseng, Department of Neurology and Neurological Sciences, Stanford University School of Medicine, Stanford, CA 94305.

We developed an in vitro model of chronic epileptogenesis by taking advantage of the hyperexcitability and epileptogenesis known to develop in chronically isolated in vivo. Partially isolated neocortical islands were prepared in P5-P42 rats. 17-122 days later, slices were prepared through the previous lesion. Orthodromic stimuli in lesioned but not control cortex evoked long and variable latency field potentials that had the characteristics of epileptiform events. Discharges were maximal in layer 2-3 and propagated into deeper layers and across the slice. Bath application of APV blocked the long latency discharges. Intracellular recordings showed that epileptiform field potentials were accompanied by polyphasic EPSPs without prominent inhibitory events. In both postnatally and adult-lesioned animals biocytin-filled layer V pyramidal neurons showed remarkable elaboration of recurrent axonal collaterals extending to supragranular layers. Some neurons showed the typical picture of recurrent axonal sprouting described in immature isolated cortex by Cajal and Purpura and Housepian (Exp. Neurol. 4:377, 1961). These preliminary results suggest that chronically undercut cortex studied in vitro is an excellent model for examination of development of chronic epileptogenesis after brain injury. Alterations in pyramidal cell membrane properties, development of new excitatory connectivity, and depression in inhibitory synaptic electrogenesis may play a role in the abnormal discharges generated by this tissue. Supported by NIH grants NS06477 and NS12151 from the NINDS, and a Pimley Postdoctoral Fellowship.

596.3

FORNIX LESIONS ALTER EPILEPTIFORM ACTIVITY IN HIPPOCAMPAL SLICES. *P.A. Rutecki.* Sec. of Neurophysiol., Dept. of Neuro., Baylor Col. of Med., Houston, TX 77030.

Chronic (10-90 day), unilateral fornix lesions in rats result in hippocampal slices that have a propensity for epileptiform activity in 5 mM $[K^+]_o$ saline. To assess effects of fornix lesions on local CA3 synaptic circuitry, epileptiform discharges were produced by 50 μM picrotoxin (picro) or by 8.5 mM $[K^+]_o$ saline and recorded using extracellular techniques. The duration and frequency of occurrence of epileptiform discharges in slices from control animals and from hippocampi ipsi- and contralateral to the lesion were compared. In picro, no difference in the rate of discharges in the three groups was observed, but the duration of the discharges was significantly ($P \leq 0.05$) longer in slices ipsi- and contralateral to the lesion compared to control slices (120.7±8.4, 116.9±7.6, and 93.9±5.0 ms respectively). In 8.5 mM $[K^+]_o$ saline, discharges from slices ipsilateral to the lesion were longer than those from contralateral slices (80.0±3.7 vs. 63.6±3.3 ms) and occurred at a faster rate than those from control animals (0.68±.06 vs. 0.45±.09 Hz). The effects of the NMDA antagonist APV (50 μM) on convulsant induced bursting was studied. On average, APV slowed the rate of picro induced bursting in slices from lesioned animals by greater than 50%, but did not affect control slice discharge rate. APV tended to shorten burst duration, but this did not reach statistical significance. In 8.5 mM $[K^+]_o$ saline, APV slowed significantly the epileptiform discharges in slices ipsilateral to the lesion, but did not significantly change the rate in slices taken from control or contralateral hippocampi. The duration of high $[K^+]_o$ bursts was shortened by APV in all three groups. These results suggest that deafferentation of the hippocampus by fornix lesioning altered the response of the CA3 region to convulsants and enhanced the NMDA-mediated component of epileptiform activity. (Supported by NIH grant NS-28580 and the Klingenstein Fund.)

596.4

AMYGDALA KINDLING ALTERS PROTEIN KINASE C (PKC) ACTIVITY AND THE LEVEL OF A 17 KD PKC SUBSTRATE IN DENTATE GYRUS S.J.Chen,E.Klann,M.A.Desai,J.D.Sweatt, and P.J.Conn
Div. of Neurosci., Baylor Coll. of Med., Houston, TX 77030 and Dept. of Pharm., Emory U., Atlanta, GA 30322.

Kindling is a model of epilepsy and neuronal plasticity in which repeated electrical stimulation of certain CNS structures gradually leads to development of generalized clonic convulsions. Evidence suggests that PKC plays an important role in various forms of neuronal plasticity, including LTP in the hippocampus and activity-dependent cortical development. To investigate the possible role of PKC in kindling-induced epileptogenesis, we determined the effect of amygdala kindling on PKC activity in various regions of rat brain. We first assayed for changes in kinase activity by assaying post-hoc phosphorylation of endogenous proteins. We observed that kindling markedly elevated basal ($-Ca^{2+}$) and Ca^{2+}-stimulated phosphorylation of an endogenous protein in dentate gyrus which we have termed P17. Increased phosphorylation of P17 was observed 2 hours after the last stimulus-induced seizure and did not occur in limbic regions other than the dentate gyrus. P17 phosphorylation was decreased in dentate gyrus 24 hours after seizure. Assay of the amount of P17 protein revealed a transient increase in P17 protein 2 hours after the last seizure. Addition of a competitive peptide inhibitor (PKC_{19-36}) for C kinase greatly inhibited the phosphorylation of P17 while a peptide inhibitor ($CaMKII_{273-302}$) for CaM kinase II has no effect on P17 phosphorylation, suggesting that P17 is a fairly selective substrate for PKC. PKC activity, assayed in vitro using an exogenous peptide substrate, also showed a transient increase in both basal and Ca^{2+}-stimulated activity in kindled dentate gyrus, 2 hours after kindling stimulation. These results document a kindling-associated alteration in PKC activity. Interestingly, these observations also suggest that there is a coordinate up-regulation of PKC and the level of one of its substrates in the kindled dentate gyrus.

596.5

POSTICTAL CHANGES IN GABA METABOLISM IN SUBSTANTIA NIGRA C.F. Baxter, C.G. Wasterlain, C.C. Oh and R.A. Baldwin Epilepsy and Neurochem. Labs, VAMC, Sepulveda, CA 91343 and Dept. of Neurology, Psychiatry and BRI, UCLA Sch. Med., Los Angeles, CA.

Seizures are invariably followed by profound behavioral depression and by enhanced inhibitory post-synaptic potentials, but the responsible mechanisms are unknown. We investigated GABA metabolism in the substantia nigra (SN) of rats kindled by stimulation of medial septum. SN GABA concentration was 93±11 μmole/g prot in untreated controls, 87±11 μmole/g prot. in naive rats subjected to septal stimulation, 107±7 μmole/g prot in kindled unstimulated rats, and 138±11 μmole/g prot in kindled rats stimulated to a stage 5 seizure 60 minutes before SN tissue was sampled (Gp 4). The corresponding rate of GABA synthesis in SN of these 4 groups of rats was respectively 57±8, 69±14, 68±7, and 116±20 μmole/g prot/hr. Only the GABA metabolism in SN of Gp 4 was significantly different from controls. The increase of GABA concentration and synthesis in SN was observed post-ictally; 5 to 60 min after all seizure activity had ceased, the rate of GABA synthesis in SN was 160 μmole/g prot/hr compared to 68 μmole/g prot/hr pre-ictally. These changes may provide a potential explanation for the post-ictal depression and enhancement of inhibitory processes. Supported in part by funds from the V.A. Research Service and by grant NS13515 from NINDS.

596.6

LONG-LASTING DECREASES OF TYPE II CALMODULIN KINASE EXPRESSION IN KINDLED RAT BRAINS. J.M. Bronstein, P. Micevych, P. Popper, G. Huez, D.B. Farber, and C.G. Wasterlain. Depts of Neurology, Anatomy, and Ophthalmology, UCLA School of Medicine and Dept of Biological Chemistry, ULB, Brussels, Belgium.

The kindling model of epilepsy is associated with long-lasting changes in Type II Calmodulin kinase (CaM kinase) activity and immunoreactivity. In order to determine the mechanism of these alterations, we measured gene expression of CaM kinase using in situ hybridization in septally kindled rat brains and paired controls using a 35-S labeled riboprobe for the CaM kinase beta subunit. We found the mRNA concentrated in the hippocampus and other limbic structures in a similar pattern of staining observed immunohistochemically. Kindling resulted in significant decreases in hippocampal CaM kinase mRNA: 30% in CA1, 34% in CA2, 35% in CA3, 41% in CA4, and 29% in the dentate gyrus. Hybridization was also decreased by 21% in the cerebral cortex but not in the lateral septum. These changes are similar in distribution and direction as those measured immunohistochemically. These data suggest that altered CaM kinase activity and immunoreactivity associated with kindling reflect long-lasting alterations in gene expression of this synaptic protein and provides further evidence for its possible importance in the kindling phenomenon.

596.7

KINDLING REDUCES THE SENSITIVITY OF PRE- BUT NOT POSTSYNAPTIC $GABA_B$ RECEPTORS IN THE BASOLATERAL AMYGDALA (BLA). E.K. Asprodini, D.G. Rainnie, & P. Shinnick-Gallagher, Dept. of Pharmacology, Univ. of Texas Medical Branch, Galveston, TX 77550.

Kindling results in an enhancement of excitatory and a loss of inhibitory responses elicited by stimulating the stria terminalis (ST) in BLA neurons. Since excitatory transmission is negatively regulated by $GABA_B$ receptors on excitatory terminals, we tested whether alterations in presynaptic $GABA_B$ receptors contributes to the kindling-induced enhancement in excitatory responses using standard intracellular recordings. Superfusion of baclofen (0.1nM-100μM) depressed ST evoked synaptic responses in control (C) and kindled (K) BLA neurons. The EC_{50} for this action of baclofen was shifted 100-fold from 5nM in control to 500nM in kindled neurons (C:n=29; K:n=23). When $GABA_A$ and postsynaptic $GABA_B$ receptors were blocked with bicuculline (bic, 30μM), CNQX (10μM, to block the bic-induced bursting), and intracellular GTP-γ-S (10mM), baclofen (20nM) reduced the evoked slow EPSPs to a greater extent in control than in kindled neurons (C:55.2±4.9%, n=3; K:10±5.6%, n=4; $p<0.01$). Baclofen (20nM) had no effect on NMDA (n=3) or AMPA (n=3) responses recorded with a GTP-γ-S filled electrode. The EC_{50} for postsynaptic $GABA_B$ receptors was 1μM in control and in kindled neurons (C:n=31; K:n=24). 2-OH-Saclofen (2-OH-sac, 100μM) blocked baclofen-induced hyperpolarizations in control (n=7) and kindled (n=10) neurons; however, in 2-OH-sac baclofen (100nM) still reduced EPSP amplitude by 45.7±3.4% in control and by 28.8±5.6% in kindled neurons.

These data suggest that the kindling-induced changes in baclofen sensitivity presynaptically cannot be due to a loss of $GABA_A$ inhibition, or an effect on postsynaptic glutamate or $GABA_B$ receptors. The differential change in the sensitivity of $GABA_B$ receptors, and the response to 2-OH-sac may indicate distinct subpopulations of $GABA_B$ receptors whose long-lasting response to the kindling stimulus is different. Supp. by NS 24643.

596.8

FINE STRUCTURE AND SYNAPTIC CONNECTIONS OF THE MOSSY CELLS IN THE RAT AND MONKEY FASCIA DENTATA: M. Frotscher, L. Seress*, W. K. Schwerdtfeger* and E. Buhl*. Inst. Anatomy, Univ. Freiburg, D-7800 Freiburg, FRG

The functional role of the mossy cells in the hilar region of the fascia dentata is poorly understood. In this study commissural mossy cells in rats were identified by retrograde horseradish peroxidase (HRP) tracing combined with Golgi impregnation and, alternatively, by retrograde fluorescent tracing (Fast Blue, FB) combined with intracellular Lucifer Yellow (LY) staining. For an electron microscopic analysis the retrogradely HRP-labeled and Golgi-impregnated commissural mossy cells were gold-toned, and the LY-stained neurons were photoconverted. Mossy cells in the fascia dentata of primates (Papio anubis, Macaca mulatta, Saimiri sciureus) were, like mossy cells of rats, either Golgi-impregnated and gold-toned or intracellularly injected with LY but not retrogradely labeled. No major differences were found between mossy cells of rats and monkeys. Giant mossy fiber boutons formed synapses not only with the large excrescences on proximal dendritic portions of the mossy cells but also with peripheral mossy cell dendrites lacking excrescences. Impregnated or intracellularly labeled mossy cell axons traversed the hilus and penetrated the granular layer. These data demonstrate that the mossy cells are commissural/associational neurons that are controlled by the granule cells. This massive excitatory input from the granule cells may cause their degeneration in epileptic disorders.

(Supported by DFG grant Fr 620/1-5)

596.9

ADENOSINE KINASE AND ADENOSINE DEAMINASE INHIBITION IN RAT PREPIRIFORM CORTEX CONFERS PROTECTION AGAINST BICUCULLINE METHIODIDE-INDUCED SEIZURES G. Zhang and T. F. Murray, College of Pharmacy, Oregon State University, Corvallis, OR 97331.

Our previous studies have demonstrated the proconvulsant effects of the adenosine receptor antagonist 8-*p*(sulfophenyl)theophylline and the anticonvulsant effects of the adenosine transport blocker dilazep, suggesting that adenosine may function as an endogenous antiepileptic substance in rat prepiriform cortex (PPC). The aim of this study is to examine the influence of an adenosine kinase inhibitor and an adenosine deaminase inhibitor on bicuculline methiodide (BMI)-induced generalized seizures in this brain area.

All drug solutions were unilaterally microinjected into the PPC. We found that focal injection of the adenosine kinase inhibitor, 5′-amino-5′-deoxyadenosine (ADADO), protected rats from BMI seizures in a dose-dependent fashion (ED_{50}=2.6±0.8 nmol). A dose of 68.4 nmol of ADADO afforded complete protection against BMI seizures. In contrast, the adenosine deaminase inhibitor, 2′-deoxycoformycin (DCF), elicited anticonvulsant effects with less potency and lower efficacy showing a maximal response of 30% protection. When a dose of ADADO which was inactive when administered alone was co-administered with DCF, the DCF dose-response curve was dramatically shifted to the left with an increased maximal effect. These findings suggest that accumulation of endogenous adenosine may effect seizure suppression, and further that adenosine kinase may play a pivotal role in the regulation of extracellular levels of adenosine in the CNS. (Supported by USPHS Grant NS23227).

596.10

EPILEPTIC DISCHARGES TRIGGER SLOW EPSPS IN IMMATURE AND ADULT NEOCORTEX. L.S. Benardo. Dept. of Pharmacology, S.U.N.Y.-H.S.C.A.B., Brooklyn, NY 11203.

Slow EPSPs can be triggered in rat layer V somatosensory neocortical neurons by repetitive stimulation as early as postnatal day 6. The role of such events in epilepsy is unknown. Blocking $GABA_A$-mediated IPSPs causes epileptic discharge by releasing local recurrent excitation. Whether similar release phenomena occur for slow EPSPs was tested by exposing slices to picrotoxin (25-50 uM) to induce epileptic discharge by blocking fast IPSPs. This maneuver allowed triggering of slow EPSPs at lower stimulus intensities, with fewer stimuli. Epileptic discharge occurred spontaneously and triggered slow IPSP-EPSP sequences (most prominent at depolarized potentials), similar to those elicited by electrical stimulation. Seizure discharge in younger animals has a characteristic ontogeny, being most robust at 15-30 days of age. As expected the amplitude and duration of slow EPSPs seen under these conditions varied in relation to ontological changes in epileptic events. Atropine and atenolol in combination, blocked slow EPSPs triggered by epileptic discharge. Eserine or cocaine enhanced slow EPSPs generated by epileptic events. This supported the notion that these slow EPSPs were also subserved by cholinergic and noradrenergic transmission. A non-conventional mechanism for synaptic transmisssion is postulated to account for triggering of slow EPSPs by epileptic discharges. (Supported by CIDA NS01386-01)

596.11

RELATIONSHIP OF IMMEDIATE EARLY GENE EXPRESSION TO ELECTROENCEPHALOGRAPHIC (EEG) CHANGES DURING PENTYLENETETRAZOL SEIZURES IN THE RAT. **P. Lanaud[1], A. Depaulis[2]*, C. Bugnon[1]* and M. Vergnes[2]*. [1]Laboratoire d'Histologie-Embryologie-Cytogènètique Faculté de Médecine, 25000 Besançon, France, and [2]Centre de Neurochimie, 67084 Strasbourg Cedex, France.**

Pentylenetetrazol (PTZ) was given to rats at 3 doses to evoke qualitatively distinct seizures, based on behavioral and EEG changes. Using in situ hybridization to examine mRNA encoding immediate early genes (IEG) in brain sections, we evaluated the relationship between the seizure characteristics and regional changes in IEG expression.

At 30 min after i.p. saline (control) or PTZ (20, 40 or 80 mg/kg), mRNA encoding c-fos, c-jun, jun-B and zif/268 was evaluated. No IEG expression above basal and no EEG modification occurred after saline. After 20mg/kg, spike and wave discharges (6-7 c/s) regularly appeared on the EEG, concomitant with behavioral arrest but no modification of IEG expression was observed. At 40 mg/kg, myoclonic and clonic seizures occurred concomitant with spike discharges (3-4 c/s) and significant increases in expression of all four IEGs occurred in neocortex, hippocampus, piriform cortex and amygdala. After 80 mg/kg, severe tonic-clonic seizures were associated with a general increase of IEG expression throughout the forebrain.

Our results suggest that PTZ-evoked increases in IEG expression in brain can be detected only with convulsive seizures and not with nonconvulsive spike-and-wave discharge. Moreover, selective regional changes in IEG expression occurred with a moderate dose of PTZ; increased IEG expression had relatively little regional selectivity after the high dose. Some of the changes in IEG expression with the high PTZ dose may reflect direct effects unrelated to seizure activity.

596.12

FUNCTIONAL CAPILLARY DENSITY IS REDUCED IN BRAIN OF PATIENTS WITH MERRF (MYOCLONUS EPILEPSY, RAGGED-RED FIBER SYNDROME) L. Ribeiro, S.F. Berkovic, H. Kuwabara, F. Andermann, A. Gjedde. Montreal Neurological Institute, Montreal, Quebec H3A 2B4 Canada.

MERRF is a disorder of the mitochondrial respiratory chain, sometimes associated with a mutation in mitochondrial tRNA. The energy state in muscle of these patients is abnormal, but the cerebral metabolic consequence are poorly understood. Our previous studies using magnetic resonance spectroscopy and PET showed globally reduced cerebral metabolism for glucose and oxygen with no evidence of intracerebral acidosis. Here we applied our technique for measuring functional capillary density (D_{cap}) in these patients to further elucidate the cerebral metabolic profile in MERRF. We analysed 3 patients with MERRF, and 5 normal volunteers. The global averages were (means±SEM; CBF, cerebral blood flow; CMR_{glc}, cerebral metabolic rate for glucose; T_{max}, maximal transport rate for glucose; K_t, half-saturation constant):

Variable	Unit	Control (n=5)	MERRF (n=3)
CBF	(ml/hg/min)	31 ± 2	40 ± 5*
K_t^*	(ml/hg/min)	9.6 ± 0.6	7.3 ± 0.6*
CMR_{glc}	(μmol/hg/min)	32 ± 2	17 ± 1**
D_{cap}	(mm^{-2})	255 ± 17	157 ± 13**
T_{max}	(μmol/hg/min)	82 ± 8	52 ± 14*
K_t	(m*M*)	2.3 ± 0.2	3.2 ± 0.5*

*$P < 0.05$, **$P < 0.005$

The data show that, despite elevated CBF, there is a marked reduction in CMR_{glc} together with a parallel reduction in D_{cap}. While it is possible that reduced capillary density limits the diffusion of glucose and oxygen, it is more likely that the reduction of D_{cap} and CMR_{glc} are chronic consequences of the intracellular mitochondrial defect.

597.1

ANXIOLYTIC-LIKE EFFECTS OF THE NON-COMPETITIVE NMDA ANTAGONIST MK-801 ON CONFLICT BEHAVIOR IN THE RAT. Z.C. Xie and R.L. Commissaris. Dept. Pharmaceutical Sci., Coll. Pharmacy & AHP, Wayne St. Univ., Detroit, MI 48202.

The present study determined the effects of the non-competitive NMDA antagonist MK-801 on behavior in the conditioned suppression of drinking (CSD) paradigm, a repeated measures conflict task. In daily 10-minute sessions, water-restricted rats drank from a tube which was occasionally electrified (0.25 mA shocks signaled by a tone). Trained subjects (4 weeks of CSD testing) exhibited stable baselines for both punished (41 $\pm$ 5 [Mean $\pm$ SEM] shocks received/session and unpunished (14.0 $\pm$ 0.7 ml/session) responding. Phenobarbital (5 - 40 mg/kg, IP) or chlordiazepoxide (2.5 - 20 mg/kg, IP) administration with a short (10- or 30-minute) pre-treatment resulted in a dose-dependent increase in punished responding, whereas (+) MK-801 did not increase punished responding when administered using a 10-minute or 4-hour pretreatment. However, at a 24-hour pretreatment (+) MK-801 (0.01 - 0.4 mg/kg, IP) produced a dramatic and dose-dependent increase in punished responding. The "inactive" isomer (-) MK-801 did not produce an anxiolytic-like effect in the CSD paradigm at any pretreatment interval. These data suggest that the anti-convulsant (+) MK-801 also may possess anti-anxiety effects in humans. (Supported in part by MH 47181.)

597.2

EFFECTS OF ANTI-SEIZURE AGENTS ON CONFLICT BEHAVIOR IN THE RAT. T.J. Hill, D.J. Fontana and R.L. Commissaris Dept. of Pharmaceutical Sci., College of Pharmacy, Wayne State University, Detroit, MI 48202.

Traditional anti-seizure agents recently have been used in psychiatry for the treatment of a number of disorders, including anxiety disorders. The present study examined the effects of acute treatment (10- to 120-minute pretreatment) with several anti-seizure agents in the conditioned suppression of drinking (CSD) conflict paradigm, an animal model for the study of "anxiety" and anti-anxiety agents. The agents tested were diazepam (DZ), phenobarbital (PhB), carbamazepine (CBZ), phenytoin (DPH), valproate (VPA), and ethosuximide (ESI). Consistent with their anxiolytic effects in man, DZ (1.25-20 mg/kg) and PhB (5-40 mg/kg) produced dose-dependent and robust (maximal effect 50 - 70 shocks above control) increases in punished responding. CBZ (2.5 - 40 mg/kg) and VPA (50 - 200 mg/kg) produced dose-dependent increases in punished responding, but the maximal effect was less than that seen with PhB or DZ. Neither ESI (25 - 100 mg/kg) nor DPH (0.6 - 20 mg/kg) increased punished responding. Thus, not all anti-seizure agents exert anxiolytic-like effects on conflict behavior. (Supported in part by MH 47181.)

597.3

BEHAVIORAL EFFECTS OF INTERACTIONS AMONG D1 AND D2 DOPAMINE RECEPTOR AGONISTS AND ANTAGONISTS. J. L. Katz and J. M. Witkin. Psychobiology Laboratory, NIDA Addiction Research Center, Baltimore, MD 21224, U.S.A.

The effects on schedule-controlled operant behavior of the D1 receptor agonist, SKF 38393, and the D2 agonist, quinpirole, were assessed alone and in combination with selective dopamine-receptor antagonists. Squirrel monkeys (*Saimiri sciureus*) were trained to press a response key under fixed-interval and fixed-ratio schedules of food reinforcement. Quinpirole increased rates of responding under the fixed-interval schedule at doses (0.1 - 1.0 mg/kg) that decreased rates of responding under the fixed-ratio schedule. Under the fixed-interval schedule, the D2 antagonists, spiperone (0.003 - 0.006 mg/kg) and to less of a degree haloperidol (0.003 - 0.01 mg/kg), and the D1 antagonist, SCH 23390 (0.03 mg/kg), shifted the quinpirole dose-effect curve to the right. At the highest doses of the antagonists the maximal effects of quinpirole were decreased. However, only the D2 antagonist, spiperone, antagonized the effects of quinpirole under the fixed-ratio schedule. The D1 agonist, SKF 38393, dose-dependently (1.0 - 10.0 mg/kg) decreased rates of responding under both schedules. Those effects were not antagonized by any doses studied of either spiperone (0.003 mg/kg) or SCH 23390 (0.003 - 0.3 mg/kg). SCH 23390 produced a dose-related enhancement of the effects of SKF 38393.

597.4

DIFFERENTIAL EFFECTS OF D_1 AND D_2 ANTAGONISTS IN COMBINATION WITH THE D_2 AGONIST (+)-PHNO IN SQUIRREL MONKEYS. Sharon Rosenzweig-Lipson, Sara Johnson*, and Jack Bergman. Harvard Medical School/NERPRC, Southboro, MA.

Behavioral effects of the dopamine D_2-selective agonist (+)-PHNO were determined alone and after pretreatment with the D_2-selective antagonist eticlopride, the D_1-selective antagonist SCH 39166, and the nonselective dopamine antagonist clozapine in squirrel monkeys responding under a 30-response fixed-ratio schedule of stimulus-shock termination. When administered cumulatively, (+)-PHNO (0.001 - 0.3 mg/kg) produced dose-dependent decreases in rates of responding, with ED_{50} values ranging from 0.002 to 0.014 mg/kg for individual monkeys. Eticlopride (0.001 - 0.006 mg/kg) produced graded rightward shifts in the dose-response curve for (+)-PHNO; ED_{50} values for (+)-PHNO were increased by as much as 30-fold for individual monkeys. In contrast, SCH 39166 did not antagonize the behavioral effects of (+)-PHNO at any dose. Instead, increasing doses of SCH 39166 (0.01 - 0.1 mg/kg) produced leftward and downward shifts in the dose-response curve for (+)-PHNO. Clozapine (1 - 5.6 mg/kg) also resulted in graded leftward and downward shifts in the dose-response curve for (+)-PHNO. These results show that the rate-decreasing effects of (+)-PHNO can be surmountably antagonized by the D_2 antagonist eticlopride but are modified differently by the D_1 antagonist 39166 and the atypical neuroleptic clozapine. (Supported by USPHS grants DA03774, DA00499, MH07658, and RR00168).

597.5

EFFECTS OF CHRONIC MDMA (METHYLENEDIOXYMETHAMPHETAMINE) ADMINISTRATION ON RHESUS MONKEY PERFORMANCE IN AN OPERANT TEST BATTERY (OTB). M.G. Paule, M.P. Gillam*, and R.R. Allen*. Div. Reprod. Development. Toxicol. & Computer Based Systems, Inc., National Center for Toxicological Research, Jefferson, AR 72079-9502.

MDMA profoundly depletes serotonin (5-HT) in nerve terminals in primate brain following several repeated doses. The present studies were conducted in adult male monkeys (n=3) to determine the effects of such 5-HT depletion on complex brain functions including learning, short-term memory and attention, time perception, color and position discrimination, and motivation to perform for food. Daily (M-F) performance in a multiple schedule OTB was used to model these functions with response rate, accuracy, and percent task completed serving as indicators of functional ability. Each dose of MDMA [0.0 (saline), 0.1, 0.3, 1.0, 3.0, 5.6, 10.0 and 20.0 mg/kg] was given intramuscularly twice daily for 14 consecutive days in ascending order. Although there were clear acute effects of MDMA (generally at $\geq$ 1.0 mg/kg) to disrupt performance of all tasks 45 min after administration, marked tolerance to these effects was demonstrated. OTB performance generally returned to predrug values a few weeks after chronic treatment ended, when 5-HT systems are presumably severely depleted. Thus, 5-HT systems would not appear to be of fundamental importance for the performance for these specific brain functions.

597.6

THE LACK OF SEDATIVE PROPERTIES OF CI-988, A SELECTIVE CCK_B RECEPTOR ANTAGONIST. Lakhbir Singh, Ryszard J. Oles and Geoffrey N. Woodruff. Parke-Davis Research Unit, Addenbrookes Hospital Site, Hills Road, Cambridge CB2 2QB, U.K.

Previously it has been reported that the selective CCK_B receptor antagonist CI-988 (previously PD 134308) produces anxiolytic-like action in a number of animal models of anxiety (Hughes *et al.*, 1990; Singh *et al.*, 1991). We now show that CI-988 does not induce sedation/ataxia or potentiate the action of CNS depressants.

Chlordiazepoxide (CDP) or CI-988 were administered i.p. 40 min before test to male TO mice (25-30g). CDP dose-dependently (1-30 mg/kg, i.p.) impaired rotarod performance and increased sleeping time induced by sodium pentobarbitone (60 mg/kg, i.p.) with minimum effective dose (MED) of 10 mg/kg. CDP also potentiated the ability of ethanol to impair rotarod performance. In contrast, CI-988 up to 30 mg/kg which is 300-3000 fold higher than the anxiolytic dose was found to be inactive in all these tests.

Thus, it is suggested that CI-988 should have a better side-effect profile in man than the benzodiazepines.

Hughes *et al.*, (1990). *Proc. Natl. Acad. Sci., U.S.A.* **87**, 6728.
Singh *et al.*, (1991). *Proc. Natl. Acad. Sci., U.S.A.*, **88**, 1130.

598.1

SPROUTING IN THE NEUROMUSCULAR SYSTEM: EARLY SIGNALING BY IGF1 FROM INACTIVATED MUSCLE FIBERS. Caroni, P. and Schneider, C*. Friedrich Miescher Institute,P.O.Box 2543,CH-4002 Basel

Upon lesion- or toxin-induced paralysis processes in the inactive muscle fibers and in nearby cells are initiated to bring about restoration of muscle activity. Among these is the local stimulation of nerve growth.Insulin-like growth factors (IGFs) can induce neurite growth in vitro, and contents of IGFs mRNA in muscle correlate with the presence of nerve growth inducing activity in fast skeletal muscle (Ishii, PNAS (1989)86:2898-2902). We are therefore investigating the possible roles of IGFs in neuromuscular regeneration, including intramuscular nerve sprouting.We found that: 1.)embryonic motoneurons in vitro respond to IGFs in the subnanomolar range with a vigorous neurite growth and ramification reaction; 2.) non-lesioning application of IGFs to adult rodent skeletal muscle in vivo specifically elicits processes that are observed in denervated or paralyzed muscle, including a marked nerve sprouting reaction (Caroni and Grandes, JCB (1990) 110:1307-1317); 3.) in the adult rat, paralysis leads to rapid (detectable after 15h) and transient (peak at about 48h) induction of IGF1 mRNA in muscle fibers (in situ hybridization data; DIG-alkaline phosphatase method), followed by a slower and persistent increase of IGF1 and IGF2 mRNA in muscle fibers and in muscle interstitial cells. For comparison, increase in muscle fiber N-CAM mRNA was detectable 3d after paralysis; 4.) adult rat motoneurons in vitro express IGF1-receptor mRNA (method as above). Since elevated IGF1 levels are sufficient to trigger sprouting and intramuscular cell proliferation, and since muscle fiber IGF1 mRNA levels are rapidly elevated upon paralysis, IGF1 is likely to initiate restorative responses in paralyzed skeletal muscle, possibly including a direct stimulation of nerve sprouting.

598.2

INSULIN, IGF-I, IGF-II AND NGF ACTIVATE A COMMON BIOCHEMICAL PATHWAY DURING NEURITE GROWTH: ROLE OF NEUROFILAMENT AND TUBULIN GENE EXPRESSION. D.N. Ishii, C. Wang*, Y. Li*, B. Wible*, and K.J. Angelides. Physiol. Dept., Colorado State Univ., Ft. Collins, CO 80523; Physiol. & Molec. Biophysics Dept., Baylor Univ., Houston, TX 77030.

We tested the hypothesis that insulin, insulin-like growth factors (IGFs) and nerve growth factor (NGF) can all increase the expression of genes encoding major structural proteins of axons, particularly 68 and 170 kDa neurofilament (NF) genes, in human neuroblastoma SH-SY5Y cells. We also studied whether more than one transmembrane signal might be involved.

Insulin, IGF-I, and IGF-II increased 68 and 170 kDa NF mRNA content relative to total RNA, poly(A)$^+$ RNA, and histone mRNA during neurite formation. Insulin and IGFs had little effect on stability of NF mRNAs, whereas insulin increased transcription of 68 and 170 kDa NF mRNAs. Similar effects are reported for NGF (Lindenbaum et al., 1988). In contrast, our previous studies showed that insulin, IGF-I, and NGF all stabilize α- and β-tubulin mRNAs, but have no detectible effect on transcription. We conclude that insulin, IGF-I and IGF-II can selectively increase NF gene expression, and that insulin, IGFs and NGF can activate two branches of a common biochemical pathway regulating neurite formation. (Supported by NIH grants NS24327 and NS24606).

598.3

SEPTAL DENERVATION OF THE HIPPOCAMPUS IS REQUIRED FOR SYMPATHETIC AXON INGROWTH. B. N. Saffran and K. A. Crutcher. Department of Neurosurgery, University of Cincinnati, Cincinnati, OH 45267.

Sympathetic sprouting into the rat hippocampal formation (HF) after a medial septal lesion (MSL) may occur as a result of accumulation of NGF. Intraventricular infusion of NGF in the absence of septal denervation, however, does not elicit sympatho-hippocampal sprouting even though there is proliferation of vascular sympathetic fibers (Brain Res. 492: 245). Furthermore, NGF infusion in the presence of a MSL results in reduced sympathohippocampal sprouting, perhaps due to competition with perivascular collaterals (Brain Res. 525: 11). We tested whether intraventricular NGF infusion would promote sympathetic sprouting into the HF from autologous superior cervical ganglion transplants in the presence or absence of a MSL. Female SD rats received 2 week infusions of NGF beginning at the time of transplantation of the SCG to the HF. Four rats received a MSL, six did not. Animals were killed at 2 (n=2) or 4 (n=8) weeks after surgery and the brains were examined for sympathetic ingrowth (SPG method), septal denervation (AChE histochemistry) and NGF content (2-site ELISA). Although extensive ingrowth was found in septal-denervated regions of the HF, no ingrowth was observed in animals without evidence of septal denervation (n=4). Furthermore, in animals where there was partial septal denervation (n=2), sympathetic axons were found only in denervated areas. In NGF-infused animals, contralateral sympathetic hyperinnervation of the internal carotid artery occurred. In 7 animals with transplants and NGF infusion, fibers extended from the transplants through the fimbria and corpus callosum to surround the ventricle where the cannula was placed. These results indicate that infused NGF stimulates sympathetic fiber outgrowth from transplanted ganglia but does not permit sympathetic sprouting in the HF in the presence of septohippocampal fibers. (Supported by NIH #NS-17131.)

598.4

STAUROSPORINE NEUROTROPIC EFFECTS IN PC12 CELLS ARE INDEPENDENT OF PROTEIN KINASE C. D. Rasouly[a*], D. Lester[b*], Y. Matsuda[c*] and P. Lazarovici[a], [a]Dept. of Pharmacology, Hebrew University, Jerusalem, Israel, [b]Dept. of Membrane Res., Weizmann Institute, Rehovot, Israel and [c]Kyowa Hakko Kogyo Co. Ltd., Tokyo, Japan.

Nerve growth factor (NGF) is a polypeptide orchestrating a number of sympathetic neuronal phenomena including differentiation and survival. In PC12 cells, NGF induces differentiation expressed by neurite-outgrowth and neuronal network formation.

We have found that Staurosporine (St), an alkaloid known to inhibit Protein Kinase C (PKC) in vitro, induces neurite outgrowth in PC12 cells within 6 hours at non toxic, 10-30nM, concentrations. Appearance of neurites induced by St was similar to that induced by NGF but differed in the ruffling property. Down regulation of PKC (assessed by protein phosphorylations, ^{3}H-St binding and fluorescent phorbol ester imaging) by TPA (500nM/24 hrs) or inhibition of kinases by K-252a family of compounds, had no effect on neurite outgrowth in PC12 cells. This suggests that the neurotropic effect of St (at doses that inhibit PKC) is unrelated to the inhibition of PKC.

Elucidation of these mechanism(s) has clinical implications and is important in exploring NGF action in the nervous system.

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598.5

IMMUNOCHEMICAL EVIDENCE FOR NGF-LIKE MOLECULE(S) IN THE CENTRAL NERVOUS SYSTEM OF LYMNAEA. R.L. Ridgway, G.C. Hauser, N.I. Syed and A.G.M. Bulloch. Neuroscience Research Group, Dept. of Med. Physiol., Univ. of Calgary, Calgary, Alberta, Canada T2N 4N1.

We have recently reported that murine 2.5S Nerve Growth Factor (NGF) stimulates neurite outgrowth in vitro of identified motor neurons and interneurons of the pond snail, Lymnaea stagnalis (Ridgway et al., J. Neurobiol., in press). Antisera generated against NGF was found to block not only the sprouting activity of 2.5S NGF but also of Lymnaea brain-conditioned medium (CM), raising the possibility that endogenous NGF-like molecule(s) might be active in the molluscan CNS. Here we examined this possibility further using immunoblotting and immunoaffinity chromatography methods.

Culture medium conditioned with Lymnaea central ganglionic rings (2/ml) for 72 h was concentrated ~30-fold via 10kD cut-off (Amicon YM-10) filtration membranes, subjected to gel electrophoresis, and then transferred to nitrocellulose for immunoblot analysis. Prominent polypeptide bands with relative molecular weights (M_r) of ~140, ~100, ~62, ~27, and ~15kD (under reducing conditions) were identified using 3 different antisera to NGF; some bands may represent proteolytic fragments of a larger protein. Chromatography of concentrated CM via an anti-NGF affinity column resulted in the elution of several polypeptides, the most prominent being at M_r ~15kD. In preliminary bioassays, the elution volume was found to contain neuritogenic activity on Lymnaea Pedal A (Pe.A) cluster motor neurons. These results, together with those of the companion abstract (Bulloch et al.), strongly support the hypothesis that molecule(s) resembling mammalian NGF are present in the CNS of Lymnaea. Supported by AHFMR and Canadian Centre of Excellence in Neural Regeneration and Functional Recovery.

598.6

GLUTAMATE MODULATES A NGF-like PROTEIN AND A NGF-like mRNA IN THE LYMNAEA CNS. A.G.M. Bulloch, R.L. Ridgway, K.C. Harrington and W. Tetzlaff. Neuroscience Research Group, University of Calgary, Faculty of Medicine, Calgary, Alberta, Canada, T2N 4N1.

This study continues our recent examination of neurotrophic molecules in the nervous system of the snails Lymnaea and Helisoma. We recently reported that glutamate enhances the release and/or production of factors that are responsible for the conditioning of medium required for neurons to sprout in vitro. We have also shown that a mammalian neurotrophic factor, Nerve Growth Factor (NGF), can induce sprouting by specific Lymnaea neurons. In the adjacent presentation (Ridgway et al.) we show that Lymnaea brain-conditioned medium (CM) contains a NGF-like immunoreactive molecule. Here we demonstrate that the concentration of this molecule in CM can be enhanced by glutamate. One of the key questions arising from these observations is the nature of the mechanism by which glutamate enhances CM (e.g., does it increase the synthesis and/or release of conditioning factors by CNS tissues?). In this study we have assayed the CNS of Lymnaea and Helisoma for NGF-like mRNAs using a P^{32}-cDNA probe derived from the human NGF sequence. Northern blots of RNA from both Lymnaea and Helisoma have shown that this probe hybridizes with a 1.35 kB mRNA under low stringency conditions. Furthermore,the level of this NGF-like mRNA is increased by axotomy and also by the presence of glutamate during the conditioning period. These results suggest that the molluscan nervous system contains a NGF-like protein and a NGF-like mRNA, both of which can be modulated by glutamate. Supported by the Canadian Centre for Exellence in Neural Regeneration and Functional Recovery.

598.7

Mn^{2+} INDUCES NEURON-LIKE MORPHOLOGICAL CHANGES IN PC12 CELLS. W.H.Lin*, K.A.Marcucci* and J.A.Roth, Dept. of Pharmacology and Therapeutics, School of Medicine and Biomedical Sciences, SUNY at Buffalo, Buffalo, NY 14214

Divalent cations in the culture medium have been shown to affect the attachment of PC12 cells to the substratum. However, their effect on the process of neuronal differentiation have not been characterized. When cells were treated with 0.3 - 2.0 mM of $MnCl_2$, we observed morphological changes similar to those induced by NGF treatment, i.e. flattening of cell bodies and extension of neurites. These responses were dose-dependent and observable within 6 hours after treatment. The effects of Mn^{2+} were enhanced in medium containing 1% serum and in the presence of NGF. Of the other divalent cations examined, only Co^{2+} was able to simulate the effects of Mn^{2+}. When compared to the morphological changes observed with forskolin, the Mn^{2+}-treated cells were appreciably flatter, with cell body diameters up to 30 μm. While forskolin alone induced neurite outgrowth maximally in only 60% of the cells, co-incubation with submaximal concentration of $MnCl_2$ increased the response rate to over 90%, with a profound increase in the size of the cell bodies. In addition, Mn^{2+} was also able to induce similar responses in a mutant PC12 cell line deficient in protein kinase A activity. These observations suggest that Mn^{2+} induces neurite outgrowth in PC12 cells through a mechanism independent of cAMP. Questions remain as to whether NGF and Mn^{2+} share a common signal transduction pathway which results in morphological differentiation of PC12 cells. (Supported by NIH grants NS 20530 and ES 04249)

598.8

ELEVATED POTASSIUM MAINTAINS NEURITE STABILITY IN A PC12 MUTANT CELL LINE AFTER NGF WITHDRAWAL. KENNETH K. TENG AND LLOYD A. GREENE*. Department of Pathology and Center for Neurobiology and Behavior, College of Physicians and Surgeons, Columbia University, New York, N.Y. 10032.

We have isolated a clonal PC12 mutant, designated as Clone 41 (C41), with heightened responses to NGF. Compared to wild type PC12 cells, the C41 line extends neurites in significantly greater abundance in the presence of NGF. Biochemically, NGF-differentiated C41 cells differ from their PC12 counterparts in showing more pronounced changes in cytoskeletal proteins. In characterizing the C41 line, we observed that elevated K^+ (20-80mM) can maintain neurites for at least 3 weeks after NGF withdrawal; however although they are maintained, the neurites do not continue to elongate. Without either NGF or K^+, the neurites degenerate. Cells in which NGF is replaced with K^+ also retained a "primed" state characterized by capacity for rapid NGF-dependent neurite regeneration. The K^+-induced effect is inhibited by Ca^{+2} channel blockers (Verapamil and/or Nitrendipine). This neurite-stabilizing effect is accompanied by an alteration in several cytoskeletal components. Amongst these, phospho-β-tubulin decreases to undifferentiated levels while the microtubule associated proteins MAP1.2 and chartins remain highly phosphorylated as in NGF-treated controls. The data presented here suggest that depolarization may stabilize neurites in the absence of neurotrophic factors, at least in part by affecting the cytoskeleton.

598.9

INDUCTION OF GROWTH CONE COLLAPSING ACTIVITY IN CULTURED ASTROCYTES BY FGF AND INTERLEUKIN 1. Alan R. Johnson*, James W. Fawcett*, Roger J. Keynes and Geoffrey M.W. Cook*. Physiological Laboratory and Department of Anatomy, University of Cambridge, Downing Street, Cambridge, U.K.

Mature astrocytes and astrocytes derived from injury-induced glial scars may be inhibitory for axon growth. We have explored astrocyte-growth cone interactions by assessing the capacity of detergent extracts of cultured astrocytes to cause growth cone collapse *in vitro*. Astrocytes prepared from neonatal rat brains were cultured for 2 weeks, purified, and subsequently homogenised in 2% CHAPS in PBS in the presence of protease inhibitors. The detergent extracts were then incorporated into liposomes and added to cultures of embryonic chick and rat dorsal root ganglia for 1 hour. Detergent extracts of untreated astrocyte cultures produced only modest levels of growth cone collapse; extracts prepared from cultures of Schwannoma cells also lacked activity. Some cultures were treated with bFGF and Interleukin 1 for 24 hours before extraction, and now posessed potent growth cone collapsing activity. It is known that both these molecules can produce reactive-type changes in astrocytes. Similarly, axons growing directly on monolayers of astrocytes exhibited a collapsed growth cone morphology, while axons growing on Schwannoma cells had fully spread growth cones. These observations raise the possiblity that reactive astrocytes possess surface-bound molecules that inhibit axon regeneration.

598.10

A NEURITE PROMOTING ACTVITY FROM AN IMMORTAL SCHWANN CELL CLONE. L.M. Bolin and E.M. Shooter, Dept. of Neurobiology, Stanford University School of Medicine, Stanford, CA,94305

Regeneration of the peripheral nervous system requires the precise reformation of synaptic connections between motor and sensory neurons and the tissues that they subserve. Schwann cells are essential to this process. To explore the Schwann cell - neuron interaction, we have established primary cultures of adult rat Schwann cells from crushed sciatic nerves. From these primary cultures, we have isolated a spontaneously immortal Schwann cell clone (iSC), whose conditioned media (CM) contains a neurite pro-moting activity that is not active on PC12 cells. Embryonic chick ganglia explants were incubated with CM to determine the neuronal specificity of this activity. This activity is not selective for a general class of neurons, neurites are elicited from CNS (spinal cord, brain and retina) and PNS (dorsal roots and sympathetic chain ganglia). We have characterized this activity after elution from a cation exchange column. In a dilution of 1:50, this eluant retains its neurite promoting activity with embryonic neurons. Neither the column profile nor the types of embryonic neurons supported by this eluant are consistent with it being CNTF. This activity does not bind to heparan and antibodies to bFGF do not inhibit neurite promotion. Although poly-adenylated RNA from iSC gives a positive signal when probed with cDNA for NGF, blocking antisera to NGF does not inhibit neurite formation. This Schwann cell clone has been transformed at a time of trophic activity and may provide new information on the regenerative milieu.

598.11

PURIFICATION AND BIOCHEMICAL CHARACTERIZATION OF RAT AND BOVINE CNS MYELIN ASSOCIATED NEURITE GROWTH INHIBITORS NI-35 AND NI-250. C.E. Bandtlow* and M.E. Schwab, Brain Research Institute, University of Zurich, 8029 Zurich, Switzerland.

Rat and bovine CNS myelin contain two proteins of 33-35 kDa and 250 kDa which exert potent inhibitory effects on extending neurites (neurite growth inhibitor NI-35 and NI-250). SDS-PAGE eluted NI-35 and NI-250 revealed several active spots in the pI range of 4-6 on 2D-gels. NI-35 and NI-250 are hydrophobic proteins; so we further purified this material on a C4 reverse phase HPLC column. The chromatograms, showing two major protein peaks (I and II) were identical for both, NI-35 and NI-250. Peaks I and II, derived from NI-35 or NI-250 migrated at 33 kDa on SDS-PAGE. The amino acid compositions of peak I and II were determined and found to be very similar. Initial N-terminal and internal amino acid sequence data of peak II derived from NI-35 were identical to the corresponding sequences of peak II of NI-250. The amino acid sequences available so far show no homologies to known proteins.

These results show that NI-35 and NI-250 are closely related proteins and that NI-250 is most likely a complex containing the 35 kDa protein. NI-35 is microheterogenous consisting of 2 subforms differing slightly in their hydrophobicity.

598.12

LOCAL CONTROL OF NEURITE OUTGROWTH OF DORSAL ROOT GANGLIA AND SPINAL CORD NEURONS BY ACTH ANALOG ORG 2766, BIM 22015 AND NGF. S.J. Lee, T.S. Lee and F.L. Strand. Department of Biology and Center for Neural Science, New York University, Washington Square, New York, New York 10003.

The dorsal root ganglia and spinal cord of 14-15-day old Sprague-Dawley rat embryos were removed and plated into the center chamber of three compartment culture dishes.[1] The center chamber neurons were maintained for 3-14 days with 100ng/ml 7S nerve growth factor (GIBCO). The left compartments contained the peptide analogs, Org 2766 or BIM 22015, while the right chambers contained the NGF, as a control. The neurite outgrowths can be seen extending from the center compartment to the side chambers containing NGF. This extension can also be seen in side chambers containing peptide analogs (100ng/ml) without NGF. The local withdrawal of NGF in the side chambers prevented further neurite elongation but maintained their survival for 5-7 days. Peptide withdrawal from the side chambers also caused cessation of outgrowth but the survival rate is questionable. Neuronal survival of DRG and spinal cord cultures was also determined after peptide treatments.

Nerve growth factor is required for survival, differentiation and neurite outgrowth. Treatment of the DRG and spinal cord neurons with ACTH analogs *in vitro* seemed to delay neuronal cell death, a characteristic of normal development in the mammalian nervous system.[2] The effects of Org 2766 and BIM 22015 on neurite outgrowth have been compared and preliminary studies show that they may possess neurotrophic properties in the culture system. [1] R.B.Campenot. *Proc.Natl.Acad.Sci.USA* 74: 4516-4519, 1977. [2] D.K.Berg. *Neuronal Development.* Plenum Publ.Corp. 297-331, 1982. Supported by Council for Tobacco Research.

599.1

TISSUE SPECIFIC EXPRESSION OF VGF8a, A GENE INDUCED BY NGF

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We have analyzed the tissue specific expression of VGF, a secretory protein similar to secretogranin proteins, that is highly induced by NGF in PC12 cell line. Immunohistochemistry, in situ hybridization, northerns and RNAse protection have been used to identity the protein and the mRNA in different tissues. In particular, we have focused our attention on neuronal and endocrine tissues. Previous work has shown the expression of VGF in localized area of the brain (van Den Pol et A J. Neurosci. 1989, 9, 4122). The protein, as well as the mRNA, is present in a sub-population of cells of Dorsal Root Ganglia, Sympathetic Ganglia, Trigeminal Ganglia, Spinal Cord, Adrenal Medulla, Adenohypophysis, and in some neurons of the Myoenteric Plexus. We could not detect the protein or its mRNA in muscle, liver, spleen and other tissues. Furthermore, we have studied the regulatory regions of the VGF8a gene and the tissue specific expression of the promoter in different cell lines. Nuclear extracts from different cell lines show distinct patterns of band shifts and foot printings which map to two different region. CAT activity with partial deletion of the promoter shows that the region to -78 can act as a minimal promoter, albeit without NGF inducibility.

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599.2

SELECTIVE EFFECTS OF NERVE GROWTH FACTOR DEPRIVATION ON DORSAL ROOT GANGLION CELLS IN EMBRYONIC RATS.

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Although embryonic dorsal root ganglion (DRG) neurons in general are known to require nerve growth factor (NGF) for their survival and function, the degree to which the numerous classes of these neurons depend on NGF is unsettled. We have assessed the effects of embryonic NGF deprivation by labelling the characteristic central axonal arborizations of the various classes of DRG neurons. Embryonic rats (E15) received individual, *in utero* systemic injections of goat anti-mouse NGF serum. The embryos were then taken by Caesarean section on E18 and perfusion fixed. The central axonal arborizations of DRG neurons were visualized utilizing the lipid-soluble tracer DiI placed either within individual DRGs or in the skin covering the dorsum of the embryo.

NGF deprivation in this prenatal period was selective with regard to which classes of sensory neurons were affected. Embryonic exposure to NGF antibodies prevented the ingrowth of the fine cutaneous (pain, thermoreceptive) primary afferents which occupy Rexed's lamina I and the most superficial part of lamina II in the dorsal horn. On the other hand, the "flame-shaped" axonal arborizations characteristic of the thicker hair follicle afferents, which occupy normally laminae IV, III, and only the inner part of lamina II in the dorsal horn, not only survived but now sprouted into the unoccupied superficial laminae. In many cases, their terminals reached nearly to the surface of the spinal cord. The central arborizations of other classes of primary afferents, including Type I muscle afferents, appeared unaffected by NGF deprivation during the E15-E18 period.

We conclude that DRG neurons differ in their requirement for NGF, and that the small diameter cutaneous afferents are the most sensitive.

599.3

COMPARISON OF RABBIT FACIAL NERVE REGENERATION IN NGF-CONTAINING TUBES TO AUTOLOGOUS NERVE GRAFTS.

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Previous reports suggest that NGF enhances rabbit facial nerve regeneration. We compared facial nerve regeneration in NGF or Cytochrome C (Cyt. C)-containing silastic tubes to autologous nerve grafts. A 4-5 mm segment of the buccal division of the rabbit facial nerve was removed, the nerve ends were inserted into a 10 mm tube and sutured to create an 8 mm nerve gap. For nerve graft repair, an 8 mm autologous segment of the buccal nerve was removed, rotated 180° and sutured to bridge the nerve gap. Five weeks post-implantation, the animals were processed for morphological evaluation. NGF treated nerves recovered significantly higher percentage of their preoperative number of myelinated fibers than Cyt. C treated nerves (46% vs 18%). The number of myelinated fibers at the nerve grafts midpoint was significantly greater than at the NGF tube midpoint. However, the number of myelinated fibers reaching the distal nerve stump was proportionately greater in NGF treated nerves than in nerve grafts. In nerve grafts, the percentage of intrafascicular myelinated fibers was inversely related to the amount of myelin debris and the majority of fibers (66%) were extrafascicular. Thus, although nerve grafts contain larger number of myelinated fibers than NGF-containing tubes, functional recovery and elimination of aberrant reinnervation may not be superior to entubation repair with NGF.

599.4

INHIBITION OF NGF-STIMULATED NEURITE OUTGROWTH BY AMINE-MODIFIED α_2-MACROGLOBULIN.

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Alpha-2-macroglobulin (α_2M) is a rather ubiquitous protein in extracellular spaces of mammals. It is an inhibitor of endopeptidases and a carrier for a number of cytokines/hormones such as β-nerve growth factor (NGF) (P.H. Koo and R.W. Stach, J. Neurosc. Res. 22:247, 1989). The objective of this study is to determine whether α_2M and its modified forms may affect the biological activity of NGF. Alpha-2-M was purified from human plasma and then modified by either methylamine or trypsin. Our results clearly show that methylamine-modified α_2M (MA-α_2M) exerted a dose-dependent inhibition on the NGF-stimulated neurite outgrowth by embryonic chicken dorsal root ganglia whereas normal α_2M and trypsin-modified α_2M had little or no effect. MA-α_2M is not toxic to neurons and the inhibition can be overcome by higher NGF concentrations. As determined by gel filtration, native polyacrylamide gel electrophoresis and SDS-urea gel electrophoresis, both normal α_2M and MA-α_2M carry >90% of NGF noncovalently. Although MA-α_2M carries about twice as much NGF, much higher doses of normal α_2M had little or no effect on the biological activity of NGF. This suggests that amine-modified α_2M may inhibit neurite growth via an active process not involving passive absorption of NGF.

599.5

NGF FACILITATES THE RECOVERY OF NIGRO-STRIATAL DOPAMINERGIC NEURONS AFTER A LESION INDUCED BY MPTP.

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Nerve growth factor (NGF) promotes the development and survival of sympathetic and sensory neurons in the periphery, however, it is reported to be neurotrophic for cholinergic but not catecholaminergic neurons of the brain. We administered NGF 7S ICV for 2 weeks to mice pretreated with MPTP, 30 mg/kg i.p. for 7 days, and found that NGF facilitated the recovery of the nigrostriatal dopaminergic neurons. In our model, administration of MPTP results in about a 60% decrement of striatal dopamine (DA), tyrosine (TH) activity, and DA uptake, which is accompanied by loss of TH-immunopositive cells in substantia nigra. The administration of NGF 24 hr after completing the MPTP treatment restored DA, TH activity and DA uptake in a dose-dependent manner. Moreover, NGF restored the number of TH-immunopositive cells in substantia nigra of MPTP-treated mice. NGF had no effect on the above neurochemical parameters in control animals.

599.6

CHRONIC TREATMENT WITH NGF ALTERS MONOAMINE METABOLISM IN RATS WITH LESIONS OF THE NBM.

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Rats received bilateral lesions of the nucleus basalis magnocellularis by infusion of ibotenic acid (28 nmoles in two injections per side). Fourteen days later, osmotic minipumps, releasing human recombinant NGF (0.3 µg/day) were implanted subcutaneously. Pumps were replaced every 14 days. After 6 weeks the rats were sacrificed and the levels of monoamines and their metabolites were determined in the frontal and parietal cortex and hippocampus. Treatment with NGF led to a 50% reduction in the levels of 5-HIAA in cortical areas and DOPAC in the hippocampus. NGF did not affect the levels of DOPA, NE, MHPG, DA, HVA and 5HT in any of the brain regions studied. These results suggest that chronic treatment with NGF influences the turnover of serotonin and dopamine in selected brain regions. These non-cholinergic effects may play a role in the amelioration of behavioral deficits by NGF in rats with lesions of the nucleus basalis.

599.7

NERVE GROWTH FACTOR PREVENTS TOXIC NEUROPATHY. M. E. Moran*, S. C. Apfel, J. C. Arezzo, and J. A. Kessler. Albert Einstein College of Medicine, Bronx, N.Y. 10461

Nerve Growth Factor regulates the survival and function of subpopulations of dorsal root ganglia sensory neurons in vitro and in vivo. We have previously demonstrated the ability of NGF to prevent the small fiber sensory neuropathy caused by administration of the experimental anti tumor agent taxol. In this study we sought to determine whether NGF is also capable of preventing the large fiber sensory neuropathy caused by the widely used anti tumor agent cisplatin. Cisplatin was administered intraperitoneally to adult mice at a dose of 10 mg/kg once a week for eight weeks. The animals were concurrently injected subcutaneously with either NGF (5 μg/g) or control buffer three times a week. After the eight week administration period peripheral nerve function was examined using carefully selected behavioral, biochemical, and electrophysiological techniques. Cisplatin administration impaired proprioception as measured by the ability to balance on a rotating dowel ($p<0.005$), reduced sensory ganglion levels of the peptide transmitter calcitonin gene related peptide ($p< 0.02$) and slowed nerve conduction in the tail ($p <0.006$). NGF coadministration prevented all of these abnormalities, indicating that the factor was effective in averting cisplatin induced neuropathy. We are currently testing the ability of NGF to prevent other diseases of the peripheral nervous system. These observations suggest that clinical trials of the growth factor are now warranted.

599.8

CHROMATOLYTIC-LIKE ALTERATIONS AND ABERRANT NEUROFILAMENT PHOSPHORYLATION IN SENSORY NEURONAL PERIKARYA FOLLOWING NGF ANTISERUM INJECTION. B.G.Gold, D.R. Austin*, W.C. Mobley^ and T. Storm-Dickerson*. Center Res. Occup. and Environ. Toxicol., Oregon Health Sci. Univ., Portland, OR 97201 and ^Depts. of Neurol. and Pediat., UCSF, San Francisco, CA 94143.

The mechanism by which the neuronal perikaryal response to injury (axon reaction) is initiated is unknown. Our previous study utilizing nerve growth factor (NGF) antiserum injections for 2 weeks (Gold et al., J. Neurosci. 11:943,1991) demonstrated that loss of NGF triggers two components of the axon reaction (i.e., somatofugal axonal atrophy and nuclear eccentricity). In the present study, three-week-old male rats were given daily injections of goat-antimouse NGF antiserum (0.5 μl/gm) into the left footpad for 4 weeks; age-matched controls received normal goat serum. At 7 weeks of age, the rough endoplamic reticulum was displaced to the periphery of the cell body in some (5-10%) L4-L6 DRG neuronal perikarya. Eccentric nuclei demonstrated prominent nuclear membrane infoldings. Some neurons showed accumulations of lysosomes and lipofusin granules. Axonal atrophy, present only at the level of the DRG at 2 weeks, was observed in the distal portion of the dorsal roots, demonstrating its somatofugal progression. Preliminary immunocytochemical studies (peroxidase-antiperoxidase method) revealed light to modest immunoreactivity in neuronal perikarya (approx. 10%) to phosphorylated neurofilament (pNF) epitopes (antibodies 6-17 and 7-05). The results suggest that interruption of target-derived supply of NGF initiates chromatolytic-like alterations and aberrant expression of pNF epitopes in DRG neurons. Supported by NS26265.

599.9

NERVE GROWTH FACTOR (NGF) CHANGES IN RAT EXPERIMENTAL DIABETIC NEUROPATHY.

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NGF-responsive peripheral neurons with small and unmyelinated fibers are affected at an early stage of diabetes mellitus. As revealed by NGF enzyme-linked immunoassay, streptozotocin (STZ)-induced destruction of insulin-producing pancreatic islets, which causes diabetes mellitus led to up to a 3-fold increase of NGF levels in most NGF-producing tissues (Iris, heart atrium and ventricle, spleen, vas deferens), but to a 50% decrease in the NGF-dependent sympathetic superior cervical ganglion[1]. This decrease in the superior cervical ganglion was reversed by successful pancreatic islet allotransplantation. Since NGF is known to exert its effects after retrograde axonal transport together with NGF receptor, we have studied this axonal transport in the rat sciatic nerve 8 weeks after the induction of diabetes with STZ. The overall retrograde transport of NGF decreased to 50% of untreated controls ($p<0.001$ in a 2-sided Student' t-test), and the amount of retrogradely transported NGF in the STZ-treated animals was negatively correlated with the individual blood glucose concentration ($r=0.68$, $p<0.002$).

Taken together, our results could suggest a pathophysiological role for NGF in diabetic neuropathy which may be due to a decreased neuronal capacity of NGF uptake and retrograde transport. Thus, prevention of diabetes-related NGF deprivation in NGF-dependent peripheral neurons could play an important role in the future therapy of diabetic neuropathy.

[1]Hellweg, R. and Hartung, H.D. J. Neurosci. Res. 26:258-267, 1990

599.10

THE EXPRESSION OF NERVE GROWTH FACTOR RECEPTOR AND HEAT SHOCK PROTEIN mRNA IN ALS SPINAL CORD. J. L. Seeburger, S. Tarras, M.E. Lewis, E. Robbins, and J.E. Springer, Dept. of Neurology, Hahnemann University, Philadelphia, PA 19102, and Cephalon, Inc. 145 Brandywine Pkwy, West Chester, PA 19380 (MEL and ER).

The trophic actions of nerve growth factor (NGF) are necessary for the development and maintenance of neural crest derived sensory, sympathetic and basal forebrain cholinergic neurons. Recently it has been shown that alpha motor neurons may also utilize NGF. These neurons express nerve growth factor receptors (NGFR) developmentally and in response to mechanical injury to peripheral axons. Because of these findings, we were interested in the role of NGF in the alpha motor neuron response to pathological degenerative processes. We also analyzed whether alpha motor neurons may express heat shock protein (HSP) mRNA, which may identify vulnerable cells. An in situ hybridization procedure was used to localize alpha motor neurons expressing NGFR and HSP mRNA in postmortem spinal cords from patients with amyotrophic lateral sclerosis (ALS). Cervical, thoracic, and lumbar sections were processed with a radioactive 2.5 kb cRNA probe complimentary to rat NGFR mRNA, or a 30-mer oligonucleotide probe complimentary to HSP mRNA. Additional sections were processed using cresyl violet or silver degeneration staining procedures. The expression of NGFR mRNA in the ventral horn was greatest at those spinal cord levels which exhibited the most extensive cell loss and agyrophilia, and which were associated with the most extreme clinical symptomatology. In addition, HSP mRNA was localized primarily to a few ventral horn cells in all segments analyzed. We hypothesize that as the disease process advances, some motor neurons start to re-express growth related proteins in an attempt to compensate for the progressive degenerative state. This is supported by EMG and histochemical evidence demonstrating the reinnervation of denervated muscle via collateral sprouting in ALS patients. Supported by PHS grant AG-08969 (JES).

599.11

FLUORESCENT AUTOMATED SEQUENCING OF PCR AMPLIFIED DNA CORRESPONDING TO THE PROTEIN CODING REGION OF THE HUMAN B NERVE GROWTH FACTOR GENE.

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PCR primers with added restriction enzyme sites were synthesized that flanked the protein coding region of the human β nerve growth factor (NGF) gene (Ullrich et.al., Nature, 1983). After the primers were used to PCR amplify normal and schizophrenic human genomic DNA, the resultant double-stranded DNA was restriction-digested and ligated into Bluescript. The circularized vector with NGF insert was transfected into competent E. coli. Resulting white colonies were screened for NGF insert by amplifications of DNA with universal and reverse M13 primers. The positive NGF PCR products were purified using Centricon 100 filters and used for DNA sequencing. Sequence analysis was carried out with fluorescent-labeled universal and reverse primers, using the Applied Biosystems, Inc. cycle sequencing linear amplification protocol. Sequencing reactions were analyzed on an ABI 373 DNA Sequencer. Automated DNA sequencing of the NGF gene as described here has the potential to identify changes in the primary sequence of NGF that may be associated with specific disease states. This work was supported by the VA Medical Research Service and USPHS Grant No. P50 MH44212-02.

599.12

DELETION ANALYSIS OF MOUSE NERVE GROWTH FACTOR

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Nerve growth factor (NGF) is a trophic peptide responsible for the survival, development, maintenance and regrowth of neural-crest derived sensory and sympathetic neurons as well as basal forebrain cholinergic neurons. Binding sites and mRNA for NGF have been detected throughout the developing avian and mammalian central nervous system, indicating that NGF may affect other cell types early in development. The primary structure of NGF from several different species has been determined by amino acid and nucleotide sequencing, however, as yet, resolution of the three-dimensional structure has not been completed. NGF is initially synthesized as a 33.8 kDa precursor, from which the mature form is cleaved by one or more trypsin-like enzymes. In mouse, active NGF appears to comprise a non-covalently bound homodimer of 118 residue chains, each with three internal disulfide bridges. In an attempt to define a minimal structure of NGF with biological activity, a series of deletion mutants were constructed, using DNA *in vitro* mutagenesis. These mutants were expressed in COS-7 cells and analysed for biological activity. The yields of two mutants were significantly reduced in comparison to wild type recombinant NGF, and no activity could be detected in a neurite outgrowth assay. Though the third mutant, in which amino acids 3-14 were deleted, was produced in relatively high amounts, it was not correctly processed to the mature, active form. Treatment with trypsin does not appear to activate this mutant precursor. Therefore, this region may be critically involved in the specific processing of NGF from its precursor. We are currently examining this region in greater detail by further *in vitro* mutagenesis.

599.13

A RAT CELL LINE PRODUCING RECOMBINANT HUMAN NERVE GROWTH FACTOR (rhNGF)

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By grafting genetically modified cell lines that secrete neurotrophic factors to the CNS, the problems of long term delivery of trophic proteins across the blood brain barrier can be overcome. In the present experiments we wished to obtain a cell line which secreted rhNGF and could be grafted to Sprague-Dawley rats without immunosuppression. The coding portion of the human NGF gene was therefore introduced (using MRE(4X)-OVEC vector) into a rat cell line immortalized from E15 Sprague-Dawley rat muscle tissue through 47 passages. Clones were tested for NGF production (approximately 0.5-1.0 ng/ml of conditioned medium) by a biological assay using fibre outgrowth from embryonic sympathetic ganglia, as well as a two-site enzyme immunoassay for NGF. Pieces of collagen gel containing rhNGF-secreting or parental cells were grafted to unilaterally fimbria-fornix lesioned adult Sprague-Dawley rats. Grafts of these cells survived well and rescued axotomized cholinergic neurons in the medial septum, as compared with non-transfected, parental cell lines. These studies indicate that cell lines established from the recipient strain survive grafting without immunosuppression and can secrete amounts of biologically active NGF sufficient to exert tropic effects on damaged CNS neurons in vivo.

599.14

A FUNCTIONAL DOMAIN OF THE LOW AFFINITY NGF RECEPTOR (LNGFR).

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Regions of the cytoplasmic domain of LNGFR which might interact with G protein subunits were identified by comparison with the cationic amphiphilic tetradecapeptide mastoparan, which catalyzes nucleotide exchange on G proteins similar to that of receptors. The program PRISM was used to identify αhelical regions and BIOGRAF was used to construct and minimize these regions using an AMBER force field with the αhelix constrained and the amino acid side chains free to find their most stable conformations. Synthetic peptides were tested for effects on trophic factor induced survival/differentiation responses of rat PC12 cells, chick DRG neurons and chick ciliary neurons. A peptide representing residues 370-383 of human LNGFR accelerated the neurite growth response to NGF of PC12 cells and DRG neurons but not the survival effect of CNTF on ciliary neurons. Mastoparan and a hybrid peptide had no effect. Millimolar Mg^{2+} and benzalkonium chloride blocked the effect of the peptide on PC12 cells, and, of three bacterial toxins tested, only diphtheria was inhibitory. The LNGFR may modulate the biological effect of NGF on responsive cells mediated by the high-affinity NGF receptor by influencing an effector system coupled to a diphteria toxin sensitive G protein.

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599.15

RECEPTOR RADIOAUTOGRAPHY AND IN SITU HYBRIDIZATION FOR NGF RECEPTORS IN PRIMARY SENSORY NEURONS.

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Individual neurons in adjacent sections of rat lumbar DRG, nodose ganglia, and trigeminal ganglia have been examined by receptor radioautography for high-affinity NGF receptors (HNGFR) and in situ hybridization with an oligonucleotide probe for the low-affinity NGF receptor (LNGFR). In normal lumbar DRG, all neurons with HNGFR plus some additional neurons have relatively high levels of LNGFR mRNA. In nodose ganglia, many neurons lacking HNGFR have abundant LNGFR mRNA. Intrathecal infusion of NGF increases expression of the LNGFR gene in lumbar DRG of otherwise normal rats and, for neurons with HNGFRs only, counteracts a sharp down-regulation of LNGFR mRNA which follows sciatic nerve transection. The observations are consistent with the hypotheses that i) the HNGFR consists of the NGF-inducible LNGFR plus some unidentified component, ii) the previously reported down-regulation of the HNGFR following peripheral injury results from interruption of NGF support for the LNGFR, iii) LNGFRs in some sensory neurons lacking HNGFRs could constitute one component of BDNF receptors.

599.16

A RAPID AND HIGHLY QUANTITATIVE SOLUTION HYBRIDIZATION METHOD FOR DETECTING NERVE GROWTH FACTOR RECEPTOR MESSENGER RNA.

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Currently the most sensitive method of detecting a specific species of messenger RNA (mRNA) from total cellular RNA is by nuclease protection assay (NPA). Though highly sensitive, the accuracy of the NPA is limited by variations in efficiency per sample in 1) ethanol precipitation, 2) dissolution and gel-loading of hybridized fragments, and 3) densitometric analysis. Furthermore, NPA analysis of mRNA is slowed by the extended time often necessary for autoradiography and the limited number of samples allowable on a standard electrophoresis apparatus.

We report the application of a solution hybridization technique (SHyT), described by Franklin et al. (Molec. Brain Res. (1991) in press), to quantitate levels of mRNA for nerve growth factor receptor (NGF-R) derived from rat brain. With the SHyT, protected riboprobe fragments are TCA precipitated, harvested over glass-fiber filters, and quantititated immediately by scintillation spectroscopy. Utilization of the SHyT led to a marked increase in sample reproducibility, linearity over two orders of magnitude, and approximately a twofold increase in assay sensitivity. Moreover, direct counting of samples and the lack of sample number limitation allowed RNA analysis of multiple experimental groups in days instead of weeks. Hence the SHyT provides a significant increase in the efficiency and accuracy of mRNA analysis compared to previously described methods.

(Support: NINDS, NICHD, NIDA, and McKnight Foundation).

599.17

CALCIUM ANATGONISTS PREVENT SMOOTH MUSCLE HYPERTROPHY AND NEURAL PLASTICITY ASSOCIATED WITH BLADDER OUTLET OBSTRUCTION.

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Calcium influx has been implicated as an initial signal triggering smooth muscle hypertrophy and growth factor production. Rats with bladder outlet obstruction were maintained for 3 weeks on verapamil (V) (75mg/kg/da PO) and diltiazem (D) (100mg/kg/da PO). Both drugs significantly prevented the increase in bladder weight following obstruction [397mg (no drug) to 277mg (V) and 237mg (D), ($p<0.05$)]. Bladder tissue from obstructed rats maintained on calcium antagonists contained less smooth muscle. The calcium antagonists also reduced the neuronal hypertrophy of pelvic ganglion cells (525um^2 to 411um^2 ($p<0.001$)) and nerve growth factor levels (45.2pg to 23.1pg (V)). Calcium antagonists did not prevent the increased voiding frequency (1.25 vs 1.18 (V) and 0.98 (D) hr^{-1}) associated with obstruction. Prazosin (10mg/kg/da) did not affect bladder enlargement (396mg) or neuronal hypertrophy (522um^2). However, this alpha-1 adrenergic blocker prevented the increase in voiding frequency (0.89hr^{-1}, $p<0.05$). These data suggest that calcium is involved in the muscle growth and neural plasticity associated with obstruction. Furthermore, alpha adrenergic antagonists abolish symptoms of bladder hyperactivity independent of the relief of obstruction.

599.18

GLUCOCORTICOIDS REGULATE mRNA EXPRESSION FOR NEUROTROPHIC FACTORS IN THE RAT BRAIN

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The hippocampus is the major target region for glucocorticoid action in the nervous system. Adrenalectomy results in a nearly complete cell loss of hippocampal granule cells 3 to 4 months after surgery. In the present study we investigated the effect of glucocorticoids on neurotrophic factor mRNA expression. In the rat brain NGF mRNA expression in the hippocampus was found to be significantly decreased 3 days after adrenalectomy compared to sham-operated animals. Corticoid replacement by daily s.c. dexamethasone injections (1mg/kg) restored NGF mRNA levels to normal. The same effect was seen for BDNF and NT-3 mRNAs. Sham animals recieving s.c. dexamethasone injections showed no significant changes in neurotrophic factors mRNA expression.

The effect of adrenalectomy on neurotrophic factor mRNA expression in the cortex was also assesed and the results showed a decrease in the level of mRNA for all three factors. As in the hippocampus, the increase in the cortex was also prevented by dexamethasone replacement. The effect of a single dose of dexamethasone (5mg/kg) administered i.p. to intact animals on the expression of neurotrophic factors was also analysed. NGF mRNA in the cortex was significantly increased after a single i.p. injection of dexamethasone . The mRNA for NT-3 showed only a sligth increase and BDNF mRNA was the same as in control animals recieving vehicle injection.

600.1

MORE REGENERATED AXONS IN THE DISTAL STUMP OF TRANSECTED SCIATIC NERVE TREATED WITH GLIAL MATURATION FACTOR ß. K. Harman*, J. Katnick*, R. Lim, J.C. de la Torre. Univ. of Ottawa, Ontario Canada KIH 8M5

Sixteen female Sprague-Dawley rats were anaesthetized and prepared for bilateral sciatic nerve transection. The transection and entubulation repair involved: pre-treating the nerve with 15% polyvinyl alcohol:chlorpromazine solution, to reduce post-transection extrusion of the nerve tip; rapid cooling with fluorimethane spray, to render the nerve firm; transection with an ultrafine-edged razor blade. In each animal, the 2 mm (± 0.25 mm) interstump gap created was filled with either collagen only (COL) or a collagen / neurotrophic (COL/NTF) factor mixture. Each NTF held the prospect of exerting a stimulatory or protective effect on regenerating axons when applied at the transection and repair site and were chosen because they were as yet untested in the sciatic nerve. The concentration of each NTF/ml collagen was: 4-aminopyridine (4-AP) 0.2 mg; lipid angiogenic factor (LAF) 2.0 mg; leupeptin (LEU) 0.2 mg; ß-GMF 20 ng. After six weeks the number of regenerated myelinated axons in the distal stump were counted using our line-sampling quantification technique, and compared to the controls (COL). We found that nerves treated with 4-AP, LAF and LEU had both greater and fewer axons. However, in the distal stumps of the nerves treated with ß-GMF, there were consistently more axons (48-52%). We therefore conclude that ß-GMF may stimulate regeneration in the peripheral nervous system. The implantation of a COL/NTF mixture in our entubulation preparation provides a useful and quantifiable technique for screening potential enhancers of peripheral axon growth.

Funded by the Easter Seal Research Institute (Ont.).

600.2

MODULATION OF 2-DEOXYGLUCOSE UPTAKE IN CULTURED ASTROCYTES. D.L. Davies and T.M. Ross*. Dept. of Anatomy, Univ. of Arkansas for Med. Sci., Little Rock, AR 72205.

Reactive differentiation of astrocytes is a key component of the cerebral response to injury. As part of an endeavor to define the metabolic changes associated with cellular differentiation, 2 deoxyglucose (2DG) uptake was measured in cultures treated with agents known to induce morphologic reconfiguration or impede proliferation. Secondary rat astrocytic cultures were treated with dibutyryl cyclic AMP (dBcAMP), a cerebral tissue extract with morphogenic properties or ethanol levels sufficient to diminish proliferation. The 2DG uptake was measured using the method of Brooks and Yarowsky (1985, J. Neurochem. 44:473-479). Increased 2DG uptake was detected in control cultures at 3 and 6 hrs after replenishing the nutrient medium, but uptake values returned to baseline at 24 hr. Treatment with either dBcAMP (1mM) or cerebral extract (1mg/ml) enhanced 2DG uptake at 3, 6 and 24 hrs. These changes in 2DG were noted prior to qualitative changes in astrocytic morphology. The increase in 2DG uptake induced by treatment with dBcAMP and cerebral extract (0.5 mg/ml) was sustained at 24 and 48 hrs of treatment. Simultaneous exposure to 0.2% or 0.5% (w/v) ethanol augmented this response to dBcAMP and cerebral extract at 24 hr. These data suggest that metabolic changes requiring glucose consumption preceed alterations in astrocytic morphology and may have a role in reactive differentiation.

Supported by NIAAA grant AA07145.

600.3

NEURONAL SURVIVAL OF CEREBELLAR GRANULE CELLS *IN VITRO* IS REGULATED BY LEVELS OF INTRACELLULAR CALCIUM. T.Koike. Department of Natural Science, Saga Medical School, Nabeshima, Saga 84001, JAPAN.

We have previously proposed a calcium set-point hypothesis for the degree of neuronal dependence on trophic factor *in vitro* (T.Koike et al. PNAS, 86:6421, 1989), and provided evidence for this hypothesis in sympathetic neurons (T.Koike and S.Tanaka, ibid, 88, in press,1991). Here, as an extension of this study, we examined the relationship between intracellular calcium ([Ca2+]i) and survival of cerebellar granule neurons. The granule neurons isolated from 7-day-old postnatal rats undergo massive degeneration around DIV 5, which appears to mimic neuronal death due to unavailable trophic factor that occurs in development. Chronic depolarization with high potassium prevents this cell death. The neurons were grown in various concentrations of potassium for 1 or 2 days, loaded with fura-2 (2µM), and their [Ca2+]i levels were measured using digital fluorescence imaging. Neuronal survival was determined by measuring release of lactate dehydrogenase activity into medium or counting the number of viable neurons. By combining these two data we generated a correlation curve between [Ca2+]i and neuronal survival similar to that for sympathetic neurons; the result indicates that neurons with [Ca2+]i =30nM at 5mM K did not survive and the compete survival occurred at 150nM (at 25mM K). Moreover, NMDA is known to mediate neuronal survival of granule cells. We found that NMDA elicited a sustained level of [Ca2+]i which is pertinent to neuronal survival. These results demonstrate the feasibility of this hypothesis in neurons from the central nervous system as well as in peripheral neurons.

600.4

NICOTINIC AGONISTS, PHORBOL ESTERS AND GROWTH FACTORS ACTIVATE TWO DISTINCT MYELIN BASIC PROTEIN KINASES IN BOVINE CHROMAFFIN CELLS. B. Pavlovic-Surjancev*, A.L. Cahill, R.L. Perlman. Depts. of Pediatrics and Pharmacol. and Physiol. Sciences, The University of Chicago, Chicago, IL 60637

Insulin-like growth factor I (IGF-I) increases protein kinase activity against microtubule associated protein-2 and myelin basic protein (MBP) in bovine chromaffin cells (Cahill and Perlman, J. Neurochem., in press). To characterize the MBP kinases in chromaffin cells, we separated chromaffin cell proteins by electrophoresis on SDS-polyacrylamide gels into which MBP had been incorporated, allowed the proteins to renature and then assayed MBP kinase activity by incubating the gels in solution containing [γ-^{32}P]ATP. We have identified six MBP kinases, with M_r of 116, 80, 60, 53, 46 and 42,000. The MBP kinases of M_r 46,000 and 42,000 (PK46 and PK42) were activated by treatment of the cells with the nicotinic agonist dimethylphenylpiperazinium (DMPP), phorbol 12,13-dibutyrate (PDBu) or IGF-I. The involvement of protein kinase C (PKC) in the activation of PK46 and PK42 was examined. Down regulation of PKC by incubation of the cells with PDBu abolished the activation of both PK46 and PK42 by DMPP, PDBu and IGF-I. Staurosporine, a PKC inhibitor, prevented the activation of PK46 and PK42 by DMPP and PDBu, but did not block the activation of these kinases by IGF-I. Thus, chromaffin cells appear to contain two MBP kinases that can be activated by both PKC-dependent and PKC-independent mechanisms. These kinases may participate in protein kinase cascades activated by various agonists. (Supported by research grants from NIH and NSF)

600.5

CHOLINERGIC DIFFERENTIATION OF CULTURED RAT SYMPATHETIC NEURONS BY FACTORS APPLIED TO DISTAL NEURITES. D. R. Ure, R. B. Campenot, and A. Acheson. Dept. of Anatomy and Cell Biology, Faculty of Medicine, Univ. of Alberta, Edmonton, Alta., Canada T6G 2H7.

The development of cholinergic properties in rat sympathetic neurons in vivo is believed to occur in response to factors derived from target tissues. Although the cholinergic switching event in mass cultures occurs in response to a variety of factors, only one model, the rat sweat gland innervation, has directly supported the theory of target-mediated cholinergic differentiation. The results of our studies using a variety of factors applied to rat sympathetic neurons in a compartmented culture system also support that theory. When 600 units/ml leukaemia inhibitory factor (LIF; Dal.a cell line proliferation assay), 50% heart cell conditioned medium, or 50% dermal fibroblast conditioned medium are supplied to only distal neurites from Days 7-21 in culture, a many-fold increase in specific choline acetyltransferase (ChAT) activity and a decrease in tyrosine hydroxylase immunoreactivity are observed. The increase in ChAT activity is time- and concentration-dependent. Our results suggest that LIF and the factor(s) in conditioned media could act as target-derived differentiation factors in vivo.

600.6

NOT ALL CNS TRANSPLANTS CAN RESCUE AXOTOMIZED CLARKE'S NUCLEUS NEURONS. B.T. Himes[1], P. Baker*[1], M.E. Goldberger[1], and A. Tessler[1,2] Dept. of Anatomy and Neurobiology, The Medical College of Pennsylvania[1], and VA Medical Center[2], Philadelphia, PA.

Axotomy of Clarke's nucleus (CN) results in a 50% loss of CN neurons in neonatal rats and a 30% loss in adults 2 months postoperatively. This retrograde degeneration is a reaction to the loss of target derived neurotrophic factors. Grafts of fetal spinal cord, cerebellum, or neocortex all prevent lesion-induced cell death and maintain normal numbers of CN neurons for at least two months following surgery (Himes et al.'90, Soc. Neurosci. Abst. 16:818). These three areas of embryonic CNS produce a neurotrophic factor, neurotrophin-3 (NT-3). NT-3 provided by the grafts may be responsible for the survival of axotomized CN neurons. We therefore transplanted into P2 rats an area of the CNS that does not contain NT-3, the striatum (Maisonpierre et al.,'90 Neuron 5:501-509). Grafts of E14 striatum were placed into a spinal cord hemisection cavity at T8. The transplants survive, although they are not well integrated with the host spinal cord. CN in the L1 spinal cord segment was analyzed 2 months postoperatively. The mean number of surviving CN neurons ipsilateral to the lesion is reduced by 50% (n=3). This cell loss is equivalent to that seen in the neonate after hemisection alone. Transplants of striatum appear to be unable to rescue axotomized CN neurons, perhaps because they do not provide NT-3.

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600.7

LAMININ B2 mRNA IS UP-REGULATED IN SENSORY NEURONS AND SCHWANN CELLS DURING PERIPHERAL NERVE REGENERATION. J. M. Le Beau[1] and F. J. Liuzzi[2]. Diabetes Institute[1] and Dept. of Anatomy and Neurobiology[2], Eastern Virginia Medical School, Norfolk, VA. 23501

Laminin, a large glycoprotein consisting of three polypeptide chains, is an extracellular protein that has been implicated in promoting Schwann cell-neuron interactions. Laminin functions as a potent stimulator of neurite extension and can enhance neuronal survival *in vitro*. *In vivo*, its presence on the surface of Schwann cells has been correlated with the advancement of neurites regenerating across a 10 mm gap (Longo et al., Brain Res. 309:105-107). We examined regenerating rodent peripheral nerves and their associated sensory neurons for changes in laminin mRNA expression by *in situ* hybridization with sense or anti-sense laminin B2 RNA probes. Regenerated sciatic nerves showed an up-regulation of laminin B2 mRNA in Schwann cells compared to control sciatic nerve. More suprisingly, we detected laminin B2 mRNA in L4 and L5 DRG neurons and this mRNA appeared to be up-regulated during regeneration. Increased laminin B2 mRNA expression was also observed in the satellite cells of those DRGs after sciatic nerve injury. These findings suggest that neurons, as well as supporting cells, may be capable of regulating the synthesis and secretion of a glycoprotein implicated in their own as well as neighboring cells differentiation, migration and in the case of neurons, neurite elongation. Immunohistochemical studies are currently in progress to determine the relationship between the synthesis and deposition of laminin during peripheral nerve regeneration.

600.8

SCHWANN CELLS PROMOTE SURVIVAL AND MORPHOLOGY OF NEURONS FROM POSTNATAL LOCUS COERULEUS, SUBSTANTIA NIGRA, AND CEREBRAL CORTEX. M.B. Clark and C. Chui. Dept. Anatomy, Univ. MD. Sch. of Med.,Baltimore MD 21201.

We studied dissociated cell cultures from the locus coeruleus, substantia nigra and cerebral cortex of postnatal rats to determine if Schwann cells (SCs) and SC products promote survival and regeneration of specific types of postnatal CNS neurons (NCs). We used an image analysis system to measure several morphometric parameters of NCs to assay for the effects of SCs or SC conditioned medium (SCM) on the regenerative capacity of surviving NCs. NCs were identified by immunostaining for neurofilament (Nf), Tau protein, and tyrosine hydroxylase (TH). Two populations of NCs were identified by their immunoreactivity for Tau and/or NF: Tau positive (Tau+) alone or both Nf and Tau positive (Nf/Tau+). In addition, a third population of NCs which was both TH and Nf immunoreactive (TH/Nf+) was identified in cultures from brainstem nuclei. Neuronal survival of all three populations was increased at least 1.5 fold in the presence of living SCs and in SCM as compared to appropriate controls, including neurons grown in the presence of fibroblasts. Interestingly the greatest effect of SCs and SCM was seen on the NF/Tau+ subpopulation. SCs or SCM increased the number of these NCs by at least 10 fold. Image analysis of NF/Tau+ NCs revealed an increase in somal perimeter, mean number of primary neurites, mean number of processes, total neurite length, and the extent of neuritic field in the prescence of SCs or SCM. Filtration and dialysis experiments appear to indicate that the active components in SCM are ≥ 10kDa. Our results suggest that SCs and soluble SC products support both the survival and regenerative capacities of several specific classes of postnatal CNS neurons. (Supported by NS24252).

600.9

SAFETY TESTING OF INTRATHECAL URIDINE AND OROTIC ACID IN RABBITS. C.K. Haun, G. Goubran*, W.K. Engel and I. Tan*, Depts. of Anatomy & Cell Biology and Neurology, USC School of Medicine, Los Angeles, CA 90033.

In an ongoing study of agents with potentially trophic effects upon spinal cord neurons, we have safety-tested intrathecal uridine (U) and orotic acid (OA), two precursors of RNA via the salvage & de novo paths, respectively.

In uM/Kg/d, doses of U ranging from 410 to 1640, and OA, from 440 to 3520, were administered to young male and female rabbits with Alzet osmotic pumps, through a catheter inserted into the spinal subarachnoid space at the L7-S1 interspace. Freshly loaded pumps were reimplanted at two-week intervals for as long as tolerated.

Of the two, OA seemed better tolerated: viz. 3520 uM/Kg/d for over five mos., and one rabbit tolerated 880 uM/Kg/d for over 9 mos., without discernable side effects. U was well tolerated for over 20 wks. at 820 uM/Kg/d; but with 1640 uM/Kg/d, diarrhea and weight loss occurred after 9 wks. The animals continued to maintain normal growth and behavior throughout the treatment periods, but at a reduced rate with the higher dosages of OA & U, respectively 3520 and 1640 uM/Kg/d.

Thus, OA & U will probably be safe, within the dose ranges noted above, for intrathecal administration as potential therapeutic agents for human neurological disorders.

600.10

S100β, A GROWTH FACTOR WITH NEUROTROPHIC ACTIVITY, STIMULATES INCREASES IN INTRACELLULAR CALCIUM IN NEURONAL AND GLIAL CELLS. S.W. Barger and L.J. Van Eldik. Depts. of Cell Biology and Pharmacology, Vanderbilt Univ., Nashville TN 37232.

Recent data have suggested that S100β is a bifunctional growth/differentiation factor, stimulating proliferation of glial cells and survival and morphological differentiation of immature neurons. We demonstrate here that dimeric S100β can evoke increases in free, intracellular calcium concentrations ($[Ca^{2+}]_i$) in both glial and neuronal cells. The S100β-induced $[Ca^{2+}]_i$ increase included a rapid, transient component that was independent of extracellular calcium and a sustained, reversible elevation that was dependent on calcium influx. The monomeric form of S100β appeared to lack the ability to evoke this response. The S100β-induced $[Ca^{2+}]_i$ increase was blocked by an inhibitor of phospholipase C, consistent with the ability of S100β to stimulate the hydrolysis of phosphatidylinositol. In at least one neuronal cell type, S100β stimulated increases in $[Ca^{2+}]_i$ synergistically with high K^+. These data demonstrate that while the biological responses of neuronal and glial cells to S100β are quite different, transduction of the S100β signal in both cell types may involve changes in $[Ca^{2+}]_i$. (Supported by Muscular Dystrophy Association.)

600.11

RELEASE OF OTOCYST-DERIVED TROPHIC FACTORS IS DEVELOPMENTALLY REGULATED. L.M. Bianchi and C.S. Cohan, Dept. of Anatomical Sciences, SUNY ay Buffalo, Buffalo, N.Y. 14214

During the early stages of auditory development, the otocyst releases a diffusible trophic factor which promotes the outgrowth of neurites from statoacoustic ganglia (SAG). The present experiments evaluated the developmental time course of this trophic influence by coculturing E4-E15 SAG explants with otocysts of various developmental stages. In the absence of otocysts, little neurite outgrowth was seen from SAG explants at all stages. However, extensive neurite outgrowth occurred when E4-E6 SAG were cultured in the presence of otocysts of the same age. The amount of neurite outgrowth observed in cocultures steadily decreased at later development stages. E7-E9 cocultures produced less outgrowth and E10-E13 cocultures produced the least outgrowth compared to E4-E6 cocultures. Additionally, otocysts from older stages (E9) were unable to promote outgrowth of E4 SAG. Thus, the level of the trophic factor released by the otocysts declines during development. In contrast, the ability of SAG neurons to respond to the trophic factor was maintained throughout development. Neurite outgrowth from E10-E15 SAG was promoted in the presence of the conditioning factor released by younger stage (E4) otocysts. The results of our experiments indicate that there is a developmentally-regulated release of a diffusible chemotrophic factor from the otocyst which influences neurite outgrowth from SAG. (Supported by grants from the Deafness Research Foundation and NIH #NS25789)

600.12

AN IMPROVED METHOD FOR POSTNATAL RAT HIPPOCAMPAL CULTURE. **L.T. Happel[1], J.M. Lyles[2], G.D. Clark[2] & C.F. Zorumski[3]. [1,2]Dept. of Neurol., [1]Neurosurg. & [1]Physiol., LSU Med. Ctr., New Orleans, LA 70112 & [3]Depts. of Psych., Anat. & Neurobiol., Wash. Univ., St. Louis, MO 63110.**

Current methods of postnatal rat hippocampal culture lead to inconsistent neuronal survival prompting efforts to improve techniques. Hippocampi from 1-4 d rat pups were sliced transversely and placed in an oxygenated L-15 media containing .2 mg/ml bovine serum albumin and 15 U/ml papain for a 30 minute incubation period. Hippocampal neurons were gently dissociated by trituration in Eagles minimal essential media containing 5-10% (v/v) fetal calf serum, 5-10% (v/v) horse serum, 200 μM glutamine, 50 μg/ml streptomycin, 50 U/ml penicillin, 6 mM glucose, 10 μg/ml insulin, 10 μg/ml human transferrin and 10 ng/ml selenium. The resultant cell suspension was placed in 35 mm culture dishes coated with a reconstituted basement membrane substance, Matrigel (Collaborative Research). Though neurons on standard collagen matrix were still attaching and had few processes within 12 h of plating, neurons on Matrigel had extended processes. Little difference in neuronal yield was noted in dishes coated with Matrigel concentrations of ≥ 1:5. Fewer neurons were noted at ≤ 1:10 Matrigel and even fewer on collagen. Matrigel improves survival and enhances development of hippocampal neurons.

600.13

THE DISTRIBUTION OF THE CHICK PRION-LIKE-PROTEIN IN THE NERVOUS SYSTEM OF THE CHICKEN F. A.Johnson, and G.D.Fischbach
Department of Neurobiology, Harvard Medical School, Boston,MA 02115

A protein purified and cloned in our laboratory based on its copurification with the 42kD Acetylcholine-Receptor-Inducing-Activity shares a 33% homology to the mammalian prion protein (Falls et al, CSH Sym, 55 1990). We report here the distribution of this chick prion-like-protein (ch-PLP) in the chicken spinal cord and brain.

A monoclonal antibody raised to a peptide of amino acids 213-224 of ch-PLP labels neurons in both the spinal cord and brain of chickens, and this labelling is significantly reduced by the specific peptide, but not by another peptide. Motoneurons in the anterior horn chicken cord are very intensely labelled. Some neurons in the dorsal and medial gray matter are also labelled, but less intensely. The anti-ch-PLP antibody also labels neurons in the embryonic cord, with labelling in the anterior horn visible as early as E6. Many neurons in the brain are labelled, with intensely labelled neurons visible in the Edinger-Westphal nuclei, oculomotor nuclei, the isthmus nuclei, magnocellular and parvocellular parts, the Purkinje cell layer in the cerebellum, and cells in layer SGFSi of the Optic Tectum. Many of these nuclei, with the exception of the Purkinje cells, are probably cholinergic as they are labelled both by acetylcholinesterase histochemistry and an anti-choline acetyl-transferase antibody (kindly provided by Miles Epstein). We are currently investigating if the labelling in the brain and cord is restricted to cholinergic neurons. This is important in light of the possible trophic activity of this molecule.

600.14

SURVIVAL OF LOCUS COERULEUS NEURONS IS PROMOTED BY ELEVATING INTRACELLULAR CYCLIC AMP LEVEL. L.Sklair and M.Segal. Center for Neuroscience, The Weizmann Institute, Rehovot 76100, Israel.

Central noradrenergic (NA) neurons from the locus coeruleus (LC) differ from other neurons in their extensive morphology and outstanding regenerative capacity. The aim of our study was to identify agents that specifically regulate the survival of tissue cultured rat LC neurons. The numbers of NA and total neurons were quantitated using tyrosine hydroxylase (TH) and NSE immunocytochemistry, respectively. Following exposure of LC cultures to cAMP analogs or to forskolin, a dramatic increase, up to to 4 fold, in the number of NA cells was seen. The non-NA cells, in the same culture, were not affected. The increase in the number of TH cells in LC cultures resulted from a selective trophic effect on the survival of NA neurons. Glial cells and calcium were not required for the expression of the trophic effect. NGF, EGF and FGF did not affect the survival of TH neurons. However, the beta-adrenergic agonist isoproterenol (10uM) significantly increased the number of NA neurons. Therefore, cAMP may be the messenger by which norepinephrine exerts its trophic effect on central NA neurons.

600.15

A PURIFIED DOPAMINE-RELEASING PROTEIN (DARP) FROM MESENCEPHALIC CELL CULTURES STIMULATES *IN VITRO* DA RELEASE FROM CORPUS STRIATUM AND AN ANTI-DARP REDUCES THE NUMBER OF NEURONS AND NEURITE OUTGROWTH. K. Sachchithanantham, Y. Park*and V. D. Ramirez, Dept. Physiology, University of Illinois, Urbana, Illinois, 61801.

Previously we reported that a partially purified DARP from adrenal gland is a 54 kD glycoprotein that stimulates *in vitro* DA release from corpus striatum. To purify DARP from CNS, mesencephalic cells (M-cells) derived from E17 rat fetuses were cultured. DARP was purified from media of 12 day old M-cell cultures using ammonium acetate/ethanol precipitation and anti-DARP affinity chromatography column. The DARP preparation from ammonium acetate/ethanol precipitation stimulated *in vitro* DA release in a dose-response manner. For instance, a 200 µg dose stimulated DA release from 22.6 ± 3.5 pg/mg/min, basal to 54.5 ± 8.7, peak level, n=9, $p < 0.01$. In contrast, a similar preparation from media alone was not effective (22.1 ± 3.5, vs 23.2 ± 5.5, n=4). After affinity chromatography, highly purified DARP at an estimated protein concentration of 100-200 ng also increased *in vitro* DA release (8.7 ± 1.6, basal level, vs 23.0 ± 3.8, peak, n=6). In addition, a monoclonal anti-DARP (Ig-M type) was added once to 9 day old M-cell cultures. Anti-NGF was used as a control. On day 12, 14 and 16, cell numbers were counted. 35 and 70 µg of Anti-DARP clearly decreased the number of cells as well as neurite outgrowth, while Anti-NGF did not affect either of them. These results suggest that DARP is produced from M-cells in culture, and seems to be a neurotrophic factor for these cells.

600.16

REGULATION OF THE EXPRESSION OF G41, A NOVEL LAMININ-LIKE CDNA CLONE, IN INJURED RAT BRAIN AND IN PC12 CELLS. T.T.Quach, S.A. Tijoe and A.M. Duchemin. Department of Pharmacology, The Ohio State University College of Medicine, Columbus, Ohio, 43210 USA.

G41 clone was isolated from a cDNA library constructed in plasmid pGEM, from poly (A^+) RNA extracted from lesioned rat brain. Sequence comparison of a partially sequenced insert revealed more than 53% homology with the nucleic acid sequence coding for laminin. A cRNA probe was used to study G41 expression in northern blot analyses. An hybridizing band was detected at a position corresponding to approximately 3.8 Kb. In the rat, the strongest signal was found in the brain. We failed to detect G41 expression in kidney, lung, muscle and liver, under identical hybridization conditions. A low level of G41 mRNA was detected in the heart. In preliminary experiments, we found that 2 days after injury to the cerebral cortex of the rat brain the expression of G41 was increased more than 300% in the tissues surrounding the injury site. We also detected high levels of G41 expression in tumors and in PC12 cells. In PC12 cells, NGF negatively regulates the expression of G41 gene: G41 mRNA levels decreased more than 60% after incubation of the cells with 10 nM NGF for 4 hours. These preliminary results suggest that G41 gene is regulated during brain injury and cell differentiation.

600.17

GLYCYL-L-GLUTAMINE REGULATES THE EXPRESSION OF ACETYLCHOLINESTERASE ASYMMETRIC FORMS IN CULTURED FETAL CARDIAC MYOCYTES. C.N. Battie, K. Hagler* and W.R. Millington. School of Basic Life Sciences, University of Missouri-Kansas City, Kansas City, MO 64108.

ß-endorphin and other opioid peptides have been identified in rat heart, although their role in cardiac function is poorly understood. We examined whether glycyl-glutamine (Gly-Gln; ß-endorphin$_{30-31}$), a dipeptide formed during ß-endorphin processing, regulates the expression of acetylcholinesterase (ACHE) forms in rat cardiac myocytes as it does in skeletal muscle and sympathetic ganglia. Globular (G) and asymmetric (A) AChE forms are present in the ventricles of neonatal rats; however, neonatal ventricular myocytes cultured in a defined medium for 72h do not synthesize A-forms of AChE. Dissociated cells from ventricles of neonatal rats were preplated in M199 and 0.5% fetal calf serum to remove non-muscle cells, then transferred to laminin- coated culture dishes (1.5×10^5 cells/35mm dish) in a defined medium with or without 10^{-4} or 10^{-6} M Gly-Gln. Gly-Gln did not change the specific activity of cell-associated AChE or the amount of secreted AChE, but Gly-Gln did alter the relative proportions of AChE forms, inducing the expression of asymmetric AChE. A_{12} AChE was barely detectable in control cultures but was clearly present, constituting 15 ± 3% of cell associated activity, in those with the dipeptide. These results suggest that Gly-Gln may have trophic activity towards the postsynaptic cholinergic system in developing rat heart.

600.18

EXPRESSION OF c-FOS IN MONKEY AND HUMAN CEREBELLUM AND HIPPOCAMPUS.
K. A. Maguire-Zeiss, L. M. Nakayama* and R. W. Hamill. Dept. of Neurology & Neurobiology and Anatomy, University of Rochester Medical Center, Monroe Community Hospital, Rochester, NY 14620.

The proto-oncogene c-fos is able to form complexes with the transcription factor c-jun and these dimers bind with high affinity to the AP1 consensus site thereby regulating the expression of a number of genes (ie. beta amyloid precursor protein, tyrosine hydroxylase, SV40). In addition, the expression of the c-fos is regulated by a number of physiological pertubations: induction of c-fos occurs following membrane depolarization and calcium influx, and exposure to nerve growth factor and cholinergic agonists. Since proto-oncogenes represent a third messenger system and participate in key cellular regulatory phenomenon, alterations of which may occur in the brain regions involved with neurodegenerative disorders, we sought to determine whether c-fos might be examined in postmortem human brain. We have used semi-quantitative in situ hybridization (ISH) to localize c-fos messenger RNA (mRNA) in postmortem monkey (M. *nemestrina*) and normal human cerebellum and hippocampus. In order to compare c-fos expression with Fos protein, we utilized immunocytochemical techniques. The data demonstrate detectable c-fos mRNA and Fos protein in dentate gyrus of hippocampus as well as hippocampal subfields, and the purkinje cell layer of cerebellum. Thus, the role of proto-oncogenes in the pathophysiology of human neurological disorders may now be characterized utilizing postmortem human brain. (Supported by NIH grants AG03644 and NS20832).

600.19

MODULATION OF CALBINDIN-$D_{28k/29K}$-LIKE GENE EXPRESSION BY RETINOIC ACID IN NEURONAL CELLS OVER-EXPRESSING A RETINOIC ACID RECEPTOR.

A. K.Hall, Y-Z. Wang*, R. K. Gill*, and S. Christakos, New Jersey Medical School, Newark, NJ 07103.

It is a well established fact that the calbindin-D_{28k} gene can be influenced by vitamin D in intestine and kidney. However, attempts to modulate this gene within the brain using vitamin D and its metabolites, even at pharmacological doses, have been unsuccessful. Whilst screening for retinoid-sensitive genes in B104 neuroblastoma cells transiently transfected with an expression vector (pSG5RAR) harboring a human retinoic acid receptor (RAR), we found that retinoic acid ($2x10^{-7}$M for 3 days) enhanced the relative abundance of an mRNA which cross-hybridized with human brain calbindin-D_{28k} cDNA. A 5-8 fold enhancement was observed using both slot blot and Northern blot analyses and this enhancement was detectable only in cells over-expressing RAR mRNA. Cross hybridization was observed in non-transfected cells. However, the signal was weak and no change was observed after treatment with retinoic acid. Western blot analyses using an antibody specific for rat calbindin-D_{28k} indicated the stimulated presence of an immunologically similar protein with an apparent molecular weight of 29,000 in retinoic acid-treated, retinoic acid receptor transfected cells. Collectively, these data suggest that the nuclear retinoic acid receptor can target "calbindin-like" genes in cells derived from the neural crest.

600.20

POSSIBLE INVOLVEMENT OF THE ODC/POLYAMINE PATHWAY IN NEURONAL DEGENERATION.

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The ornithine decarboxylase (ODC) / polyamine pathway has been involved in cell growth and differentiation in many tissues. ODC activity and levels of polyamines are high during the postnatal period and decrease gradually to adult levels during the fourth week after birth. Kainic acid (KA) produces seizure activity in neonate and adult rats. Despite prolonged seizure activity, young rats (< 21 days old), are less susceptible to the histopathological damage due to KA than adults. In adults, KA-induced seizure activity is accompanied by ODC induction, increased levels of putrescine and spectrin breakdown product (bdp) in hippocampus and piriform cortex. Furthermore, putrescine levels are positively correlated with increased bdp levels and pretreatment of the animals with the irreversible ODC inhibitor DFMO reduces both putrescine and bdp levels. During the postnatal period, KA-induced seizure activity did not produce ODC induction and increased spectrin breakdown before PND 21. On the other hand, chronic DFMO treatment between PND 6 and 11 decreased both polyamine and spectrin bdp levels measured at PND 11. These results indicate that polyamines participate in the *in situ* regulation of calpain activity, both in adult and in developing brain. Moreover they indicate that the seizure-induced triggering of the ODC/ polyamine pathway may represent a critical step in the susceptibility to neuronal injury.

600.21

INHIBITION OF TROPHIC FACTOR-MEDIATED CHOLINERGIC DIFFERENTIATION BY CHRONIC PHORBOL ESTER TREATMENTS IN SYMPATHETIC NEURONS.

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Cholinergic differentiation of cultured rat sympathetic neurons is promoted by several factors including ciliary neurotrophic factor (CNTF), leukemia inhibitory factor (LIF), and a membrane associated factor (MANS). However, their post-receptor signalling mechanisms are unclear. Here, phorbol 12, 13-myristate acetate (TPA) was used to implicate calcium, phospholipid-dependent protein kinase (PKC) in neurotrophic factor action. Short-term TPA treatment (0.5 μM, 15 min) reduced cytosolic PKC levels by 70% with a corresponding 91% increase in membrane enzyme levels. This redistribution of PKC was followed by down-regulation of total cellular enzyme levels in chronic TPA-treated (30 nM, 1-2 wks) cultures to 14% of control levels. CNTF (0.5 ng/ml) and LIF (0.5 ng/ml) both significantly increased choline acetyltransferase (ChAT) activity by 4.5 and 1.7 fold, respectively. Additionally, neurons grown at double cell density exhibited a 1.9 fold increase in ChAT activity. In cultures depleted of PKC activity by chronic TPA treatment, co-treatment with LIF (0.5 ng/ml), CNTF (0.5 ng/ml) or increasing cell density failed to increase ChAT levels. An inactive phorbol, 4α-phorbol, did not affect PKC levels or trophic factor-mediated increases in ChAT activity. Phosphatidylinositol 4, 5 bis-phosphate (PIP_2) hydrolysis and the generation of inositol triphosphate (IP_3) and diacylglycerol (DAG) is thought to be an initial step in PKC activation. However, LIF or CNTF did not stimulate PIP_2 hydrolysis as compared to carbachol which increased PIP_2 hydrolysis by 268%. These results implicate PKC in neurotrophic factor signalling processes and cholinergic development in sympathetic neurons but activation of PKC is likely to involve alternative sources of DAG such as phosphatidylcholine.

600.22

TCP-TREATED HUMAN SPINAL CORD CULTURES: AN IMMUNOCYTOCHEMICAL AND ULTRA-STRUCTURAL STUDY.

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Experimental evidence supporting for the excitotoxic theory involving the excitatory neurotransmitters came from cell culture models. NMDA antagonists are considered as potential neuroprotective agents in neurodegenerative disorders related to excitatory mechanisms. The results reported here show that TCP (N,1-(2-thienyl) cyclohexyl) piperidine, a non-competitive NMDA antagonist enhances the survival of human fetal spinal cord cells in cultures. The spinal cords from 7-10 week-old human fetuses were cultured as either dissociated cells or organotypic explants. Both sets were treated at 3 weeks with 2-8.10-6M TCP. After 10 weeks, progressive neuronal necrosis was observed in control whereas TCP-treated cultures survived up to 6 months. In all models, neurons were immunoreactive for GABA, CAT and N. S. enolase. However, the TCP-treated neurons showed at the ultrastructural level a marked increase in the length of the processes, in the number and size of growth cones and in the degree of maturation of synapses. To conclude, TCP appears as a promising drug for the protection and long term survival of neurons.

600.23

PRIMATE SCHWANN CELLS EXPRESS MULTIPLE GROWTH FACTORS IN VITRO.

M.F.D. Notter, D.L. Hurley,[1] D.M. Gash, and J.T. Hansen, Department of Neurobiology and Anatomy, University of Rochester Medical Center, Rochester, NY 14642 and Department of Anatomy,[1] Tulane University Medical Center, New Orleans, LA 70112

Aged primate Schwann cells have been shown by us to respond to specific mitogens in vitro and express appropriate cell markers such as nerve growth factor receptor after two weeks of culture (Soc. Neur. Abst. 16:341, 1990). In this study we have begun to examine the neuronotrophic factor profile of these cells important in neural regeneration.

Adrenal medullary cells from the same aged Rhesus monkeys were cocultured with sciatic nerve explants in the absence or presence of neutralizing levels of antiserum to nerve growth factor (NGF). After immunostaining for tyrosine hydroxylase and scoring for neuritogenesis, chromaffin cells were shown to extend neurites on the surface of Schwann cells and fibroblasts even in the presence of antiserum to NGF. In order to screen for the presence of other factors a radioactive RNA probe to acidic fibroblast growth factor (afgf) cDNA was generated and used for in situ hybridization with explant cultures of monkey peripheral nerve. Moderate levels of mRNA were expressed in monkey as well as rat Schwann cell cultures when compared to negative and positive expressing cell cultures. Furthermore, immunocytochemistry employed for detecting the presence of insulin-like growth factor-I (IGF-I) in primate Schwann cells revealed positive staining of these cells with only background levels detected in contaminating fibroblasts. Therefore, although primate Schwann cells exhibit NGF receptors indicative of NGF activity in these cells, the presence of afgf and IGF-I in Schwann cells may contribute to axonal repair during injury. (Supported by NIH NS25778).

600.24

NORMAL AND SEAD RAT SCHWANN CELLS ARE IMMUNOREACTIVE FOR INSULIN-LIKE GROWTH FACTOR-1 AND NERVE GROWTH FACTOR RECEPTOR.

E.L. Imperato, M.F.D. Notter, and J.T. Hansen. Department of Neurobiology and Anatomy, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642.

Neurotrophic factors, nerve growth factor (NGF) and insulin-like growth factor (IGF-1), are important in peripheral nerve regeneration and are expressed in Schwann cells *in vivo*. In the present study, we determined trophic factor expression in two types of rat Schwann cells, SEAD and normal, after culture. SEAD cells represent a rat clonal cell line established by gene transfer of the E1A gene. Therefore, these cells are permanently growth-stimulated. Normal neonatal rat Schwann cells were harvested from sciatic nerve, cultured, and partially purified. Both cell types were cultured for 2-3 weeks in serum-containing medium. IGF-1 (gift from R. Furlanetto) and NGF-receptor (NGF-rec; 192 IgG) immunocytochemistry was done for both groups of Schwann cells; the normal rat Schwann cells were immunoreactive for NGF-rec and IGF-1. Fibroblasts did not exhibit NGF-rec-like immunoreactivity, but a very faint IGF-1-like immunoreactivity was observed. SEAD cells also were immunoreactive for NGF-rec and IGF-1. In control samples, elimination of the primary antibody showed no immunoreactivity in all cases. This is the first example of *in vitro* staining of IGF-1 in both types of Schwann cells. The results of this study suggest that normal and genetically altered Schwann cells may express important trophic factors. (Supported in part by NS25778).

600.25

REGULATION OF SUBSTANCE P IN SYMPATHETIC NEURONS BY ENDOGENOUS IL-1β. M. Freidin*, E.Goldensohn, & J.A. Kessler. Dept. Neurosci. & Neurol., AECOM, Bronx, NY 10461.

The peptide neurotransmitter, substance P (SP), is regulated and distributed differently than coexisting transmitters in sympathetic neurons. Denervation of the rat sympathetic superior cervical ganglion (SCG) elevates levels of the peptide within the ganglion, but not in the nerve terminals or in target organs. Further, the cytokine, interleukin-1β (IL-1β) stimulates levels of SP and preprotachykinin mRNA in SCG culture. These observations suggest that SP may help regulate intraganglionic responses to injury, which in turn may be mediated by endogenous cytokines. To test this hypothesis, cultures of the SCG were treated with recombinant IL-1 receptor antagonist (IL1ra) derived from activated human monocytes. IL1ra largely blocked the increase in SP normally seen in explants and dissociated neuronal cultures grown in the presence of ganglion nonneuronal cells. Levels of SP were the same as controls values when excess IL-1β was added to IL1ra treated cultures, indicating both the specificity and lack of toxicity of IL1ra. Further, IL1ra treatment did not inhibit the stimulation of the peptide by another SP-acitivating cytokine, D-factor. In conclusion, these results suggest that endogenous IL-1β plays a role in mediating the regulation of SP expression in the SCG and supports the hypothesis that the peptide is involved in ganglion responses to injury.

600.26

THE EXPRESSION OF PLATELET-DERIVED GROWTH FACTOR (B-CHAIN) IN THE DEVELOPING BRAIN AND TRANSPLANTED OLFACTORY BULB OF THE RAT. [1]J.N. Kott, [3]A. Sasahara*, [3]M. Sasahara*, [3]R. Ross*, and [1,2]L.E. Westrum. Depts. of [1]Neurol. Surg., [2]Biol. Struct., and [3]Pathol., University of Washington, Seattle, WA 98195.

Platelet-derived growth factor (PDGF) was originally recognized as a major mitogen in human platelets. This growth factor exists as hetero- and homodimers of two separate but related chains (PDGFA and PDGFB). PDGF B-chain has been localized to specific neurons and fibers of the monkey CNS (Sasahara et al., Cell, 64, 1991).

We have examined the distribution of PDGF B-chain-like immunoreactivity (PDGFB-I) in the CNS of rats at various embryonic (E) and postnatal (PN) ages. At E 14, PDGFB-I is seen associated with specific developing fiber systems especially the main and accessory olfactory nerves (ON), but not generally with perikarya. Heavily labeled ON fibers persist through all stages examined and into adulthood. This persistent and particularly heavy reactivity may be related to the fact that ON neurons are continuously generated throughout life and suggests that PDGFB may serve in some way to support the regenerating fibers. While many reactive cell bodies are visible in the periventricular proliferative zone at E14, reactive cells identifiable as neurons by their size and position are not apparent until PN 1.5 when lightly reactive pyramidal and Purkinji cells are seen in the developing hippocampus and cerebellum. The intensity of staining of these neurons as well as that in certain other nuclei gradually increases with age.

As the above findings suggest that PDGFB may have roles in both the development and maintenance of the CNS, we are undertaking studies of the expression of PDGFB-I in donor olfactory bulbs which have been transplanted to the site vacated by removal of a host OB. These data along with general anatomical localization will be presented. (Supported by NIH Grant NS 09678, NS07144 and HL18645. L.E.W. is an affiliate of the CDMRC).

NUTRITIONAL AND PRENATAL FACTORS

601.1

IDENTIFICATION OF NUCLEAR RETINOIC ACID BINDING ACTIVITY AND RETINOIC ACID RECEPTOR TRANSCRIPTS IN A HUMAN NEUROBLASTOMA CELL LINE (LA1-15n). M. Clagett-Dame[1], J.L. Biedler[2*] and T.J. Verhalen[1*]. School of Pharmacy, Univ. of Wisconsin-Madison, WI[1] and Cellular and Biochemical Genetics, Memorial Sloan-Kettering Cancer Ctr., NY.[2]

Retinoic acid (RA) can influence nervous system development and may do so by binding to one or more nuclear receptor proteins. We have identified nuclear RA receptors (RAR's) in a clonal line of human neuroblastoma (NB) cells that differentiate in response to RA. Specific [3H]-RA binding sites were detected in both nuclear and cytosolic fractions with the cytosolic RA binding protein sedimenting at ~2S on sucrose density gradients. In contrast, the nuclear protein sedimented at ~4S on gradients which is consistent with the Mr of ~50,000 deduced from RAR cDNA's. The specific [3H]-RA binding activity in the nuclear fraction was saturable and of high affinity (Kd~0.2 nM). The nuclear [3H]-RA binding protein bound to DNA-cellulose, an activity characteristic of other members of the steroid hormone receptor superfamily. Northern blotting experiments suggest the nuclear protein represents a RAR of the α subtype. The expression of RARα mRNA was unaffected by treatment of cells with RA (100 nM). In contrast, mRNA for RARβ was initially absent but was induced within four hours after treatment of cells with RA. These results support the hypothesis that RAR's are involved in human NB cell differentiation.

601.2

INCREASED REPEATED ERRORS IN RHESUS MONKEYS WITH OMEGA-3 FATTY ACID DEFICIENCY (FAD). S. Reisbick, M. Neuringer and W.E. Connor. Or. Health Sci. Univ., Portland, OR 97201 & Or. Reg. Primate Res. Center, Beaverton OR 97006

Omega-3 fatty acids are major structural components of neural and retinal membranes. In primates, perinatal omega-3 FAD has been associated with lowered levels of omega-3 fatty acids in cerebral cortex and retina, abnormal electroretinograms and delayed development of visual acuity. Altered performance (higher response rates to both S+ and S-) of operant learning tasks has been reported in rats with long-term omega-3 FAD.

In the current study, monkeys with combined pre- and postnatal omega-3 FAD and their controls learned a series of operant visual discriminations using a correction procedure. Monkeys discriminated pairs of visual stimuli including horizontal and vertical stripes in a series of pairs of decreasing size. There were no group differences in speed of learning the pairs (trials to criterion) or in % repeated errors (repeated errors/ total errors x 100) while learning new pairs (first 5 days with a new pair). However, when tasks were well-known (last 5 days to criterion), deficient monkeys made a higher % repeated errors (24.1 $\pm$ 3.3 vs 6.4 $\pm$ 0.7 for the controls: Mean $\pm$ SEM, $p<.01$). Such perseverative errors may reflect deficits in attention or memory, or difficulty in inhibiting a previous response.

601.3

THE EFFECTS OF DIETARY N-3/N-6 RATION ON BRAIN DEVELOPMENT IN THE MOUSE: A DOSE RESPONSE STUDY WITH LONG-CHAIN N-3 FATTY ACIDS. P.E.Wainwright, Y.S.Huang*[1], B.Bulman-Fleming, D.Dalby*, D.E.Mills*, P.Redden*[1] and D.McCucheon*. Department of Health Studies, University of Waterloo, Waterloo, Ontario N2L 3G1. [1]Efamol Res.Ins., Kentville, NS B4N 4H8.

A dose-response study was conducted to examine the effects on brain development in mice of maternal dietary ratios of n-3/n-6 fatty acids (FA), where the n-3 FA were supplied as the preformed long-chain compounds. From conception until 12 days after birth, B6D2F$_1$ mice were fed dietary n-3/n-6 ratios of 0, 0.25, 0.5, 1.0, 2.0 and 4.0, with an n-6 content of greater than 1.5% of energy in all diets, and similar levels of total polyunsaturated FA. Biochemical analyses were conducted on pup brains, as well as on samples of maternal milk. There were no obvious effects on overall pup growth and development, apart from a smaller litter size in ratio 1. Covariance analysis indicated that increasing the n-3/n-6 ratio was associated with slightly smaller brains, relative to body weight. Increasing dietary n-3/n-6 ratios generally resulted in an increase in n-3 FA, with a corresponding decrease in n-6 FA. In the milk 18:2n-6 was the predominant n-6 FA, and 20:5n-3 the predominant n-3 FA; in the brain these were 20:4n-6 and 22:6n-3 respectively. The n-3/n-6 ratio of the milk lipids showed a strong linear relationship with the diet, but in the brain the rate of increase tended to decrease beyond 0.5 (phosphatidylcholine, PC) and 0.25 (phosphatidylethanolamine, PE), such that there was a significant quadratic contribution to the relationship. These results indicate that the n-3/n-6 ratio of the phospholipids in the developing mouse brain respond maximally to maternal dietary long-chain n-3/n-6 ratios of between 0.25 and 0.5, which are equivalent to a n-3/n-6 ratio of approximately 0.25 in maternal milk.

Supported by a grant from NSERC (Canada).

601.4

COMBINED Mg^{2+} AND Ca^{2+} DEFICIENCY DURING FETAL AND POSTNATAL LIFE INCREASES LEVELS OF THE 28 kDa CALCIUM-BINDING PROTEIN (CALBINDIN) IN BRAIN. S. Bose, B. Cheng, B. Rychlik and M. P. Mattson, Center on Aging and Dept. of Anat. & Neurobiol., Univ. of Kentucky, Lexington, KY.

Rats were maintained on a diet deficient in Mg^{2+} (<0.003%) and Ca^{2+} (< 0.02%), or a control diet (0.065% Mg^{2+} and 0.75% Ca^{2+}), beginning on day 4 of pregnancy and through 3 weeks postpartum. Overall growth of pups from mothers on the Mg^{2+}/Ca^{2+} deficient diet was significantly retarded; in 2 week-old pups body and brain weights were reduced 40-60% and 20%, respectively. The pups of mothers on the Mg^{2+}/Ca^{2+} deficient diet developed seizures and often died within 3-5 weeks of birth. Cell counts in hippocampi revealed a >40% reduction in neuronal density in regions CA1 and CA3, and a 20% reduction in neurons in the dentate gyrus in 2-3 week-old pups of mothers on the Mg^{2+}/Ca^{2+} deficient diet. Immunocytochemical localization of calbindin in brain revealed an increase in both the number of calbindin immunoreactive neurons, and in their relative staining intensities in both hippocampus and cerebral cortex. Western blots of whole brain demonstrated a large increase in calbindin levels in pups from mothers maintained on the Mg^{2+}/Ca^{2+} deficient diet. Since calbindin may play a role in protecting neurons against excitotoxicity (*Neuron*, 6:41), the increased levels of calbindin in Mg^{2+}/Ca^{2+} deficient pups may represent an attempt to compensate for the increased vulnerability to seizures resulting from the Mg^{2+} deficiency. (we thank S. Christakos for anti-calbindin; supported by the International Life Sciences Institute and NS29001).

601.5

QUANTITATIVE STUDY OF CELL DENSITY IN NEONATAL RAT BRAINS. S. Zamenhof. Dept. of Microbiology and Immunology, and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024

This work is a continuation of studies of brain parameters quantitatively correlated with behavioral performance. In previous work we have studied one such parameter, brain cell number or brain DNA (Life Sci 18:1391, 1976; Nutr. Res., in press). In the present work we have studied another parameter: brain cell density (D). The rationale is that lower D allows for more voluminous future development of dendritic tree, hence possible superior behavioral performance (Physiol. Behav. 23: 945, 1979). However, lower D could be also caused by lower brain cell number (undesirable), and it is not known which of the two parameters is more important for behavioral performance. We limited our search to those brains which have cell number (DNA) not lower than the mean for the group. Because of the inhomogeneity associated with different cell types, and quantitatively difficult brain geometry, we studied instead the ratio of brain cell number to brain volume (DNA/brain weight, as index of D). We found that among the total of 1948 neonatal rats there were 22 (1.1%) outstanding individuals (0) that had D more than 2 std. deviations (significant) below the mean, while also having DNA not below its mean. Of these 0 individuals, 71% came from the litters that had at least one more such 0 individual (suggesting common factors, such as prenatal nutrition). The mean number of fetuses per litter was n=9.4± 2.5 for the entire group, and 6.3 ± 3.2 for litters with at least one 0 individual (difference significant at $P<0.01$), which suggests that low n is one of the factors contributing to the favorable low brain cell density.

601.6

EARLY DEFICITS IN BRAIN WATER RESULT IN REDUCED BRAIN GROWTH. E J Moore* and J Diaz. Dept of Psychology University of Washington, Seattle, WA 98195.

The procedure of artificially rearing rat pups results in brain weight deficits as large as 7-10% compared to mother reared siblings Despite these differences, brain water content is comparable on day 18 and as early as day 10 even with extreme undernutrition, indicating that brain water content is well defended. Given the osmotic density of the formula, the possibility of an acute hyperosmotic insult must be considered, especially since brain weight deficits have been reported after only 24 hours of artificial rearing (Br. Res. Bull., 11, 1983). The purpose of this experiment to test the hypothesis that brain water content cannot be altered acutely

Four day old rat pups were randomly assigned to one of five groups 1) mother reared (MR); 2) gastrostomized, given formula (AR-F); 3) gastrostomized, given only water (AR-W); 4) gastrostomized, given hypertonic saline (AR-S); and 5) removed from mother (ISO). After 24 hours, all animals were sacrificed and wet whole brain weights were recorded. Subsequent dry brain weights were collected.

Brain water content can be altered acutely. Compared to the MR group, brain water content was significantly lower in the AR-F and ISO groups; higher in the AR-W group; and lowest in the AR-S group

Brain water content is well defended over the long term, but even this fundamental rate-limiting factor of brain growth is susceptible to acute early challenges. Accelerated brain growth cannot be maintained without sufficient water. Relative dehydration may thus lead to a deceleration in the long term trajectory of brain growth.

601.7

SENSITIVITY TO COCAINE IN RAT PUPS GIVEN PRENATAL COCAINE EXPOSURE. J.S. Meyer, J.D. Sherlock*, and N. MacDonald*. Dept. of Psychology, Neuroscience and Behavior Program, University of Massachusetts, Amherst, MA 01003.

This study determined whether prenatal exposure to cocaine alters behavioral sensitivity to postnatal cocaine administration. Pregnant Sprague-Dawley rats were divided into 3 groups: Cocaine-treated, Saline-treated, and Untreated. The cocaine group was injected s.c. 2X/day with 20 mg/kg cocaine HCl from gestational day 11 to day 20. Saline controls were pair-fed to the cocaine females, whereas untreated controls were undisturbed. Litters were culled to 8 pups (normally sex-balanced) and were fostered to normal lactating dams on postnatal day (PD) 1. On PD11, subjects were given either saline or cocaine (1.25, 2.5, or 5.0 mg/kg), returned to the nest for 15 min, and then removed for testing. Distress vocalizations and other behaviors were recorded during a 5-min test session. The results indicated that while acute cocaine treatment markedly suppressed distress vocalizations, there was no shift in the dose-response curve as a function of prenatal condition. In contrast, cocaine-induced activation of wall-climbing was influenced by prior cocaine exposure. Both control groups displayed a near-maximal increase in wall-climbing to the lowest cocaine dose, whereas the cocaine-pretreated pups showed little response except for the high (5.0 mg/kg) dose. Thus, prenatal cocaine treatment alters the sensitivity of some but not other behaviors to subsequent cocaine exposure. Supported by NIDA Grant DA-06495.

601.8

EFFECTS OF MATERNAL ALCOHOL EXPOSURE ON PERINATAL ORNITHINE DECARBOXYLASE LEVELS. A. CHICZ-DEMET, B.D. NGUYEN*, B.S. STOVEKEN*, S. TEWARI*, E.M. DEMET. Dept. Psychiatry, Univ. California, Irvine, CA 92717 and State Developmental Research Institutes, Costa Mesa, CA 92626

The effects of maternal alcohol ingestion on ornithine decarboxylase (ODC) and organ weights were examined in 8 day old rat pups. Control dams were fed a similar isocaloric diet without alcohol. Elevated ODC levels may reflect stress induced growth processes responsible for a disordering of normal developmental events. Treated animals had elevated ODC levels in 8 brain regions, heart and liver. Decreases were found in whole body, brain, liver, and heart weights although the latter was not significant. Alcohol treated female pups had larger ODC increases in all tissues than their male counterparts, and had greater decreases in whole body, and brain weights. In contrast, no gender differences in body or organ weights were evident in controls. The results suggest a relationship between ODC increases and organ weight decreases and imply a greater susceptibility to prenatal alcohol in female rats.

601.9

EFFECT OF PRENATAL ETHANOL EXPOSURE ON ETHANOL TOLERANCE IN ADULT OFFSPRING IS DEPENDENT ON BLOOD LEVEL AFTER CHRONIC ETHANOL EXPOSURE. H.C. Becker and R.L. Hale. VA Medical Center and Medical University of South Carolina, Charleston, SC 29403

We previously found that prenatal ethanol (EtOH) exposure renders adult offspring less tolerant to the motor incoordinating effects of EtOH following chronic exposure to the drug. The purpose of this study was to examine whether this effect is influenced by the degree of chronic EtOH intoxication prior to tolerance testing. Female C3H mice derived from dams maintained on isocaloric liquid diets containing 25% or 0% EtOH-derived calories, or standard lab chow were used as subjects. Offspring were placed in inhalation chambers and exposed to either EtOH vapor or air for 48 hrs. Six hours following withdrawal, all mice were injected ip. with 3 g/kg EtOH and tested for motor coordination on a horizontal dowel. Immediately after fall, a sample of cortex was collected. Results indicated that EtOH-exposed offspring fell off the dowel at significantly lower brain EtOH levels than control offspring following chronic exposure that yielded blood EtOH levels of 185.0 ± 4.7 mg% (i.e., prenatal EtOH offspring exhibited less tolerance). This prenatal treatment difference was not observed with chronic exposure that resulted in blood EtOH levels of 93.1 ± 3.0 and 149.3 ± 1.7 mg%. Blood EtOH levels did not differ among prenatal treatment groups in any of teh exposure conditions. Results from studies varying the duration of chronic EtOH exposure will be presented as well.
Supported by NIAAA and VA Medical Research Service.

601.10

A STEREOLOGICAL STUDY OF THE EFFECT OF EARLY POSTNATAL ALCOHOL EXPOSURE ON THE NUMBER OF GRANULE CELLS AND MITRAL CELLS IN THE RAT OLFACTORY BULB. N.E. Bonthius, D.J. Bonthius, R.M.A. Napper and J.R. West. Department of Anatomy, University of Otago, Dunedin, New Zealand.

This study examined the effect of early postnatal alcohol exposure on the total number of olfactory bulb mitral cells and granule cells. Sprague-Dawley rat pups were reared artificially over postnatal days (PD) 4 through 9, a period of rapid brain growth comparable to the human third trimester. Alcohol-exposed pups received a daily alcohol dose of 4.5 g/kg, administered as a 10.2% solution in 2 of the 12 daily feedings. Gastrostomy and suckle control pups were also reared. Pups were sacrificed either on PD 10 (to determine the acute effects) or on PD 105 (to determine the long-term effects). The total number of granule cells and mitral cells from one bulb in each subject were determined using unbiased stereological methods and systematic sampling. Control rats had an average of 2,200,000 granule cells and 53,000 mitral cells on PD10. By PD 105, granule cell number had increased to 5,060,000, while mitral cell number had decreased to 43,000. On PD 10, immediately following the alcohol exposure, the alcohol-exposed subjects had a 33.1% deficit in granule cells and a 36.7% deficit in mitral cells, relative to gastrostomy controls. On PD 105, both the granule cells and mitral cells were still significantly reduced in number, by 19.3% and 26.6%, respectively. These results demonstrate that, while there may be some rebound in neuronal number following alcohol exposure, the deficits induced in some neuronal complements are permanent.

601.11

THE EFFECT OF CHRONIC IN UTERO ALCOHOL EXPOSURE ON DEVELOPING NEURONS IN CULTURE. M. L. Weaver*, C. S. Cretan*, D. A. Ciraulo* and S. Jacobson. Depts. of Clinical Pharmacology and Anatomy and Cell Biology, Tufts Univ., and Dept. of Veterans Affairs Outpatient Clinic, Boston, MA.

The effects of in utero ethanol exposure on the growth of neurons in culture were examined. CD-1 female mice consumed liquid diet containing 3.2 % or 20 % ethanol derived calories (EDC) or a control diet. The mice were maintained on the diet until fetal day 17 when they were sacrificed. The pups were removed, the cerebellum and cerebrum where mechanically separated in incomplete F12 media, and the neuroblasts plated on thermanox coverglasses. Cells were grown for 6-29 days in culture. Cells from animals given control diet developed into mature appearing neurons with a well differentiated dendritic tree. Neurons from animals exposed to the 20% EDC diet had a grossly simplified and abnormal dendritic tree. Neurons from the 3.2% EDC group exhibited stunted growth as demonstrated after the images were digitized and evaluated.

601.12

EFFECT OF PRENATAL EXPOSURE TO ETHANOL ON GLUCOSE UTILIZATION IN PRIMARY SOMATOSENSORY CORTEX OF RATS: RESPONSE TO STIMULATING A ROW OF VIBRISSAE. M.W. Miller and D.L. Dow-Edwards. Dept. of Cell Biology, Univ. of Med. and Dent. of N.J.- Sch. of Osteopathic Med., Piscataway NJ 08854, and Dept. of Neuroscience and Cell Biology, Univ. of Med. and Dent. of N.J.- R.W. Johnson Med. Sch., Piscataway NJ 08854.

Prenatal exposure to ethanol produces permanent morphological and physiological changes in the neocortex of adult rats. For example, glucose utilization in all of the cortical laminae of primary motor and somatosensory cortices is depressed 20-30% following prenatal exposure to ethanol. We used [^{14}C]2-deoxyglucose autoradiography to determine whether the ethanol-induced metabolic depression disappeared when cortex was stimulated by sensory activity. The mature offspring of 2 groups of rats were examined: the pups of mothers that were fed a liquid diet containing 6.7% ethanol during the second half of gestation and the offspring of mothers which were pair-fed a nutritionally-matched liquid diet. The mystacial vibrissae of all rats, except for the C row on the right side, were shaved. As in previous studies, glucose utilization in non-stimulated regions of cortex (e.g., right non-barrel somatosensory cortex) was significantly depressed in ethanol-treated rats. Stimulation of the vibrissae produced a significant increase in glucose utilization in the C row barrels in ethanol-treated and control rats. Nevertheless, the substantial ethanol-induced difference in glucose utilization remained. Thus, prenatal exposure to ethanol depresses cortical metabolic activity regardless of the physiological state. Funded by DE 07734 and AA 06916.

601.13

EFFECTS OF EXPOSURE TO ETHANOL DURING DEVELOPMENT ON PLAY BEHAVIOR. Janie H. Wilson and Sandra J. Kelly. Department of Psychology, University of South Carolina, Columbia, SC, 29208.

This experiment examined the influence of early postnatal ethanol exposure on play behavior as measured by the number of pins and the involvement of opioid activity in this behavior. Rats were artificially reared and exposed to either 5 or 3 g/kg/day of ethanol from postnatal days 4 through 10. Control groups consisted of rats artificially reared but not exposed to alcohol and rats reared normally by dams. All rats were reared by dams from postnatal day 12 to 21 and tested between 32 and 40 days of age. The rats were habituated alone in the testing chamber for 5 min on two consecutive days and tested on the following 4 days. All subjects were housed in isolation 24 hours before the first test session and remained isolated throughout the testing period. Rats were tested with a nonexperimental same-sex rat of approximately the same weight for 10 min on 4 consecutive days. The experimental rats received subcutaneous injections of either saline, saline followed by naloxone (1 mg/kg), morphine (1 mg/kg) followed by saline, or morphine followed by naloxone before testing. The order of drug treatments was balanced across sex-by-group blocks. All sessions were taped, and the number of pins was recorded.

There was a trend for ethanol exposure to increase pinning. When the results were divided by gender, females pinned more when exposed to 5 g/kg/day dose of ethanol; however, males pinned more when exposed to 3 g/kg/day of ethanol. The effect of drug injections on pinning varied with gender but not with ethanol treatment. It was found that males pinned more than females overall, and all animals increased their pinning behavior over days. In conclusion, exposure to ethanol during the early postnatal period alters the amount of pinning behavior in rats, but this alteration in behavior does not appear to be mediated by the opioid systems. (Supported by NIAAA Grant AA08080)

601.14

ABERRANT MITOTIC ACTIVITY IN THE DEVELOPING TADPOLE CEREBELLUM FOLLOWING ACUTE ETHANOL EXPOSURE. N.J. Uray and P.S. Sexton*. Dept. of Anatomy, Kirksville College of Osteopathic Medicine, Kirksville, MO 63501

To explore the mechanisms underlying alterations in cerebellar development that were seen following chronic alcohol administration, we examined the cerebella of tadpoles, after acute exposure to ethanol.

At the ages of 6, 14 and 24 w(weeks) tadpoles of the frog Rana catesbeiana were placed in 1% ethanol and were killed 1,4,8,16,24,48,72, and 168 h (hours) later. Slides of cerebella were examined for mitotic activity. Numerous mitotic figures were present in the ventricular and subventricular zones of both control and ethanol exposed tadpoles at 6 and 14 w, but few were noted in 24 w old animals. In all age groups, ethanol treated tadpoles also contained mitotic figures in the pml (primitive molecular layer) and the subpial zone. Normal tadpole cerebella do not contain dividing cells in the pml or in the subpial position, thus, it is likely that such mitotic activity is a consequence of ethanol exposure. This may represent an aberrant secondary proliferative activity or alternately, ethanol may act as a trigger to synchronize mitotic activity that in normal animals may take days. The latter possibility would imply that external granular layer cells in tadpoles also undergo a secondary proliferation, as in other species. Supported by NIAAA Grant R01 AA97537.

601.15

EFFECTS OF TREATMENT OF PREGNANT ALCOHOLIC RATS WITH SEROTONIN$_{1A}$ AGONISTS ON OFFSPRING. M.J. Druse and N.F. Tajuddin. Molecular & Cellular Biochemistry, Loyola U. Chicago, Stritch Sch. Med., Maywood, IL 60153.

Previous studies in this laboratory demonstrated that in utero ethanol exposure results in a serotonin (5-HT) deficiency, which is detected as early as gestational (G) day 15. Because fetal 5-HT is important for normal CNS development, a 5-HT deficiency could contribute to the CNS abnormalities found in ethanol-exposed offspring. In order to test this hypothesis we treated pregnant Sprague-Dawley rats with two 5-HT$_{1A}$ agonists -- 8-OH-DPAT (3.5-4mg/kg/day) and buspirone (4.5 mg/kg/day) during the last trimester of pregnancy. 5-HT and 5-hydroxyindole-acetic acid (5-HIAA) were assessed on postnatal day 5.

These experiments demonstrated that the offspring of ethanol-fed rats had ~40% less 5-HT in the brain stem and cortex and ~30% less 5-HIAA in these regions. Treatment of pregnant alcoholic rats with 8-OH-DPAT had no significant effect on 5-HT or 5-HIAA levels in these regions, although the size of the brain stem 5-HT deficiency was reduced. In contrast, buspirone treatment resulted in brain stem 5-HT levels in ethanol-treated rats that were not significantly different from those of control rats ($p>0.05$). Additional studies will determine whether buspirone treatment of ethanol-fed dams ameliorates other 5-HT abnormalities in offspring.

This research was supported by USPHS-AA03490.

601.16

NEUROTRANSMITTER LEVELS IN LIMBIC REGIONS IN STRESSED AND NONSTRESSED RATS EXPOSED TO ALCOHOL DURING DEVELOPMENT. Sandra J. Kelly and Regina R. Dillingham*. Department of Psychology, University of South Carolina, Columbia, SC, U.S.A. 29208.

The effect of alcohol exposure during the early postnatal period on neurotransmitters and their metabolites was examined in stressed and nonstressed rats. Rats were artificially reared and exposed to either 5 or 3 g/kg/day of ethanol from postnatal day 4 through 10. Control groups consisted of rats artificially reared but not exposed to alcohol and rats reared normally by dams. When the rats were between 90 and 95 days of age, half the rats were put into wire mesh close-fitting restrainers for 30 min and then decapitated. The other half of the rats were decapitated within two min of being removed from the animal quarters. The head was immediately dropped into liquid nitrogen and then the brain was removed. The hypothalamus, hippocampal region, septal region and amgdala region were dissected free. The tissue was sonicated in 0.2M perchloric acid and frozen until the time of assay. At that time, the sonicated tissue was thawed and centrifuged for 15 min. Twenty microliters of the supernatant was injected onto a C18 reverse phase column for HPLC with electrochemical detection. The mobile phase was 700 parts 100 mM sodium monophosphate and 300 parts methanol with 0.97 mM octyl sodium sulfate, 1 mM disodium EDTA and 0.26 mM triethylamine (pH 3.2). The supernatant was analyzed for noradrenaline, dopamine, DOPAC, HVA, 5-HT, and 5-HIAA content.

Noradrenaline levels in the hypothalamus in both stressed and nonstressed conditions were increased by exposure to alcohol during the early postnatal period. In the amygdala, the stress-induced change in 5-HIAA was increased and the stress-induced change in HVA was decreased in alcohol-exposed rats. (Supported by NIAAA Grant AA08080 to S.J.K.)

601.17

CORTICAL ASTROGLIOSIS INDUCED BY EARLY POSTNATAL ALCOHOL EXPOSURE IN RATS DEMONSTRATED BY CONFOCAL MICROSCOPY OF GFAP IMMUNOFLUORESCENCE. J.T. Leo.* C.R. Goodlett, J.C. Mahoney,* and J.R. West. Dept. of Anatomy, University of Iowa, Iowa City, IA 52242.

Alcohol exposure during the early postnatal brain growth spurt in rats, in a pattern which produces cyclic blood alcohol concentrations with high peaks and low troughs each day, results in an acute reactive astrogliosis in the cerebral cortex. Glial fibrillary acidic protein [GFAP] is increased over 300% in the cortex relative to controls on postnatal day [PD] 10, and numerous, hypertrophied astrocytes are revealed by GFAP immunocytochemistry in cortical layer V. The goal of this study was to determine whether cortical astrogliosis included an increase in glial density in addition to hypertrophy of astroglia. The density (per unit volume) of astrocytes labeled by GFAP immunofluorescence was quantified in layer V of the cortex in alcohol-exposed and control pups on PD 10, via the high resolution in 3 dimensions provided by confocal microscopy. Pups from 5 litters were gastrostomized on PD 4. On PD 4-9, they were given either 4.5 g/kg of alcohol per day (delivered in 2 of 12 daily feedings as a 10.2% v/v solution in milk formula; mean peak BAC=294 mg/dl), or were given a calorically-matched diet free of alcohol. Pups were perfused on postnatal day 10, and 50-µm frozen sections were processed for GFAP immunoreactivity using a biotinylated secondary antibody and FITC-conjugated avidin. Matched sections from control and alcohol-treated pups were evaluated with a Bio-Rad MRC 600 confocal microscope [60x objective, N.A. 1.4], sampling from at least 6 locations in layer V of parietal cortex in each section. Unbiased estimates of glial densities were obtained using a 95 µm-square counting frame through a z-axis depth of 10 µm (taken in 1 µm steps). The mean density of labeled astrocytes was significantly higher in alcohol-exposed pups than controls (mean±sem of 5.9±0.7 and 2.8 ±0.6 cells per 10^5 μm^3, respectively). The mean area of GFAP immunoreactivity in the optical sections was also greater in the alcohol-exposed group. Thus, the astrogliosis induced by alcohol in cortical layer V involved an increased density of astroglia as well as hypertrophy of their somas and processes. (Supported by grants #AA 07313 and #AA 05523)

601.18

DISTRIBUTION OF TAURINE, GABA AND GLUTAMATE IN DEVELOPING CAT CEREBELLUM. P. Lu and J.A. Sturman. Inst. for Basic Research, Staten Island, NY 10314.

Taurine has been shown to be involved in feline cerebellum development by *in vivo* and *in vitro* studies. In order to further understand its role we have examined its distribution in newborn, 8-week-old and adult cat cerebellum, along with that of GABA and glutamate. Antisera to taurine, GABA and glutamate were prepared by immunizing rabbits with the amino acids conjugated to bovine serum albumin with glutaraldehyde, and purified to high specificity as determined by ELISA. Clear developmental differences in the distribution of these amino acids were seen. In newborn cerebellum taurine was present in granule cells and in a band in the inner molecular layer adjacent to the Purkinje cell layer, but was not present in Purkinje cells at their dendrites, which did contain glutamate. GABA was present only in Golgi II cells in the granule cell layer. At 8 weeks after birth, taurine is present in most cerebellar cells, especially Purkinje cells and their dendrites and granule cells, which both contained glutamate as well. Stellate and basket cells contained taurine and GABA, which again was also present in Golgi II cells. In adult cerebellum taurine was located in granule and basket cells and was now virtually absent from Purkinje cells and their dendrites, which contained GABA and glutamate. GABA was also present in Golgi II, stellate and some basket cells. Glutamate was also present in granule and a few Golgi II cells. Thus at birth, Purkinje cells appear to contain only the excitatory amino acid glutamate, which is joined by taurine sometime early in development, which, in turn, is replaced by GABA during maturation.

601.19

FELINE MATERNAL TAURINE DEFICIENCY: EFFECT ON NEWBORN VISUAL CORTEX. J.A. Sturman, Y. Xu*, H. Imaki and P. Lu. Inst. for Basic Res., Staten Island, NY 10314.

Cats are dependent on an adequate dietary source of taurine to maintain their body pools and without which they become taurine-depleted. Taurine deficiency has an adverse effect on feline reproduction resulting in excessive fetal wastage and low-birth-weight live kittens. We have previously reported abnormal ontogeny of cerebellum and visual cortex, and now report quantitative morphometric and immunohistochemical evidence of abnormalities in newborn visual cortex. Kittens born alive to taurine-deficient mothers have body and brain weights approximately 70% of similar control kittens (from mothers fed the same diet supplemented with 0.05% taurine) but brain taurine concentrations less than 50% of controls. The thickness of all discernible laminae, except layer I, and total thickness are significantly smaller than control kittens. There was no significant difference in the number of cells (neurons plus glia) under 1 mm^2 of surface, either total or in individual layers, except layer V, which had fewer. Numerical density of cells was greater in layers II-III and IV. Using antibodies raised to amino acids linked to BSA with glutaraldehyde, no cells in visual cortex from taurine-deficient or control showed taurine-like immunoreactivity, although there was extracellular reactivity throughout the region. This was greater in control than in taurine-deficient kittens, and was not present when the antiserum was preabsorbed with antigen. This is not an artefact of the perfusion fixation since neurons in other regions such as frontal cortex, hippocampus and cerebellum showed clear reactivity. Visual cortex neurons did show GABA-like immunoreactivity.

601.20

CEREBELLAR CORTEX ALTERATIONS IN TAURINE DEFICIENT MICE. M.C. Márquez-Orozco, A. Márquez-Orozco, V. Gazca-Ramírez* and H. Pasantes-Morales*. Sch. of Med., Inst. Cell Physiol. Natl. UNAM. P.O.B. 70-553. México 04510 D.F. MEXICO.

Adverse effects of taurine deficiency on differentiation and maturation of cat cerebellum are well documented (Sturman et al., J. Neurosci. Res., 13, 405, 1985) but the consequences of taurine deficiency in nervous tissue in other species have not been explored. In rodents, tissue taurine depletion is obtained by treatment with guanidino ethane-sulfonate (GES), a potent blocker of taurine transport. In the present study we describe the effect of GES treatment (1% in drinking water, 4-12 weeks) on the structure of cerebellar cortex of adult mice. Brains were excised and fixed in 10% formaline pH 7.3, 7 µm-sections were stained with hematoxylin-eosin and observed in a photonic microscope. Alterations in Purkinje cells consisting in marked swelling of the soma and the molecular layer were consistently observed since the 4th week of treatment. Subsequently, picnotic and degenerated Purkinje cells appear. Empty spaces corresponding to degenerated glial cells and neuron synaptic terminals also characterize the alteration caused by GES. The observed changes my be the consequence of cell swelling occurring as result of a decrease of intracellular taurine as a large number of recent reports substantiate the involvement of taurine in osmoregulation in brain.

601.21

MINIMAL REQUIREMENTS FOR LONG-TERM SERUM-FREE CULTURING OF ELECTRICALLY ACTIVE RAT CEREBRAL CORTEX NEURONS IN A MONOLAYER G.J.A. Ramakers, Netherlands Institute for Brain Research, Royal Dutch Academy of Sciences, Meibergdreef 33, 1105 AZ Amsterdam, The Netherlands.

A method is presented and evaluated for culturing dissociated fetal rat cerebral cortex. These cultures meet the following requirements: they are maintained in a serum-free medium; (2) the cultured cells form a near monolayer allowing optimal access and visibility; and (3) neurons survive up to at least six weeks, while (4) showing a high incidence of spontaneous bioelectric activity. Series of 300 to 400 nearly identical cultures can be routinely produced from two litters of rats, and are amenable to the application of a variety of morphological, biochemical and electrophysiological techniques. The dissociated cells are grown in a central spot on poly-lysine coated round coverslips in the presence of an astroglial coculture. The medium consists of 62.5% DMEM and 22.5% Ham's F12 medium, from which glutamate and aspartate have been deleted, and is supplemented with BSA, insulin, transferrin, lipoic acid, and vitamins A and E. These factors and conditioning of this medium by (astro)glial cells are demonstrated to be essential to meet the above requirements. The medium furthermore contains for the first time ionic conditions similar to those in the brain extracellular space. These cultures appear to be especially useful for studying axonal outgrowth, neuron-glia and neuron-target interactions, myelination, transmitter release and various activity-mediated phenomena. In addition, they provide efficient models to study a wide variety of teratological and toxicological effects of chemical agents on brain development.

601.22

LHRH GENE EXPRESSION IN C57BL/6J MICE EXPOSED TO ETHANOL IN UTERO H.C. Scott, R.T. Zoeller, P.K. Rudeen, Department Of Anatomy And Neurobiology, University Of Missouri School Of Medicine, Columbia, MO 65212. The expression of LHRH mRNA was analyzed in fetal C57BL/6J mice exposed to ethanol (ETOH) on gestational day 7 (G7) or on G11. G11 corresponds to the day that LHRH neurons are first detected at the medial olfactory placode. Ethanol exposure on G7 has been shown to cause deformities in the regions where LHRH neurons originate and migrate. Our lab has previously reported that pups exposed to ethanol on G7 had a decrease in the number of LHRH neurons detected by immunohistochemistry on G18 as compared to control pups. Pregnant dams were intubated with a 25% ETOH solution (2.9 g/kg body wt.) or vehicle at 10 a.m. and at 2 p.m. on G7 or G11. Animals were sacrificed on G18 and frozen parasagittal tissue sections cut at 12 µm. In situ hybridization histochemistry localized individual neurons containing LHRH mRNA. The number of neurons expressing LHRH mRNA were counted in six neuroanatomical regions and image analysis was used to quantify the level of LHRH mRNA in neurons in those regions. Data were compared between control pups and pups exposed to ethanol on G7 or on G11. Results show no significant differences in the number of neurons expressing LHRH mRNA on G18 when given ETOH on G7 as compared to control animals. Ethanol exposure does not seem to interfere with LHRH gene expression during development. (Supported by NIAAA grants AA07458, AA05893, and AA00107).

601.23

MAMMALIAN GROWTH ENHANCED BY SUPPLEMENTAL LIGHT: FEED EFFICIENCY IS INCREASED. Patricia D. Wade+# & Ira Baum*+. +Rockefeller Univ., 1230 York Ave., N.Y., NY 10021 & #N.Y. Coll. of Pod. Med., 53 E. 124th, N.Y., NY 10035.

We are studying direct (extraocular) effects of light in the visible range on mammalian tissues. At intensities capable of penetrating the head, we have found that such light influences release of a transmitter from mammalian brain tissue in vitro (Wade et al., PNAS 1988, 85:9322). At a certain intensity it also enhances ^{14}C-2-deoxyglucose uptake into the tissue suggesting a possible effect on cell metabolism. Therefore, as a first step toward understanding what role light may have in cell metabolism, we used body growth as a monitor of metabolism and examined the extent of growth in several lighting conditions. This is based on studies in which supplemental environmental light increased body growth and/or body products (e.g., Am. J. Physiol. 1971, 220:233, J. Endocrinol. 1975, 64:549, Science 1978, 199:911).

In the present study mice pups [strain Crl:CD-1(ICR)BR)] were reared with foster mothers from postnatal day 4 to 28 in either: dark, 12h light/12h dark (standard), or 16h light/8h dark (supplemental). Details of the variation in intensity, duration and spectrum will be presented. Growth as measured by body weight (mean pup weight in grams ± S.E.) was slow in the dark (e.g., at day 28, 11.37±1.16, n=15), greater in the standard condition (13.85±0.35, n=20), and greatest in the supplemental condition (15.06±0.45, n=30). Feed efficiency of weight gain as defined by total weight gain of mother and pups together per total food consumed during the experiment was greatest in the supplemental and least in the dark condition. The increase in growth induced by the light indicates an effect on metabolism; whether it is direct and/or indirect is yet to be determined.

Supported by NSF grant BNS-8920417 to P.D.W. & NIH training grant RR07044.

AGING PROCESSES V

602.1

A MAGNETIC RESONANCE IMAGING (MRI) STUDY OF BRAIN MORPHOMETRIC CHANGES WITH HEALTHY AGING IN HUMAN MALES. D. Murphy, C.S DeCarli, W. Williams, S.I. Rapoport, M.B Schapiro, B Horwitz. National Institute on Aging, Bldg 10, Rm 6C 414 Bethesda, MD 20892.

Aging is associated wirth an increase in the prevelance in motor abnormalities. We wished to examine the effect of aging on the volume of cerebrospinal fluid (CSF) spaces, and the subcortical nuclei. We used MRI to detremine the volumes (vol) of cerebral brain matter (CBM), subcortical grey matter nuclei (GMN), and the ventricular and sulcal cerebrospinal fluid (CSF) in 27 healthy men aged 19 to 92 years. Ten subjects (YM) were below the age of 35 years (mean age 25.4 ± 4 (S.D)) and 17 (OM) were older than 60 years (mean age 73.2 ± 7.7(S.D)). We studied subjects free of primary brain disease or medical conditions that contibute to it. The MRI studies were performed on a Picker 0.5 Tesla scanner. Axial slices were analyzed, using a T1 (TR 2000 msec, TE 20 msec) sequence with 192 views and 2 repetitions. 18, 7mm thick, contiguous slices were obtained from the foramen magnum to the vertex. The results of the OM were compared to the YM with a students t test after correcting for intracranial vol. OM had significantly ($p<0.05$) less CBM than the YM. The % of intracranial vol occupied (% vol) by the CBM in the OM was 75.94 ± 3.74 (mean ± S.D), and in the YM was 80.67 ± 2.73 (mean ± S.D). The OM had significantly larger ($p<0.05$) CSF vols than the YM in every CSF measure, except the left lateral ventricle. The caudate (CN) and lenticular (LN) nuclei were significantly smaller ($p<0.05$) in the OM than the YM. In the OM the % vol of the CN and LN respectively were 0.72 ± 0.09 (mean ± S.D), and 0.90 ± 0.14 (mean ± S.D). In the YM the CN and LN volumes were 0.92 ± 0.09 (mean + S.D), and 1.22 ± 0.11 (mean ± S.D). This significant difference remained when their vol was expressed as a ratio of CBM vol. The vol of the thalamus did not differ between groups. There was no asymmetry in the vols of the CBM, GMN or CSF in OM or YM. In conclusion, the caudate and lenticular nuclei are significantly more affected by healthy aging than cerebral brain matter, this may account for some of the motor abnormalites in aging.

602.2

AGE-RELATED CHANGES IN WERNICKE'S AREA: A QUANTITATIVE DENDRITIC ANALYSIS. A. B. Scheibel and B. Jacobs. Brain Res. Inst., UCLA, Los Angeles, CA 90024.

This investigation examined age-related changes in the basilar dendrites of supragranular pyramidal cells in Wernicke's area of left and right hemispheres. Tissue was obtained from 10 male (M_{age} = 52.2; *range* = 18-78; *SD* = 17.42) and 10 female (M_{age} = 47.8 years; *range* = 20-79; *SD* = 20.47) subjects. All subjects were neurologically nomal right-handers. Using a modified rapid Golgi technique, 10 pyramidal cells were sampled from each hemisphere (total # of cells = 400; total # of segments = 16,697). These were evaluated according to total dendritic length (TDL), mean dendritic length (MDL), and dendritic segment count (DSC). A distinction was also made between proximal (1st, 2nd, and 3rd order) and the ontogenetically later developing distal (4th order and above) dendritic branches.

Despite considerable interindividual variation, the data clearly revealed an age-related shift in dendritic laterality such that individuals less than 50 years of age tended to have greater TDL values in the LH, while the trend was reversed in individuals older than 50. The data also exhibited both dendritic proliferation and degeneration with aging. There was an age-related decrease in TDL (r = -.44) and especially in MDL (r = -.69, p < .001) with increasing subject age. Age-MDL correlations were negative for all segment orders and revealed a progressive decrease in segment length in more distal branches. Although length measurements decreased with age, there was an age-related increase in the number of dendritic segments (r = .23), especially in the right hemisphere. Additional support for the age-related loss in dendritic material came from a post-hoc analysis of two 9 year old children, who had the highest TDL values of all subjects. These findings parallel the metabolic changes reported by Chugani et al. (*Annals of Neurol., 22:* 487-497, 1987) and the synaptic elimination noted by Huttenlocher (*Brain Res., 163:* 195-205, 1979).

602.3

AGE-RELATED REGIONAL DIFFERENCES IN CEREBELLAR VERMIS OBSERVED *IN VIVO*. N. Raz, K.White*, I.J. Torres*, W.D. Spencer*, and J.D. Acker*#. Dept. Psychology, Memphis State University, Memphis, TN 38152 and #Diagnostic Imaging Center, Baptist Memorial Hospital, Memphis, TN 38119.

We investigated age-related differences in cerebellar vermis. The areas of three vermal regions were estimated from digitized midsagittal MRI scans of 29 healthy Ss and 24 neurologically intact Ss free of vestibular symptoms, seizures, psychoses or alcoholism (age 18-78). The three ROIs included: 1-centralis and culmen; 2-declive, folium, tuber, and pyramis; 3-uvula and nodulus. Ventral pons was used as a control region. After covarying cranial size, we found age-related decline in the vermis area [Λ=.79; $F(3,47)=4.1$, $p<.01$]. This difference was found only for the superior segments of the vermis [$F(1,49)=6.45$ and $F(1,49)=6.75$ for ROIs 1 and 2, respectively, both $p<.015$], but not for uvula-nodulus ($F<1$). Pons was unaffected by age. No sex differences were found. The observed vermal regions of age-related loss correspond to the cerebellar areas with increased metabolic demands. Thus, selective vermal atrophy may stem from age-related decline in tissue oxygenation.

Supported by MSU Center for Applied Psychological Research.

602.4

DIFFERENTIAL CELLULAR CHANGES IN THE FRONTOPARIETAL CORTEX AFTER BASAL FOREBRAIN LESIONS IN ADULT AND AGED RATS. C.L. Wellman and D.R. Sengelaub. Program in Neural Science and Department of Psychology, Indiana University, Bloomington, IN 47405.

Because aging can be viewed as a regressive process that alters both cell numbers and plasticity of remaining neurons, aging may interact with other degenerative processes to produce unique pathological syndromes. To understand the mechanisms of aging and disorders associated with aging, we are using basal forebrain lesions in adult and aged rats to determine the contribution and potential interaction of age and depletions of cholinergic input on frontoparietal morphology.

Thirteen- and 21-month-old rats received either sham or ibotenic acid lesions of the basal forebrain. Three months after lesioning (at 16 and 24 months; adult and aged, respectively), cellular changes in the frontoparietal cortex were assessed in frozen sectioned, cresylecht violet stained material. Neuronal somas were smaller in aged sham-lesioned rats than in adult sham-lesioned rats, but cortical laminar thickness did not decrease with age. Furthermore, in both adult and aged rats, basal forebrain lesions reduced soma size in specific cortical laminae, resulting in decreased laminar thickness relative to sham-lesioned rats. However, decreases in laminar thickness and soma size were more pronounced in the 16-month-old rats compared to the 24-month-old rats. The pattern of changes also differed between the two ages: changes tended to be more pronounced in the superficial laminae of adult rats, whereas aged rats showed more changes in the deeper laminae. Thus, the magnitude and pattern of regressive changes in frontoparietal cortex after basal forebrain lesion differ in adult and aged rats, indicating that aging interacts with cholinergic depletion to produce unique neural changes.

602.5

DEGENERATIVE CHANGES IN BASAL FOREBRAIN CHOLINERGIC NEURONS IN AGED RATS: A QUANTITATIVE ANALYSIS OF CELLS STAINED FOR ChAT OR NGFr, OR RETROGRADELY LABELLED WITH FLUORESCENT TRACER OR I-125 NGF. S. de Lacalle, J.D. Cooper*, C.N. Svendsen*, S.B. Dunnett and M.V. Sofroniew. Departments of Anatomy and Experimental Psychology, University of Cambridge, England.

The effects of aging on basal forebrain cholinergic neurons were investigated in male Sprague-Dawley rats aged 3-6 or 24-33 months by immunohistochemical detection of ChAT or NGFr, and by retrograde transport of Fluoro Gold (F.G.) or I-125 labelled NGF injected into multiple sites in the cerebral cortex and hippocampus. Cell counts and surface area measurements were prepared for precisely defined subdivisions of the basal forebrain cholinergic system: medial septum (MS), vertical and horizontal diagonal band, and basal nucleus (BN). Values from about one third of the aged rats were not different from those of young adults; the remaining aged rats showed degenerative changes either in the form of cell loss or atrophy in several of the parameters tested. The degree of degenerative change varied considerably among individual rats and among different regions, the MS being affected the least and the BN the most. Some aged rats showed a decline in the ratio of F.G./ChAT labelled cells, suggesting an impaired ability to retrogradely transport tracer, while others showed reduced retrograde labelling with I-125 NGF. These findings suggest that in some but not all aged rats basal forebrain cholinergic neurons decline in number and atrophy in the form of cell shrinkage, reduced ChAT immunoreactivity, and reduced capacity to retrogradely transport tracers and I-125 NGF.

602.6

INCREASING NEURON NUMBER WITH BODY SIZE: A TEST OF THE SELECTION HYPOTHESIS. P.G.R. St. Wecker and P.B. Farel. Dept. of Physiology, Univ. N. Car. at Chapel Hill, NC 27599.

Large bullfrogs have more neurons in the hindlimb motor pool (JCN 261:125, 1986) and in dorsal root and sympathetic ganglia (Soc. Neurosci. Abstr. 15:301, 1989) than small frogs. The possibility that this association is due to counting error was eliminated by 3-D reconstructions of HRP-labeled neurons, which showed that nucleoli are not split in sectioning. A second alternative to neuron addition is that, by selection, only those frogs with many neurons survive to attain large size. Thus, the difference in mean number of neurons between small and large animals may be due to a dropping out over time of those animals with few neurons. This possibility can be tested by counting neurons from the same closed population at different points in time.

Twelve size-matched frogs were randomly divided into two groups shortly after metamorphosis. Six juvenile frogs, with a mean length of 4.0±.1 cm (mean±SEM) and a mean weight of 5.6±.2 g, were killed immediately. At the end of one year, the remaining six animals (mean length 6.7±.2 cm, mean weight 26.6±2.4 g) had substantially more lumbar sympathetic neurons (7756±571 vs 3398±413) and dorsal root ganglion neurons (9930±572 vs 5505±449).

These data show that selection cannot account for the relationship between body size and neuron number. Previous studies of ^{3}H-thymidine incorporation provided no evidence for neuron birth after metamorphosis. The explanation most consistent with these results is that immature nerve cells differentiate with increasing body size.

602.7

THE EFFECTS OF LONGTERM VOLUNTARY ETHANOL CONSUMPTION ON THE AGING OF SYMPATHETIC NEURONS IN THE RAT. P.Jaatinen[1], A. Hervonen[1], H. Alho[1] and K. Kiianmaa[2]. [1]Laboratory of Gerontology, University of Tampere Medical School, Tampere, and [2]Research Laboratories, Alko Ltd., Helsinki, Finland)

The effect of two years' voluntary ethanol intake (mean; 6.5 g/kg/day) on the aging of the nervous tissue was studied in alcohol preferring AA (Alko alcohol) rats. Here we report the ethanol-induced changes in the aging of superior cervical ganglion (SCG) neurons. The initially 3-month-old rats were given access to food, tap water, and 10% ethanol solution (ethanol group) or to food and water only (control group). At 28 months of age the rats were killed, and samples of the SCG were processed for histochemical, morphometric, and electron microscopic studies. The long-term ethanol exposure increased the heterogeneity of the aged neuron population and produced several signs of enhanced neuronal degeneration. The packing density of SCG-neurons (the number of neurons with a large nuclear profile per 90,000μm^2) decreased from 42.7±0.97 (mean±SD) at the age of 3 months to 25.5±1.34 in the controls and 21.0±3.13 in the ethanol group at 28 months. The amount of highly pigmented neurons was 39.3±2.5% in the old control rats and 59.3±4.2% in the old ethanol group. The overall intensity of TH-immunoreactivity and catecholamine histofluorescence was also lower in the ethanol rats than in the controls. The density of fluorescence intensity of the peripheral neuronal network in the submandibular gland (a target tissue of the SCG) was decreased in the ethanol-exposed rats. The monoamine contents of different brain areas did not change due to ethanol exposure. The survival of ethanol and control rats did not differ either. Thus the results may indicate a selective vulnerability of sympathetic neurons rather than a universal accelerated aging due to ethanol exposure.

602.8

PATHOLOGY OF PRE- AND POSTSYNAPTIC ELEMENTS IN AGED MOUSE SYMPATHETIC GANGLIA. R.E. Schmidt, K.G. Ruit, and W.D. Snider. Departments of Pathology and Neurology, Washington University School of Medicine, St. Louis, MO 63110.

Dysfunction of the autonomic nervous system is an increasingly recognized problem in aging animals and man, the pathological basis of which is poorly understood. In this study of young (4 month) and aged (24 month) mice we have examined the ultrastructural appearance of perikarya, dendritic processes, preterminal axons, and synapses within selected sympathetic ganglia as well as the structure of the dendritic arborizations of principal sympathetic neurons using intracellular injections of Lucifer yellow (LY).

Upon ultrastructural examination, numerous large spherical structures derived from terminal presynaptic axons and synapses were located in proximity to neurons within the prevertebral and paravertebral sympathetic ganglia of aged mice. These markedly dilated preterminal axons distorted the perikarya of sympathetic neurons and contained a variety of subcellular organelles which had the ultrastructural appearance of neuroaxonal dystrophy, a process described in a variety of clinical and experimental conditions. Dystrophic axons were rare in young animals, and, in distinction to other animal species and man, both prevertebral and paravertebral sympathetic ganglia were involved. Intracellular injections of LY revealed that the dendritic arborizations of aged sympathetic neurons were significantly smaller with regard to total dendritic length, extent, and branching when compared to those of young animals. These short, stunted dendritic processes also exhibited large, dystrophic swellings along their extent, a phenomenon not observed in young animals. The ultrastructural appearance of these dystrophic dendritic profiles is presently under investigation.

These observations may provide a neuropathological basis for changes in autonomic function observed in aged animals and man.

602.9

CELL SURVIVAL IN DORSAL ROOT GANGLION FOLLOWING SCIATIC NERVE SECTION: A COMPARISON OF ADULT AND AGED RATS. S. Croul*, E. Wolf* and I. Zimmerman. Depts of Pathology and Physiology -Biochemistry. The Medical College of Pennsylvania, Philadelphia, PA 19129.

Previous studies have quantitated the loss of dorsal root ganglion cells from the L5 dorsal root ganglion following sciatic nerve section in adult rats. In this study, we have examined the issue of dorsal root ganglion cell death in adult and aged rats. Fischer 344 rats underwent sciatic nerve section at 2, 12, 24 or 30 months of age. Animals were sacrificed at 1 month following surgery and the number of cells in the L5 dorsal root ganglion counted. we found no difference in cell survival between those age groups. The ability of dorsal root ganglion cells to respond to nerve section does not seem to change with age at this time interval.

603.1

MODIFICATION OF NON-RADIOACTIVE DETECTION OF NEUROPEPTIDES FOR IN SITU HYBRIDIZATION IN RAT BRAIN. N. Nousek-Goebl*, T.C. Cavanagh[+] and D. S. Grega[+]. R&D Division, Boehringer Mannheim Corp. and [+]Indiana Univ. Sch of Med., Program in Medical Neurobiology, Indianapolis, IN 46250

In situ hybridization histochemistry is a powerful tool to localize specific mRNAs to individual cells. This is especially important in the nervous system where the complex structural interactions of diverse neuronal and glial cell types need to be preserved. Non-radioactive detection of hybridization probes is attractive for safety and ease of use. Additionally, localization to subcellular structure is desirable.

This report describes the modification of the detection method for localizing digoxigenin-labeled oligonucleotide probes used for in situ hybridization in the rat nervous system. Ordinarily following hybridization with cellular mRNA, the 3'-end tailed oligo probe is localized using an alkaline phosphatase antibody conjugate and a subsequent color reaction. In the present study, whole anti-digoxigenin sheep polyclonal antibody is used as a primary antibody, followed by a biotinylated anti-sheep secondary. This complex is visualized using an avidin-biotinylated peroxidase or alkaline phosphatase detection system. This detection method results in a reaction product which is more immunocytochemical in appearance. This procedure has been performed with a 48mer to oxytocin which yielded crisp cellular labeling in the PVN and SON. Several other probes are under study.

603.2

A NEW METHOD FOR *IN SITU* HYBRIDIZATION HISTOCHEMISTRY TO PROCESS BRAIN MATERIAL STORED FOR SEVERAL YEARS W.X. Lu* and S. N. Haber, Dept. of Neurobiology and Anatomy, Univ. of Rochester Sch. of Med., Rochester, NY 14642.

We have developed a new protocol for *in situ* hybridization in order to label mRNA in tissue sections that have been in long-term storage and have not been treated to prevent RNase activity. Brains from rats and monkeys were sectioned at 50μ and stored free floating in an ethylene glycol based cryoprotective solution at -20 °C. Rat brain sections were stored in cryoprotective solution for 3 days, 1 month, and 2 months. Control sections were cut and mounted immediately on gelatin coated slides and stored at -80°C. Monkey brains sections were stored in cryoprotective solution for up to 5 years. 48-mer oligonucleotide probes, human preproenkephalin (sequence 130-145), rat preproenkephalin (sequence 388-435) and rat tyrosine hydroxylase (sequence1441-1488), were labeled with ^{35}S-dATP and terminal deoxynucleotidyl transferase. To prevent RNase contamination from mounting the tissue sections onto gelatin coated slides, *in situ* hybridization was performed in sterile culture dishes prior to mounting. Each solution was removed from the dish by aspiration. Prior to the hybridization step, there was no solution in the dish and the sections lay flat on the bottom of the dish. The amount of probe used for each section was 50μl (rat) and 500μl (monkey).

Using this protocol, the results in rats showed better specific labeling with less nonspecific background compared to controls. Excellent resolution was obtained from monkey sections that were stored in cryoprotectant for up to 31 months. As with conventional in *situ* hybridization methodology, the prehybridization treatment step is not necessary to reduce the background. This protocol is an efficient procedure to allow effective labeling of mRNA in brain sections that have been stored for several years in cryoprotectant solution. This protocol is suitable for various species including rat, mouse, monkey, and possibly human. Supported by NIMH MH 45573.

603.3

COMBINED IN SITU HYBRIDIZATION AND IMMUNOCYTOCHEMISTRY FOR THE ANALYSIS OF PEPTIDE AND 5-HT EXPRESSION IN THE RAT BRAIN. G. Wotherspoon*, D. Savery*, S. Averill*, J.V. Priestley and M. Rattray. Departments of Physiology & Biochemistry, UMDS St.Thomas's Campus, London, England.

To study the expression of 5-hydroxytryptamine (5-HT) and peptide mRNA, we have developed a procedure combining immunocytochemistry with in situ hybridization using ^{35}S labelled oligonucleotides. Peroxidase anti-peroxidase immunocytochemistry and immunofluorescence were tested and were applied either preceeding or following in situ hybridization. Combined 5-HT immunocytochemistry and peptide in situ hybridization was obtained using immunofluorescence followed by in situ hybridization. The fluorescence survived a post-hybridization 55°C wash and liquid emulsion autoradiography. Loss of mRNA during immunostaining was prevented by diluting antisera in 0.1% diethylpyrocarbonate treated phosphate buffered saline containing 20 mM vanadyl ribonucleoside complex. Double labelling was not as sensitive as 5-HT immunocytochemistry or in situ hybridization performed alone, but was adequate to allow localisation of 5-HT immunoreactive cell bodies and fibres combined with enkephalin, somatostatin, cholecystokinin, galanin or glutamic acid decarboxylase mRNAs. In most CNS regions 5-HT immunoreactivity and peptide mRNA occurred in separate cell populations, but coexistence was observed between 5-HT and enkephalin in raphe magnus and 5-HT and galanin in raphe dorsalis.

603.4

QUANTITATIVE AUTORADIOGRAPHY OF TYROSINE HYDROXYLASE IMMUNOREACTIVITY IN THE LOCUS COERULEUS AND THE SUBSTANTIA NIGRA OF RATS TREATED WITH RESERPINE. R. Raisman-Vozari*, V. Blanchard*, M. Savasta M., F. Javoy-Agid, E. Hirsch C., Feuerstein and Y. Agid. INSERM U 289, Hôpital de la Salpêtrière, 75651 Paris and INSERM U 318, CHU 38043 Grenoble, France.

Most of the immunohistochemical studies so far reported were only qualitative, we therefore developed a rapid and sensitive radioimmunohistochemical method for tyrosine hydroxylase (TH) quantification in fresh-frozen rat brain. The present study was performed in the substantia nigra (SNC) and ventral tegmental area (VTA), containing dopaminergic neurons and the locus coeruleus (LC) and subcoeruleus (SubC), containing noradrenergic neurons, of rats treated with reserpine. Autoradiography of TH was performed on 15 μm thick coronal sections from unfixed brain of rats treated either with reserpine (i.p.,10 mg/kg) or vehicle. Sections were incubated with TH monoclonal antibody for 2 hours and the reaction was revealed with a ^{35}S-labelled secondary antibody. TH was quantified in the different brain regions by measuring optical density (O.D.) on film or emulsion autoradiography and compared with adequate TH standards, using computer-assisted image analysis. TH concentrations (expressed in ng/mg prot) measured on films were 40% increased by reserpine in the LC and 20% in the SubC but were unchanged in the SNC and in the VTA. Microscopic examination of TH radioimmunolabeling on emulsion-coated sections showed a heavy labeling appearing as an accumulation of silver grains over cell bodies and also over processes. Grain density per cell, considered as representative of the amount of TH on individual cells, was increased in the LC and subC but not in the SNC. These results support the validity of the radioimmunohistochemical method as a tool for quantifying proteins at cellular level and confirm that TH protein content is differentially regulated in noradrenergic and dopaminergic neurons in response to reserpine.

603.5

KINETIC ANALYSIS OF SECONDARY ANTIBODY BINDING TO FLOATING BRAIN SECTIONS. A METHOD OF QUANTITATIVE IMMUNOCYTOCHEMISTRY. D.G. Morgan, D.G. Berg* and M.N. Gordon. Div. of Neurogerontology, Univ. of Southern California, Los Angeles, CA 90089-0191.

50 μm vibratome rat brain sections were incubated overnight with mouse monoclonal antibody (Ab) towards GFAP. The binding of a secondary (2°) rat monoclonal Ab conjugated to HRP was measured using the soluble HRP substrate tetramethylbenzidine. Assays were stopped with sulfuric acid, and the sections incubated by shaking to evenly distribute the reaction product before A_{450} was measured. Preliminary studies established a standard curve relating A_{450} to the molar concentration of 2° Ab. Tissue sections processed without the primary antibody were used to estimate nonspecific binding of the 2° Ab, and these values were subtracted from the "total" binding to yield specific 2° Ab binding. Rarely did these nonspecific values exceed 10% of the total binding. Specific 2° Ab binding was not affected by section thickness between 30 and 90 μm, confirming earlier observations that Ab penetration into brain sections was limited to the outer few μm.

The time course of 2° Ab association was dependent upon the concentration of 2° Ab. At high concentration, maximal binding was attained by 3 h of incubation, while 6 h of incubation was required for low 2° Ab binding. Values were lower after 23 h of incubation at all concentrations, probably because of dissociation of the primary Ab from the section. An initial saturation analysis indicated a K_d of 7 nM for the 2° Ab. These preliminary experiments validate the utility of this method for evaluating Ab binding kinetics to brain sections, and will allow quantitative analyses of primary Ab binding kinetics in future studies. Supported by AG-7892, AHA-GIA 891079, and AHA-EIA 890173.

603.6

A NEW METHOD FOR THE PREPARATION OF ANTISERUM TO LUCIFER YELLOW. C. Brandon, M.H. Criswell*, and E. Indyk*, Dept. of Cell Biology and Anatomy, The Chicago Medical School, North Chicago, IL 60064.

Intracellular injection of Lucifer Yellow (LY) has proven to be very useful for the study of cell morphology (Stewart, Cell *14*, 71 (1978)). Long periods of fluorescent observation, however, may cause fading, so it is often necessary to generate a visible marker. Photo-oxidation of LY (Maranto, Science *217*, 953 (1982)) can be used to convert it to a visible reaction product, but is tedious when applied to large numbers of cells. Immunocytochemical localization of LY has been used (e. g. Taghert, Dev. Biol. *94*, 391 (1982)), but no detailed information about the conjugate preparation and antibody production has been published. We have developed a new method for the preparation of antisera to LY, and used these sera for the immunocytochemical localization of LY in large numbers of dye-filled cells in vertebrate retina.

Covalent conjugates of the VS isomer of LY were prepared with keyhole limpet hemocyanin (KLH) or rabbit serum albumin (RSA), and used as immunogens in rabbits. Antiserum to the KLH-LY conjugate contained precipitating antibodies against KLH and LY, while antiserum to the RSA-LY conjugate had precipitating activity only against LY. When used for immunocytochemical staining of large retinal pieces containing many LY-filled cells, both antisera yielded well-stained, darkly-filled cells similar to those seen with the Golgi technique; even very fine dendritic processes of retinal ganglion cells could be followed for long distances. We used tissue that was stained *in vitro*, but the immunocytochemical approach may be especially useful for the immunocytochemical identification of cells filled with LY after tissue fixation.

Supported by the USPHS.

603.7

INTRACELLULAR INJECTIONS OF BIOTINYLATED LUCIFER YELLOW IN COMBINATION WITH RETROGRADE TRACING IN LIGHTLY FIXED SECTIONS OF THE INFERIOR COLLICULUS (IC). S.J. HILL and D.L. Oliver, Dept. Anatomy, UCONN Health Center, Farmington, CT 06030

Intracellular injections of neurons can be made even when the tissue has been previously fixed by aldehydes (Tauchi & Masland, '85; Einstein, '88; Buhl & Lubke, '89). Such cells can be converted to a permanent non-fluorescent form. In the present study of the rat, we identify cells in the IC that project to the ipsilateral thalamus by injection of rhodamine-labeled latex beads (LumaFluor) in the medial geniculate body and retrograde transport in vivo. After 1-3 days, the brains are perfused with 2.5% paraformaldehyde and 0.2% glutaraldehyde in phosphate buffer (pH 7.3). The IC is cut on a vibratome into 150-300 µM thick sections. Intracellular micropipets (100-200 MΩ, 1.0 mm borosilicate glass with capillary pulled on a Flaming-Brown P80/PC) are filled with 9% Lucifer Yellow dilithium salt (L-0259, Sigma) and 1% biotinylated lucifer yellow (L-2601, Molecular Probes). The rhodamine-labeled cells are observed with epi-fluorescent optics, impaled under visual control, and injected with 5-20 nA of negative current (100 msec duty cycle) until they appear well filled. The biotinylated lucifer yellow is then converted to a permanent stable reaction product by overnight incubation in avidin-HRP (Vectastain) in the presence of 0.1% triton X-100 at 4°C. Incubation in DAB with cobalt and nickel follows (Adams, '81).

This method produces well filled neuronal cell bodies, dendrites, and spines for light microscopic analysis. The non-fluorescent reaction product in these intracellularly filled cells will permit transmitter immunohistochemistry and synaptic fine structure to be studied in combination with neural connections and dendritic morphology.

Supported by grants from NIDOCD (R01-DC00189).

603.8

A COMBINATION OF COBALT AND IMMUNOCYTOCHEMICAL LABELING FOR ELECTRONMICROSCOPICAL STUDIES. I. Nagy*, M. Petko*, E. Polgar*, A. Sik*, M. Antal, J. D. Feigenbaum[1]. Dept. of Anat., Univ. Med. Sch. of Debrecen, H-4012, Hungary, [1]Dept. of Anat., Univ. Coll. of London, WC1E 6BT, England.

Combined methods of tracer and immunocytochemical labeling of nervous tissues for electronmicroscopical (EM) investigations described previously have various disadvantages and difficulties. We report here a new and simple EM double labeling technique using cobalt staining and peroxidase-antiperoxidase (PAP) pre-embedding or immunogold (IG) post-embedding immunocytochemical reactions, which can overcome these problems. Primary afferents of the frog spinal cord were labeled with cobalt, and GABA-, or CGRP-like immunoreactivity was shown in profiles adjacent to cobalt positive structures or in cobalt positive boutons, respectively. Our results have shown: 1. The chemical nature of profiles identified by the cobalt labelling can be studied, because the EM appearance of the endproduct of cobalt labeling is different from those of both immunocytochemical reactions. 2. Cobalt and IG labeling can be studied on the same section, because the electron density of cobalt labeling is not influenced by the osmium content of the tissue. 3. Selective and intensive dye and immunocytochemical staining can be achieved.

603.9

THE USE OF NICKEL IONS FOR TRACING CENTRAL CONNECTIONS AND PERIPHERAL PROJECTIONS OF THE LEECH NERVOUS SYSTEM. M. Nitabach and E. Macagno. Dept. of Biological Sciences, Columbia University, NY, NY 10027.

We report here on the use of Ni^{++} and Nickel-lysine (Ni-Ly) as dyes both for nerve backfilling and for direct injection of identified neurons in the leech, *Hirudo medicinalis*. The male sex nerve was backfilled with either 5% $NiCl_2$ or 250mM Ni-Ly. After precipitation of NiS and silver intensification, we saw at least 8 neuronal somata in stereotyped ganglionic positions in all cases examined. In addition, we saw ganglionic fiber tracts containing the filled central projections of peripherally located sensory neurons. We also backfilled the male sex nerve in several cases with 30% horseradish peroxidase (HRP). We always saw more backfilled neurons with Ni^{++} or Ni-Ly than with HRP. This suggests that these dyes are more sensitive for backfills than HRP.

As no previous report of direct intracellular iontophoresis of Ni^{++} or Ni-Ly into leech neurons was known to us, we examined the feasibility of these dyes for this purpose. While ~30MΩ 150mM Ni^{++} electrodes could pass positive current when the tip was in the bath, as soon as a neuron was impaled they invariably clogged. In contrast, ~30MΩ 250mM Ni/Ly electrodes could pass 300msec 1-5nA positive current pulses at 1Hz into impaled leech neurons for up to 30min without clogging. This method was used to fill identified cells in both adult and embryonic leeches. These fills were at least as complete as those obtained after intracellular HRP injection. Even very fine ramifications of embryonic sensory neurons' peripheral projections could be seen in the body wall. Interestingly, when Ni^{++} was pressure injected into a Retzius cell, it did not pass into the electrically coupled contralateral Retzius cell.

603.10

BIOTINYLATED DEXTRAN AMINE AS AN ANTEROGRADE TRACER FOR SINGLE- AND DOUBLE-LABELING STUDIES. A. Reiner, C.L. Veenman and M.G. Honig. Department of Anatomy and Neurobiology, University of Tennessee - Memphis, Memphis, TN 38163.

The utility of fluorescent dextran amines for anterograde pathway tracing has recently been reported. Fluorescent markers, however, are not always ideal for detailed anatomical mapping studies. We therefore evaluated the efficacy of biotinylated dextran amine (BDA, MW10K) for anterograde labeling in several different preparations. BDA was visualized with an avidin-biotinylated HRP (ABC) procedure followed by a DAB reaction. After iontophoretic injections of BDA into isocortex-like telencephalic regions in pigeons or into visual or somatosensory cortex in rats, we found excellent and abundant labeling of terminals in forebrain, midbrain and hindbrain target areas with one week survival times. Pressure injections of BDA into the ventral horn of embryonic chick spinal cord yielded extensive anterograde labeling of motoneuron axons in peripheral nerves. We also found that BDA labeling could be combined with a second labeling method to differentially label and distinguish two sets of projections. For these experiments, motoneuron and sensory neuron projections into the embryonic chick hindlimb were differentially labeled by HRP injection into the DRGs (visualized using a blue cobalt-DAB reaction) and BDA injection into the spinal cord (visualized with a brown DAB reaction). Taken together, these various studies show that BDA is effective for anterograde pathway tracing and can be used in double-label studies with other labeling methods.

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603.11

ANTEROGRADE NEUROANATOMICAL TRACT TRACING WITH CENTRAL NERVOUS SYSTEM INJECTIONS OF IMMUNOGLOBULIN G: A LIGHT AND ELECTRON MICROSCOPIC EVALUATION. C.F. Phelix and W.K. Paull Div. of Life Sci., Univ. of Texas, San Antonio, TX 78285, Dept. Anat. & Neurobio., Univ. Missouri, Columbia, MO 65212

Normal rabbit serum (NRS) was pressure injected into the forebrain of rats to be tested as an anterograde neuroanatomical tracing substance. Undiluted NRS was stereotaxically injected into the bed nucleus of the stria terminalis (BST) with a 1 µl Hamilton syringe. Postinjection survival times ranged from 24 hr to 14 days. An immunohistochemical method utilizing goat anti-rabbit IgG antibody was used to detect the rabbit IgG within vibratome sections. Visualization of the final reaction product (diaminobenzidine, DAB) was enhanced by a silver/gold postintensification (SGI) method. Rabbit IgG-containing neural structures were examined at both light and electron microscopic (EM) levels. At the injection site neuronal soma, dendrites and axons were filled homogeneously with the SGI-DAB at 24 hr, 48 hr, 7 days and 14 days indicating local neuronal uptake, storage and transport of rabbit IgG. In the hypothalamus many anterogradely filled axons were present and displayed short collateral branches and terminals. EM examination revealed synaptic terminals containing IgG, without signs of trans-synaptic transport after 14 days. Signs of retrograde transport of IgG were never observed. A propensity of neurons to take up, sequester and anterogradely transport IgG is indicated.

603.12

THE USE OF HERPES SIMPLEX VIRUS TYPE 1 FOR NEURONAL PATHWAY MAPPING IN MICE. G.A. Lewandowski, P.P. Sanna and F.E. Bloom. Department of Neuropharmacology, Research Institute of Scripps Clinic, La Jolla, CA 92037.

Herpes simplex virus type 1 (HSV–1) was used as a transneuronal pathway tracer in the CNS. HSV–1 was stereotaxically placed in the caudate putamen of mice and after varying survival times mice were sacrificed by cardiac perfusion with paraformaldehyde fixative. Virus was detected in brain sections by immunohistochemistry with a polyclonal antibody to HSV–1 proteins, and an antibody to mouse macrophage (Mac–1) was used to detect immune responses within the same sections. The retrograde, anterograde and transynaptic transport of the virus was observed. HSV–1 was transported from the caudate along all known major pathways and was detected in regions of the brain including the cortex, globus pallidus, substantia nigra, dorsal raphe nucleus, ventral tegmental area, and thalamus. The immunostaining patterns suggest that the virus was then transported transynaptically from these regions. The Mac–1 staining of the same sections revealed that activation of macrophages follows the same inter–neuronal pathways of the viral infection in a temporal manner.

603.13

MOUSE CORNEAL MODEL OF HERPES SIMPLEX VIRUS (HSV) INFECTION: TISSUE TARGETS AND ROUTES OF SPREAD J.R. Martin[1]*, F.J. Jenkins[2], D.B. Henken[1]* [1]Lab. of Expt'l Neuropath., NIH, [2]USUHS; Bethesda, MD, 20892

In animal models, spread of HSV-1 from epithelial replication sites to the PNS and CNS is known from analysis of individual tissues. To more completely define tissue targets of infection and infer routes of spread, corneas of adult mice were inoculated with 10^6pfu of HSV-1, F strain. One to 13 days later, groups of 4 mice were formalin-perfused. Decalcified head and neck were embedded *en block* in paraffin. At intervals, serial sections were screened for HSV antigen. On days 1 and 2, antigen was limited to cornea and conjunctiva but by days 3 and 4 was also in autonomic ganglia and the trigeminal system. On day 6, antigen reached its maximum extent; infected sites included the trigeminal complex (ganglion, ophthalmic and maxillary branches, root and spinal nucleus and tract) ethmoid sinus and olfactory bulb, visual system, and autonomic ganglia (ciliary, pterygopalatine and superior cervical). By days 8 and 10, antigen was greatly reduced, and day 13 samples were negative. This method shows a broader range of infected tissues and suggests a more complex pattern of HSV spread than has been recognized. Virus appears to reach the intracranial compartment by 4 different neural routes. Inoculum dose may alter the kinetics of virus spread to neural tissues.

603.14

TRACING OLFACTORY PATHWAYS IN THE MOUSE WITH A CORONAVIRUS. E.M. Barnett*, M. Cassell and S. Perlman*. University of Iowa College of Medicine, Iowa City, IA 52242.

Olfactory system pathways in the mouse were studied after intranasal inoculation of Mouse Hepatitis Virus (MHV) strain JHM, a neurotropic murine Coronavirus which is non-pathogenic to humans. Animals were sacrificed 4 and 5 days post inoculation and viral RNA was detected by means of in situ hybridization with a ^{35}S labeled probe. Labeling was seen in the main olfactory bulb and its afferent and efferent connections including: the anterior olfactory nucleus, insular cortex, piriform cortex, the nucleus of the diagonal band, lateral hypothalamus, the nucleus of the lateral olfactory tract, and midbrain raphe nuclei. These results are consistent with transneuronal retrograde, and possibly anterograde, movement of virus. Viral RNA was also labeled in structures such as the endopiriform nuclei, the external lateral parabrachial nuclei, the ventral tegmental area, and medullary raphe nuclei suggesting viral movement across several synapses. These findings suggest that MHV strain JHM may be a useful neural systems tracer in the mouse.

603.15

ß-GALACTOSIDASE EXPRESSING RECOMBINANT PSEUDO-RABIES VIRUS FOR LIGHT AND ELECTRON MICROSCOPIC STUDY OF RETROGRADE TRANSNEURONALLY LABELED CNS NEURONS. A.D. Loewy, P.C. Bridgman & T.C. Mettenleiter. Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110 and Federal Research Center for Virus Diseases of Animals, D-7400 Tübingen, Germany.

Bartha's strain of pseudorabies virus (Bartha PRV) is a neurotropic virus used in veterinary medicine to vaccinate pigs against Aujesky's disease (i.e., the "mad itch"). It has also been shown to be a highly specific retrograde transneuronal cell body marker (Brain Res. 491:274, 1989 and J. Neurosci. 10:2139, 1990). The latter property makes this particular virus a potentially useful tool for neuroanatomy and for other neurobiological studies.

A ß-galactosidase expressing Bartha PRV was constructed and used to transneuronally label the CNS autonomic neurons in rats that project to the sympathoadrenal preganglionic neurons and to the eye. Four days following injections of the ß-galactosidase expressing Bartha PRV, virally infected neurons were visualized with a one step histochemical reaction using the Bluo-Gal substrate (halogenated indolyl-ß-D-galactoside). In some infected neurons, a Golgi-like staining of the primary and sometimes secondary dendrites could be obtained. For electron microscopic studies, the Bluo-Gal substrate produced an electron dense reaction product that was easily identified at both low and high magnification. This virus may be useful for the study of the cell architecture and synaptic organization of transneuronally labeled neurons of functionally defined neural circuits. These results also demonstrate that it is possible to deliver foreign genes into specific chains of neurons in the mammalian CNS by means of the retrograde transneuronal viral labeling method.

603.16

SANTIAGO RAMÓN y CAJAL (1852-1934) I. EARLY ACCOMPLISHMENTS IN NEUROANATOMY AND SCIENTIFIC ILLUSTRATION. R.A. Johnson[1] and L.H. Marshall[2]. Department of Psychology[1] and Neuroscience History Program[2], UCLA, Los Angeles, CA 90024.

Cajal was born in a humble room in a stone house in the village of Petilla, in the mountainous Aragon region of Northern Spain. Medical school was attended at the insistance of his father; later, Cajal began study and research in histology at the University of Zaragova while convalascing after military service in Cuba. An appointment to the chair of anatomy at Valencia at age 32 was followed three years later by a move to the chair of histology at Barcelona. The next year, 1888, saw the beginning of his experimentation with the reduced silver method for staining the nervous system, introduced by Golgi. Cajal's facility with this and subsequent techniques employed from 1890 at the University of Madrid contributed to the revelation and faithful documentation of the histological organization of the central nervous system. The published documentation of this work reflects both technical and artistic accomplishment. Indeed, Cajal's early devotion to drawing and painting appear to have become sublimated in his later career, in the preparation of scientific illustrations. The quantity and quality of Cajal's hand-prepared drawings will be considered, with a comparison to the talents and achievements of his contemporaries. They will also be placed in perspective to the origins and continuing development of techniques for the transcription, enlargement, and reproduction of hard copy histology.

603.17

SANTIAGO RAMÓN y CAJAL (1852-1934) II. THE SPANISH TRADITION IN NEUROSCIENCE. L.H. Marshall[1] and R.A. Johnson[2]. Neuroscience History Program[1] and Department of Psychology[2], UCLA, Los Angeles, CA 90024.

Cajal and Golgi shared the Nobel Prize in Physiology or Medicine in 1906 in recognition of their work on the structure of the nervous system. Cajal's drawings of nerve cells, stained by his own and by Golgi's techniques, are marvels of precision and art. The Spanish government belatedly facilitated the achievements of the largely self-taught professor of anatomy at the University of Madrid by providing a new building in 1932. The top floor was for the master's microscopic work and the remainder accommodated programs in subspecialties beyond neurocytology, resembling the institutes established much earlier in Vienna, Leipzig, and Zurich. With the new facilities, Cajal continued to expand a school of followers, including Lorente de Nó and Rio-Ortega. His clear proof of collateral sprouting after neuronal trauma formed the basis of discoveries of reorganization of pathways in cerebral and cerebellar cortex. Cajal's emphasis on the synapse, fundamental to his demonstration of the correctness of the neuron doctrine, furthered interpretations of old work and opened the central nervous system to new understanding. A beautiful modern institute now carries forward the elegant Spanish tradition of nervous system research.

604.1

THE GENERATION OF A MOUSE MUTANT FOR THE ALPHA SUBUNIT OF THE CA/CALMODULIN KINASE II THROUGH GENE TARGETTING IN EMBRYONIC STEM CELLS.

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The brain-specific Ca/Calmodulin Kinase II (CAMKII) is thought to have a role in a myriad of phenomena ranging from synaptic transmission to neural development. In the hippocampus and forebrain, two regions of the CNS often implicated in memory, this enzyme is mostly composed of alpha subunits. Using homologous recombination, we disrupted the alpha-subunit gene of CAMKII in embryonic stem cells, and these cells were used to derive a mouse mutant for this gene. We will provide a preliminary report on our attempts to test these mice for abnormalities in development and long term potentiation, for anatomical defects in structures such as the barrel fields of the somatosensory cortex, and for spatial memory deficits in a modified Morris water maze.

604.2

CHARACTERIZATION OF FOS*LACZ* TRANSGENE EXPRESSION IN THE CNS. R.J. Smeyne, K. Schilling, L. Robertson, D. Luk*, J.G. Corbin*, J. Oberdick*, T. Curran* and J.I. Morgan. Roche Institute of Molecular Biology, Roche Research Center. Nutley, NJ 07110

Cellular immediate early genes (cIEG) are thought to orchestrate the link between transient external stimuli and long-term changes in cellular phenotype. One of these cIEGs, c- fos, is induced in specific neural regions in response to various stimuli, including seizure and light stimulation. We have constructed a transgenic mouse containing a lacZ reporter gene, fused in-frame, into the entire fos gene (FosβGal). Initiation of seizure using either kainic acid (KA) or pentylenetetrazole (PTZ) reproducibly induces expression of FosβGal in brain. The patterns of expression seen in the transgenic CNS following PTZ stimulation is identical to that previously reported using antibodies to Fos. Furthermore, subtle differences are apparent in PTZ-seizured brain compared to KA. In addition, we have found that FosβGal is expressed in the SCN following brief periods of light, delivered at night, as has also been reported using antibodies to Fos. During early postnatal CNS development, we have observed regional differences in the patterns of basal FosβGal expression; dependent on the age of the animal. Primary sites of basal expression include, but are not limited to, the olfactory bulb, hippocampus, and cerebral cortex. The versatility of this transgenic mouse has also been demonstrated through the use of characterized primary neuronal and glial cultures. Defined cells within these cultures respond to various neurotransmitters and growth factors with an induction of lacZ. In addition, we have constructed transgenic mice with specific mutations in the 5' control elements of the c-fos-lacZ promoter. Analysis of these animals will provide a picture of the in vivo utilization of these specific regulatory sequences. Supported by NRSA NS 08680-02 (to RJS) and DFG Schi 271/2-2 (to KS).

604.3

SYNAPSIN I 5' FLANKING SEQUENCES DIRECT NEURONAL LACZ REPORTER GENE EXPRESSION IN TRANSGENIC MICE.
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The synapsin I gene encodes a neuron-specific protein found in virtually all nerve cells. In order to define DNA sequences necessary for tissue-specific expression of this X-linked gene, we joined a 2.5 kb fragment of the synapsin I promoter to sequences encoding E. coli β-galactosidase. A line of transgenic mice was produced in which 10 to 20 copies of this transgene have integrated onto an autosome. Analysis of heterozygotes reveals a high degree of fidelity in the expression of the transgene. Transgene mRNA is found exclusively in the brain and testis. X-gal histochemistry stains most neurons, and the relative intensity of staining varies among neuronal cell types in a pattern similar to that observed for synapsin I immunostaining in their terminal fields. X-gal staining is high in cerebellum, olfactory bulb, dentate gyrus, hypothalamus and layers II, V and VIb of cortex, and low in hippocampal pyramidal cells. An unexplained observation is the near absence of staining in striatum. Furthermore, the amount of transgene mRNA in the brain is much lower than that of endogenous synapsin I mRNA. Our results suggest that DNA sequences required for the neuron-specific expression of the synapsin I gene are present in our construct. Nonetheless, the relatively low levels of transgene mRNA and protein suggest that additional regulatory sequences may be missing, that positional effects of the site of integration may affect expression, or that transgene mRNA stability may be different than that of the endogenous synapsin I mRNA. Supported by NS20591, NS18381 to KH, NS25050 to LD and NS27833 to LH and LD.

604.4

EXPRESSION OF A RHOMBOTIN TRANSGENE IN PHENOTYPICALLY DEFINED SUBSETS OF HIPPOCAMPAL NEURONS: ADVANTAGES OF IMMUNOHISTOCHEMICAL OVER HISTOCHEMICAL DETECTION OF ß-GALACTOSIDASE IN TRANSGENIC MICE.

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Patterns of gene expression associated with specific cell phenotypes in the CNS are not well documented. Here we report the expression in transgenic mice of a rhombotin promoter 1-lac Z fusion construct in previously described subsets of hippocampal (Hp) neurons. The rhombotin gene was identified adjacent to a T-cell tumour-associated chromosomal translocation and is believed to be involved in tumorigenesis. Histochemical staining for ß-galactosidase (ß-Gal) in these transgenic mice has previously revealed expression during development in several CNS sites including the Hp (Nature 344:158). We have now found that immunohistochemical detection of ß-Gal is both more sensitive and more precise in visualizing cell morphology than histochemical staining. The latter can give both false positive (i.e., misinterpretation of labelled terminals as cells) and false negative results. As detected immunohistochemically, ß-Gal expression was found throughout adult life in Hp pyramidal neurons of CA1 and CA2 but not of CA3 or CA4 or in dentate gyrus granule cells. Expression also occured in dentate gyrus basket cells and certain glial cells. While the cellular functions controlled by this gene are not known, rhombotin gene expression via promoter 1 occurs in certain previously defined classes of Hp neurons.

604.5

CHARACTERIZATION OF NF-H GENE EXPRESSION IN TRANSGENIC MICE.
F.Cote*(1), A.C.Peterson(2) G.A.Rouleau(1) and J.-P.Julien*(1). (1) The Montreal General Hospital Research Institute, Canada. H3G 1A4. (2) Ludwig Institute for Cancer Research. Montreal, H3A 1A1.

Neurofilaments (NFs) are the most abundant cytoskeletal elements of neurons. They are formed by the copolymerization of 3 proteins, NF-L, NF-M and NF-H. NF-H is believed to form the side arm projections seen in NFs. Various observations suggest a central role, for NFs, in the maintenance of axonal calibre. To further study the role of NF-H, we have generated transgenic mice expressing the human NF-H gene (hNF-F). A complete genomic clone for the human NF-H gene has been obtained by screening a cosmid library enriched in chromosome 22. The cosmid was linearized using the Not 1 restriction enzyme and microinjected into mouse eggs. Two founder mice with a high copy number of the hNF-H gene have been obtained. Northern blot analysis of total RNA from various tissues indicates that the human gene is specifically expressed in the mouse nervous system. Work is in progress to determine the effect of high expression of the hNF-H protein on the axonal NF architecture.

604.6

A PREPROENKEPHALIN PROMOTER SEGMENT CONFERS TRANSSYNAPTIC REGULATION AND ELEVATED TESTES EXPRESSION IN TRANSGENIC MICE.
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Transgenic mice containing a 193 bp segment of the preproenkephalin A promoter fused to a CAT reporter (pENKAT-12) or to a preproenkephalin cDNA show expression in brain and testes. CAT (chloramphenicol acetyl transferase) activity measurements from brain regions following treatments that upregulate the wild type gene show that this short promoter fragment is sufficient for correct trans-synaptic regulation in vivo during haloperidol treatment and primary afferent stimulation. However, there was no increase in hippocampal CAT activity 2 hours following seizures. These findings support differential utilization of transcriptional regulatory elements in different trans-synaptic regulatory settings.

Modest levels of brain overexpression of the preproenkephalin cDNA were insufficient to produce significant differences in baseline or opiate-altered nociception, locomotion, or respiration. However, large increases in testes enkephalin mRNA produced significant alterations in transgenic male fertility with normal reproductive behavior.

604.7

CHARACTERIZATION OF PROENKEPHALIN HETERONUCLEAR RNA IN INDIVIDUAL NUCLEI IN THE RAT BRAIN. Martin K.-H. Schäfer*[1], Yu-Fung Lin*[2] and S.J. Watson[1], [1]Mental Health Research Institute and [2]Dep. Physiology, University of Michigan, Ann Arbor, MI 48109 USA

While in vitro transcription assays have proven to be a powerful method to assess the transcriptional activity of specific genes, their application to brain tissue has been limited. An alternative approach involves analyzing changes of steady state levels of primary RNA transcripts of specific genes, which are rapidly processed in the cell nucleus by intron splicing into mature mRNA. Using probes specific for the short-lived intervening sequences, primary RNA transcripts and processing intermediates can both be localized in tissue sections and biochemically characterized in RNase protection assays. The goal of this study has been 1) to generate the specific tools for proenkephalin (PENK) RNA transcripts 2) to examine the cellular distribution of PENK hnRNA in rat brain and 3) to establish the fundamental methodology to record rapid transcriptional changes of the PENK gene. A PENK genomic clone was isolated from a rat genomic library and DNA fragments containing either intron A or intron B sequences were subcloned into pGEM4 vectors to generate intron specific cRNA probes. Use of these intron specific ^{35}S-NTP labeled cRNA probes in an in-situ hybridization assay of frozen brain sections revealed many positively labeled neurons in various brain areas after exposure time longer than four weeks. The strongest signal occurred in striatum, in which silver grains were specifically localized over counterstained cell nuclei. These nuclei were located within PENK synthesizing neurons, as identified by labeling of adjacent sections with a PENK RNA probe specific for exon 3 of PENK mRNA. This probe yielded an identical labeling pattern, but with cytoplasmic localization of the grains. PENK hnRNA levels in brain regions of known lower abundance of PENK mRNA were below our detection limit. The existence of nuclear RNA intermediates and the splicing order of PENK hnRNA is currently being assessed in RNase protection assays. Supported by NIDA #DA02265.

604.8

REGULATION OF MOUSE GLUTAMIC ACID DECARBOXYLASE GENE(Gad-1(2))EXPRESSION IN ADULT BRAIN AND IN DEVELOPING EMBRYO. G.Szabo^, Z. Katarova*, Zs. Urban*, J. Mann#, E. Mugniani" and R.Greenspan. ^*Dept.of Biochem., BRC,Szeged P.O.Box.521, H-6701, Hungary; #Div. of Biol. Beckman Research Inst. City of Hope Med.Cent. Duatre CA 91010; " Lab. of Neuromorphology, Univ. of Connecticut, Storrs, CT 06269-4154; Dept of Neurosciences, Roche Inst. of Mol.Biol.,Nutley.,NJ 07110.

In mouse Gad-1(2) gene codes for at least two mRNA-s different in size. A 3.4 kb message is predominant in adult and codes for the 66.5kD GAD isoform. Two distinct 2.2 kb transcripts are detectable as early as embryonic day 10.5. These messages have been found to be functionally bicistronic . The late and early transcripts utilize different promoters and have multiple start sites. The adult prompter is highly rich in GC and lacks TATA and CAATboxes .The embryonic transcriptional start site is 180 bp upstream from the adult ones and its 5'-flanking region contains a TATA motif. The embryonic transcripts contain additional 80 bp and 86 bp exons as a result of alternative splicing and use a different poly-A addition signal very close to the translational stop site. In an attemt to functionally disect the regulatory region of Gad-1(2) gene we studied the beta-galactosidase expression in several transgenic lines containing different parts of the regulatory region. Detailed analysis of the expression pattern will be presented.

604.9

DIFFERENTIAL EXPRESSION OF CEREBELLIN-RELATED TRANSCRIPTS IN THE ADULT AND DEVELOPING RODENT NERVOUS SYSTEM. B. Kavety*, Y. Urade*, J. Oberdick*, R. Molinar-Rode* and J.I.Morgan. Department of Neuroscience, Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110.

To investigate the molecular genetic basis of cell-specific gene expression in the developing nervous system a series of genes have been cloned that show differential expression in cerebellum. Previous studies have established that a hexadecapeptide, termed cerebellin, is highly enriched in cerebellum. Furthermore, a number of cerebellar mutant mice have reduced levels of cerebellin. To pursue the question of the regulation of this neuropeptide we have cloned a cDNA and the cognate gene that encode the cerebellin sequence. In Northern blot, the cDNA detects multiple, cerebellum-specific, transcripts that increase during postnatal development. Southern blot, suggested the presence of other genes related to precerebellin. A cDNA encoding a related gene, here termed pseudocerebellin, has been cloned using a PCR strategy from whole brain mRNA. The protein encoded by this sequence contains the cerebellin motif with one amino acid change. This transcript appears to be more ubiquitously expressed in the CNS than precerebellin. We are examining the 5' regulatory sequences of the two genes to determine the basis of their differential expression using transgenic mouse technology.

604.10

ISOLATION AND EXPRESSION OF *TRK* PROTO-ONCOGENE FAMILY MEMBERS. D.S. Battleman*, C. Lai, D.A. Fischman, M.V. Chao* and G. Lemke. The Department of Cell Biology and Anatomy, Cornell University Medical College, New York, New York 10021 and The Salk Institute, P.O. Box 85800, San Diego, CA 92186.

The product of the *trk* proto-oncogene is a low affinity receptor for nerve growth factor (NGF) that participates in the formation of the high affinity NGF receptor complex (Hempstead et al., Nature **350**, 678, 1991). *Trk* encodes a receptor tyrosine kinase which, together with p75 NGF receptor, is responsible for signal transduction by NGF. A direct prediction from these results is that the specificity of action of other neurotrophic factors is mediated by the expression of other *trk*-related receptor tyrosine kinases. We have utilized a PCR-generated fragment, tyro-10 (Lai and Lemke, Neuron, in press) as a probe for *trk*-related tyrosine kinases. A 1.75 kb cDNA clone was isolated from a two week rat brain cDNA library. Northern blot analysis with this isolate revealed an expression pattern distinctly different from both *trk* and *trk B*. PC12 cells displayed as many as four distinct mRNAs, ranging in size from 3.5 kb to 7.7 kb. In developing rat brain only two transcripts of 4.2 kb and 7.7 kb size could be detected, with the 4.2 kb mRNA being the most prominent. Expression of this 4.2 kb transcript appeared to increase during embryonic and early post-natal period and declined after postnatal day fourteen.

604.11

DEVELOPMENTAL REGULATION OF NCAM, N-CADHERIN AND ACETYLCHOLINE RECEPTOR mRNA LEVELS IN CHICK SKELETAL MUSCLE. J. Capasso, C.G. Hahn, M. Sasner, S. Tilney and J. Covault, Department of Physiology & Neurobiology, Univ. of CT, Storrs, CT 06269

Synaptic regulation of gene expression is thought to be an important process involved in neural development and in learning and memory. Neural regulation of muscle gene expression provides a simple system in which to study such trans-synaptic interactions. The cell adhesion molecules NCAM and N-cadherin are both down-regulated by innervation of skeletal muscle fibers during development as is the acetylcholine receptor in extrasynaptic regions of muscle. All three proteins are reexpressed at high levels in extrasynaptic regions following denervation of adult muscle. In order to identify mechanisms linking neural activation of muscle fibers with changes in the level of these proteins we have compared the time course during development of the down regulation of mRNA levels for NCAM, N-cadherin and the acetylcholine receptor alpha and delta subunits. We find that, in spite of exposure to the same neural stimulation, the timing of the decrease in mRNA levels for these four genes differs markedly . N-cadherin mRNA levels decline first, around 2-3 days following muscle fiber innervation. Levels of NCAM and acetylcholine receptor alpha subunit decline following an additional 4-5 days of development while decline in the acetylcholine receptor delta subunit is delayed until near hatching. Our results suggest that the neural regulation of these four genes either involves different second messenger pathways or different levels of a common second messenger signal. (Supported by grants from the March of Dimes Foundation and the NIH # NS25264 and NS10338.)

604.12

DIFFERENTIAL EXPRESSION OF THE ANDROGEN-BINDING PROTEIN GENE IN THE RAT BRAIN. Y-M. Wang, P.M. Sullivan*, and D.R. Joseph. Curriculum in Neurobiology, Departments of Pediatrics and Biology, The Laboratories for Reproductive Biology, University of North Carolina, Chapel Hill, NC 27599.

The observation that the androgen-binding protein (ABP) gene is expressed and processed differently in the rat brain (Endocrinol. 127:3124, 1990) led us to further examine its gene structure. A 20-kb fragment upstream from the testis specific exons was cloned and characterized. Three brain specific coding regions were localized on this fragment by restriction mapping and sequencing. They are approximately 15, 6.3 and 4.8-kb upstream of testicular exon 1. One kb upstream of the 5'-most exon was sequenced to locate possible promotor and regulatory regions. Analysis of the sequence shows that it is extremely G-C rich with seven GC box-like elememts. Sequences homologous with two AP2 binding sites, a GRE core sequence and an SRE were also found. Further study is underway to confirm the functional relevence of the above entities and to dissect the heterogeniety of ABP transcripts in the brain. (supported by NIH grants HD-21744 and HD-18968).

604.13

TISSUE-SPECIFIC TRANSCRIPTION OF THE RAT TYROSINE HYDROXYLASE GENE REQUIRES A SYNERGY BETWEEN AN E-BOX CONTAINING DYAD AND AN OVERLAPPING AP1 MOTIF WHICH RESPONDS TO ELEVATED LEVEL OF cAMP. Sung Ok Yoon and Dona M. Chikaraishi, Neurosci. Program, Tufts Univ. Sch. Med., Boston, MA, 02111 USA.

Tyrosine hydroxylase (TH) is the rate-limiting enzyme in catecholamine biosynthesis and is regulated in a tissue-specific manner at the transcriptional level. Using site-directed mutagenesis, we have determined that the DNA segment between -205 and -182 bp from the transcriptional start site constitutes the minimal enhancer for the tissue-specific expression of the rat TH gene in cultured pheochromocytoma cells. This segment is composed of an AP1 motif and an overlapping 20 bp dyad whose core resembles an E box consensus site (TGATTCAGAGGCAGGTGCCTGTGA). Despite the overlap, both the AP1 and the dyad elements bind proteins independently in vitro. Interaction between the two elements, however, is necessary for in vivo expression. Therefore, we conclude that the synergism between the AP1 and dyad elements is responsible for the tissue-specificity of TH. This is the first identification of a tissue-specific element in a mammalian neuronal gene, and the first known case where tissue-specificity is elicited by an obligate synergy with an AP1 motif.

Despite the 1 nucleotide difference from the consensus, the AP1 motif in TH functions as a ubiquitous activator, and mediates cAMP response upon induction with forskolin and 8-bromo cAMP. The response to elevated level of cAMP by the AP1 motif is weaker than that via the cAMP response element (CRE) located downstream at -45. Unlike other neuroendocrine genes, the CRE in TH is not essential for basal or cAMP-induced expression. The CRE in TH, however, augments the basal level of TH transcription directed by the AP1 and E-box/dyad motif.

604.14

cDNA CLONING AND mRNA DISTRIBUTION OF A NOVEL MOUSE PITUITARY HOMEOBOX-LIKE (mPHB1) PROTEIN HOMOLOGOUS TO mec-3 HOMEOBOX GENE OF C. ELEGANS.

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During our ongoing studies on the endocrine proteins expression in the pituitary, we isolated a clone containing a cDNA coding for a novel protein designated mPHB1. 1.6 kb insert was obtained from total mouse pituitary cDNA library established in pCDM8 vector. The open reading frame of the cDNA sequence codes for the last 188 amino acids, with a predicted C-terminal Cys, and the relatively long untranslated region with an AATAAA polyadenylation signal, suggesting that mPHB1 is translated into a functional protein. The alignment of the first 19 amino acids revealed that mPHB1 is highly homologous (84%) to the essential segment of the homeobox protein mec-3 found in C. elegans and also to the mouse homeobox proteins Hox 2.5; 2.9; 2.6 and 2.3.

A northern blot analysis of the total mouse RNA extracts carried out using [^{32}P]cRNA probes allowed the detection of a major 2 kb transcript in different tissues such as pituitary anterior and neurointermediate lobes. Lower levels of mRNA transcripts were found in the CNS, especially in the hypothalamus and cerebellum. Furthermore, in situ hybridization studies completed in neonatal and adult mice using [^{35}S] cRNA probes (antisense and sense) demonstrated the presence of mPHB1 transcripts within the majority of the adenohypophyseal cells and in virtually all melanotrophs of the intermediate lobe.

Based on the sequence similarity to the homeobox gene products, mPHB1 is expected to exhibit homeobox-like properties and therefore may represent a novel factor implicated in the development of particular cells within nervous and endocrine systems.

604.15

EXPRESSION OF HELIX-LOOP-HELIX TRANSCRIPTIONAL FACTOR E12/E47 HOMOLOGUES IN CHICK DEVELOPING NERVOUS SYSTEM. E.Knapik, H.O.Nornes, T.Neuman. Dept. of Anatomy & Neurobiology, Colorado State University, Fort Collins, CO 80523.

The helix-loop-helix (HLH) transcriptional factors play important roles in specifying different cell types in Drosophila neurogenesis and also in muscle differentiation of vertebrates. Using PCR and low stringency screening of cDNA libraries from chick embryonic neural tube from different stages of development, several cDNAs were isolated that have homology to the HLH region of the mouse and human E12/E47 transcriptional factor. Northern blot analyses with cloned cDNAs show expression of multiple mRNAs in the developing nervous system. Sequencing data from these clones indicate that differential splicing is involved in the generation of these transcripts. The highest level of expression was detected in early stages of development of nervous system, and the expression was weak or missing in many nonneuronal tissues at different stages. This indicates that the expression of the cloned gene is developmentally regulated. These observations further indicate that E12/E47 like transcriptional factors have a role in neuronal differentiation of vertebrates. (Funded by BRSG# 2S07RR05458-29)

POSTSYNAPTIC MECHANISMS

605.1

THREE DIFFERENT TYPES OF NEURONS IN THE RAT MEDIAL SEPTUM (MS) NUCLEUS. M.Tsurusaki* and J.P.Gallagher. Department of Pharmacology & Toxicology, Univ. of Texas Medical Branch, Galveston, TX 77550.

The role of the MS as part of a larger nuclear complex, the medial septum/diagonal band of Broca (MS/DBB) is considered essential for the generation and/or modulation of a characteristic electrical activity recorded from the brain and termed rhythmical slow wave activity (theta rhythm). The MS is thought to receive its primary afferent input from the dorsolateral septal nucleus, and serve as the major septal efferent pathway to the hippocampus. While hippocampal neurons may be the most investigated of all mammalian CNS neurons, relatively little is known of the cellular physiology and pharmacology of MS neurons in rat. Here we report on the membrane properties of rat medial septum neurons recorded in vitro. Resting membrane potentials were -60.3 ± 4.8 mV; n = 26), while individual neuronal input resistances ranged from 36 to 216 MΩ (134 MΩ ± 45 MΩ; n = 26). Within this sample of neurons three distinct populations emerged based upon: action potential firing pattern, action potential shape and amplitude, the presence of different ionic conductances,and different synaptic responses. Following a 500 msec cathodal stimulus, one population of neurons (15%) fired action potentials separated by a rapid afterhyperpolarization and exhibited no accommodation between spikes. A second group, (55%) displayed a small slow afterhyperpolarization between spikes and an early decline in firing rate. The last group (30%) of neurons exhibited strong accommodation. Synaptic responses depended on the site of stimulation and could also be divided into three types: an IPSP; a fast EPSP followed by an IPSP; or simply an EPSP. Our data suggest that the MS contains a heterogeneous population of neurons with a complex input-output relationship. (Supported by NSF Grant #BNS 89-19549)

605.2

EFFECTS OF SEROTONIN (5-HT) ON DIAGONAL BAND NEURONS OF THE RAT *IN VITRO*. Wai Ling Lee and J.P. Gallagher, Dept. of Pharmacology & Toxicology, Univ. of Texas Medical Branch, Galveston, TX 77550

The effects of 5-HT on neurons of the vertical and horizontal limb of the diagonal band of Broca (vnDBB, hnDBB) were examined using intracellular recording from rat brain slices. Bath application of 5-HT (10-30μM) produced either a membrane hyperpolarization or depolarization. In the hnDBB, a membrane hyperpolarization, 6 ± 1.4 mV (S.E.M., n = 19) was accompanied by a reduction of input resistance, 18.8 ± 4.55% (S.E.M, n = 18). This hyperpolarization was mimicked by the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT (10-30μM) or by 5-CT (1μM), and was antagonized by the 5-HT$_{1A}$ antagonist, NAN-190. 5-HT depolarized only 2 out of 25 neurons. Six neurons were studied from the vnDBB: 5 were depolarized by 5-HT; one did not respond; while no hyperpolarization was observed. Although the nDBB of the guinea pig is known to contain at least three different types of neurons (W.H. Griffith, J. Neurophysiol., 59:1590-1612, 1988), we observed no correlation between cell types and response to 5-HT in the rat.

5-HT or 8-OH-DPAT also reduced or blocked a late afterhyperpolarization which follows a train of cathodally induced spikes. Both agonists also reduced the width of an individual spike and the amplitude of its subsequent medium or slow afterhyperpolarizations; half width decreased by 7.3% and afterhyperpolarization amplitudes by 37%. Our data suggest that 5-HT has both inhibitory and excitatory effects on electrical activity within the diagonal band. (Supported by NSF #BNS 89-19549)

605.3

EFFECT OF NH_4CL ON EXCITATORY TRANSMISSION IN CA1 PYRAMIDAL NEURONS RECORDED INTRACELLULARLY. P.Fan* and J.C.Szerb, Dept.Physiol.& Biophys. Dalhousie U.Halifax,N.S. Canada B3H 4H7

We have shown that NH_4^+ blocks synaptic transmission from Schaffer collaterals to CA1 neurons without inhibiting the evoked release of glutamate, suggesting a postsynaptic block. However, NH_4^+ was only a weak inhibitor of firing induced by electrophoretic glutamate. To clear up this discrepancy, we applied 10-20 μM quisqualate (QUIS) or NMDA to slices, while the membrane current was recorded with single-electrode voltage clamp. 2-4 mM NH_4Cl induced an inward current and had opposite effects on QUIS and NMDA-induced currents: it abolished the QUIS current and greatly potentiated that of NMDA. Since transmission involves mostly QUIS receptors, block of the QUIS current explains the inhibitory effect of NH_4^+ on transmission. The action of NH_4^+ is probably due to depolarization of inadequately clamped distal dendrites, because the potentiation of the NMDA effect is not seen without Mg^{2+}. (Supported by MRC, P.F. is an MRC Fellow).

605.4

ELECTROTONIC LENGTH ESTIMATES IN NEURONS WITH SOMA SHUNT
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Previously (Soc. Neurosci. Abstr. 13:1517, 1987) we discussed the discrepancies to be expected when equivalent cylinder formulae are applied to neurons with soma shunting having L=1.0. Here we compare L estimates in hypothetical and real neurons with soma shunting having different L values.

Hypothetical cells were modeled as a cylinder with a soma when the L of the cylinder was 0.5, 1.0, or 1.5. Time constants were estimated from computed transients with the program DISCRETE. When L was 0.5 and soma shunting was large, the formula L_{τ_0/τ_1} overestimated L by 2-3-fold, but when L was 1.5, the overestimate was only 10-15% regardless of the size of the shunt.

L can be estimated graphically if τ_0 and R_N are known with and without soma shunting. If shunted and non-shunted values of τ_0 and R_N in a neuron with an extensive dendritic tree differ by a factor of four, and the only factor causing this difference is soma shunting, then L is approximately 0.5.

L_{τ_0/τ_1} estimates were computed for models of a motoneuron having different dendritic R_m and degrees of shunting. Estimates of τ_1 in these motoneuron models were complicated by the fact that no time constant after τ_0 had a coefficient larger than 2% of the signal. Overestimates of L by L_{τ_0/τ_1} were larger when dendritic R_m was large than when dendritic R_m was small, but the magnitude of the overestimates depended on the size of the shunt and on whether 2, 3, or 4 time constants could be resolved from the data.

In conclusion, L_{τ_0/τ_1} estimates computed for cells with soma shunting have overestimated L by a factor of 2 in electrotonically short cells but by only 0-20% in electrotonically long cells.

605.5

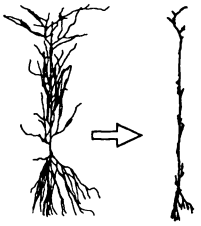
COMPUTER MODELS OF DENDRITIC EXCITABILITY. I. Segev* and W. Rall. Dept. Neurobiol. Hebrew Univ. Jerusalem, and Math. Res. Br., Bethesda, MD 20892.

Computer models with excitable membrane at different dendritic locations were used to gain insights into the possible functional roles of dendritic excitability. Simulations were on theoretical trees and on a reconstructed Purkinje cell from guinea-pig cerebellum. A previous report (Segev and Rall, Neurosci. Abstr. 12:726) focused on the "chain reaction" of action potential firing between clusters of excitable spines at distal dendritic branches. Here we modeled trees with many "hot spots" (on dendritic shafts and on spines). The main findings are: a) The chain reaction tends to spread distally; it fails toward the soma. b) The propagation delay of the chain reaction may be significant (tens of msecs), depending on the passive membrane time constant and on the kinetics of the excitable channels. c) Timing between different excitatory inputs distributed along the tree is critical for the degree of firing within the tree; when inputs to proximal regions are delayed (to allow for depolarization from distal inputs to reach the proximal sites), a larger part of the tree is more likely to fire than when all inputs are simultaneous. d) Inhibition is very effective in restricting the chain reaction to only parts of the tree. Strong somatic inhibition tends to block the spread of the chain reactions anywhere in the tree. The possible functional role for cellular plasticity will be discussed.

605.6

Electrotonic Transforms of Hippocampal Neurons. A.M. Zador, B. J. Claiborne,† and T.H. Brown, Dept. of Psychology, Yale University, New Haven, CT 06520 and †Div. of Life Sciences, Univ. of Texas, San Antonio, 78285.

The electrotonic structure of dendrites is of central importance to their potential for information processing. This structure has traditionally been analyzed using concepts like electrotonic distance and length, which have only limited applicability to cells with complex morphology. We present a new graphical method that provides direct insight into key aspects of a neuron's electrotonic structure. The method is based on the attenuation $A_{ij} = V_i/V_j$ of voltage from location i to j following current injection at i. Specifically, the length of each segment in an anatomically reconstructed neuron is replaced by the log-attenuation $L_{ij} = \log(A_{ij})$ across the segment, as calculated using a compartmental simulator. The log-attenuation is a generalization of the concept of electrotonic distance X to morphologically complex structures, and is identical to X in the case of an infinite or semi-infinite cylinder. Because the log-attenuation depends on the frequency and site of the input, many complementary views of a neuron emerge. We have extended this method to the attenuation of current and to temporal consequences of electrotonic structure.

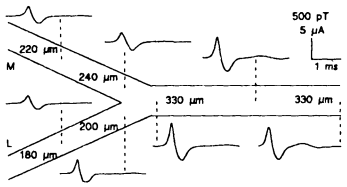


The method is illustrated at the right. The neuron on the left is a 2734-compartment 3-dimensional reconstruction of a region CA1 pyramidal neuron. On its right is a representation of voltage attenuation from the soma with respect to a 100 Hz sinusoidal current. (Supported by ONR and DARPA.)

605.7

MAGNETIC CHARACTERIZATION OF ACTION CURRENTS AT THE PRIMARY BRANCH OF THE SQUID GIANT AXON. R.N. Friedman*, J.M. van Egeraat* and J.P. Wikswo, Jr. Department of Physics and Astronomy, Vanderbilt University, Nashville, TN 37235.

Magnetic field recording provides a more direct means of measuring the action currents (ACs) in active, squid (Loligo pealei) giant axons. A room-temperature biomagnetic current probe system enables study of the spatial variations of ACs by scanning a toroidal probe along the axon. This affords acquisition of nearly continuous AC profiles. We used this system to examine the currents as the action signals approach and cross the primary branch of the stellar nerve. The figure shows the result of stimulating a 2 cm axon (1 cm shown) 3 mm distal to the ligated and cut proximal end. The axon diameters are indicated. The vertical scale represents a magnetic field strength of 500 picoTesla, corresponding to a current of 5 μA. Medial (M) and lateral (L) branches were dissected to lengths of 8 and 7 mm, respectively, and ligated and cut. Branch ACs were measured 3 mm past the bifurcation. AC amplitude increased (24%) as the depolarization wave front approached the branch and decreased 48% and 57% in the M and L branches, respectively. The conduction velocity decreased at the bifurcation from 17 m/s to 12 m/s (L) and 15 m/s (M). The late, positive deflections of the ACs in the two most proximal traces are artifacts of fin nerve depolarization. We also examined the currents resulting from antidromic stimulation.



This work was supported by a Grass Foundation fellowship to JMvE and NIH grant NS-19794.

605.8

EFFECT OF QX314 ON SOMATIC AND DENDRITIC ACTION POTENTIALS IN HIPPOCAMPAL CA1 CELLS.
S.B.COLLING*, E.W.STOCKLEY* and H.V.WHEAL.
Department of Physiology and Pharmacology, Southampton University, Southampton, SO9 3TU, UK.

Hippocampal CA1 pyramidal cells *in vitro* can be excited via stimulation of afferent fibres to the proximal and distal apical dendrites (Andersen, Storm & Wheal, 1987, J.Physiol. **383** pp.509-526). The local anaesthetic QX314 (35 mM) was injected intracellularly, and it's effects on the proximally and distally evoked synaptic responses were monitored over time. The proximally evoked action potential (AP) was totally blocked after 57±10 minutes (n=5), whereas the distally evoked AP was present up to 88±14 minutes (n=5). The excitatory postsynaptic potentials were still present after this time. In control recordings there appears to be a pre-AP component which contributes to the threshold event. This component also appears to be sensitive to QX314.

The data suggests that there is more than one triggering site for AP's in pyramidal cells and that these sites are spatially separate. The shapes of the synaptic potentials as well as their generation from these sites can be explained by the electrical properties of these cells. A compartmental cable model was used to confirm the idea that the proximally evoked AP may be triggered at either the soma or the initial segment, whereas the distally evoked somatic AP may be triggered by a dendritic spike which appears as a prepotential at the soma.

Supported by MRC and Wellcome Trust grants. The QX314 was donated by Astra Pain Control, Södertälje, Sweden.

605.9

DEPOLARIZATION-DEPENDENT STIMULATION OF POLYRIBOSOME LOADING IN SYNAPTONEUROSOMES. J.J. Weiler, W. I. Davenport, W. T. Greenough, Depts. Psychol. and Cell & Struct. Biol, Neurosci. Prog, and Beckman Inst., Univ. Illinois, Urbana-Champaign, IL 61801.

The polyribosomal aggregates observed within or at the bases of dendritic spines have suggested that some proteins, produced quickly under localized control, might contribute to synaptic plasticity. Protein synthesis has been demonstrated in synaptosome preparations by several groups. We added a depolarizing dose (40 mM) of K+ to synaptoneurosome preparations, on ice in the presence of cycloheximide. By measuring the fraction of total RNA trapped in polyribosomes at intervals (1',2',5',10',20') we were able to observe that ribosomes and some pre-formed protein rapidly associate with mRNA; this was verified by centrifugation on a continuous sucrose density gradient. Ribosomal loading peaks at 2-5 minutes and then declines. Incorporation of labelled methionine into TCA-precipitable polypeptides is transiently accelerated.

Calcium chelators reduce the steady-state size of polyribosomal complexes, but do not eliminate the response to depolarization, suggesting that there is mobilization of intracellular Ca++ stores. We suggest that depolarization-related changes may affect binding and release of translation initiation complexes with messenger RNA found near synaptic junctions. Such rapid, local protein synthesis may have implications for synaptic efficacy change.

Supported by ONR N0014-89J-1556 and MH35321.

605.10

EFFECTS OF SOMATOSTATIN ON POTASSIUM CURRENTS IN BULLFROG SYMPATHETIC GANGLION NEURONS. D.E.Kurenny*, H.Chen* and P.A.Smith. Dept. of Pharmacology, Univ. of Alberta, Edmonton, Canada.

Somatostatin (SS) increases M-current (I_M; Moore *et al.*, Science **239**, 278, 1988) in hippocampus whereas in submucous plexus, it activates an inwardly-rectifying K^+-current (I_{AIR}; Tatsumi *et al.*, J. Neurosci. **10**, 1675, 1990). Since both types of current are well characterized in amphibian sympathetic ganglia (Selyanko *et al.*, J. Physiol., **425**, 471, 1990), we used the whole-cell patch-clamp technique to examine the effects of cyclic SS (cSS) and whole SS (wSS) on isolated bullfrog paravertebral ganglia neurons. In small cells, where muscarine activated I_{AIR}, wSS (1-2 μM, 2/7 cells) sometimes activated I_{AIR} whereas cSS (1-5 μM, 8/8 cells) was ineffective. cSS was effective in modulation of I_M in the large cells in which muscarine inhibited I_M. An initial suppression of I_M by 24% was followed by a 13% potentiation (12/23 cells). Both effects were associated with shifts of the I_M activation curve. Occasionally, monophasic responses were seen: I_M suppression (6/23 and 4/23 cells for wSS and cSS, respectively) or I_M potentiation (1/23 and 2/23 cells). This implies that receptor subtypes are involved in the multiple actions of SS in this tissue (Wang *et al.*, Proc. Natl. Acad. Sci. **86**, 9616, 1989). Supported by the Canadian MRC.

605.11

THERMAL DEPENDENCE OF ACTION POTENTIALS AND AFTERHYPERPOLARIZATIONS IN RAT HIPPOCAMPAL CA1 PYRAMIDAL CELLS AT 20°C TO 25°C. M.P. Thomas* and J.M. Horowitz, Animal Physiol., Univ. Calif. Davis, CA 95616.

Previous studies over the range of 37°C to 27°C suggested that different channels have different temperature sensitivities (e.g., Thompson et al., J. Neurosci. 5:817, 1985). The intent of this study was to extend consideration of thermal effects to a lower range of temperature. Conventional intracellular recording and current injection techniques were used to elicit spike trains in CA1 pyramidal cells at 20°C to 25°C. We measured the amplitude and duration of the following slow afterhyperpolarization (sAHP). The normalized duration of the sAHP (measured at 50% of peak height) was 750 ± 301 msec/mV. The duration of single spikes (measured at 50% of peak height) was 2.3 ± 0.2 msec, and the interspike interval (measured between the first and second spikes) was 13.5 ± 1.5 msec. The duration of the sAHP is greatly extended at 25°C compared with durations measured at 37°C, an increase of approximately 750%. In contrast, the width of single action potentials did not increase as markedly as the sAHP. Data indicate that different ionic currents can have markedly different thermal sensitivities and that the sensitivity of the Ca^{2+}-activated potassium current responsible for the sAHP may reflect greatly altered calcium metabolism at low temperatures. Supported by NSF Grant BNS-88-19973.

605.12

ETHOSUXIMIDE BLOCKS CHOLINERGIC RHYTHMICAL SLOW ACTIVITY (THETA) IN CA1 REGION OF HIPPOCAMPAL SLICE. D.D. Fraser and B.A. MacVicar Neuroscience Research Group, Department of Medical Physiology, University of Calgary, Alberta, Canada T2N 4N1.

Neuronal activity resembling rhythmical slow activity (theta) can be generated by perfusion of carbachol (CCH), a non-hydrolyzable cholinergic agonist, on isolated hippocampal slices. Each burst of theta consists of 4-10 Hz oscillatory depolarizations with a rise time much slower than conventional EPSPs (MacVicar and Tse, J. Physiol. 417:197, 1989). The presence of low-threshold Ca^{2+} spikes imparts the possibility of rhythmic burst discharge patterns in neurons at a theta frequency. In the CA1 region, CCH enhances a low-threshold Ca^{2+} current in isolated lacunosum-moleculare interneurons (Fraser and MacVicar, J. Neurosci., in press, 1991). We hypothesize that CCH enhancement of a low-threshold Ca^{2+} current could underlie theta generation. Ethosuximide, an anticonvulsant used in the treatment of petit mal epilepsy, has been shown to attenuate low threshold Ca^{2+} currents at clinically relevant concentrations (Coulter et al., Ann. Neurol. 25:582, 1989). Therefore, we have examined the action of ethosuximide on CCH-induced theta in the CA1 region of rat hippocampus.

Intracellular recordings were obtained from the CA1 neurons in hippocampal slices to determine the sensitivity of CCH-induced theta to ethosuximide (700 μM). Perfusion of 50 μM CCH produced 4-10 Hz oscillatory depolarizations with amplitudes of 12-16 mV. Theta oscillations occurred repetitively for periods of several seconds separated by 20-40 seconds of quiescence. This pattern was stable in some cells for greater than one hour in the continued presence of CCH. Co-perfusion of ethosuximide reversibly blocked CCH-induced theta without altering resting potential or input resistance. The results from this study support our hypothesis that a low-threshold Ca^{2+} current underlies the generation of theta in the hippocampus. (Supported by the MRC of Canada)

CALCIUM CHANNELS: PHYSIOLOGY AND PHARMACOLOGY IV

606.1

CALCIUM INDUCED CALCIUM RELEASE CONTRIBUTES TO THE STIMULATED RISE OF INTRACELLULAR CALCIUM IN SYMPATHETIC NEURONS. D.A. Przywara, S.V. Bhave, A.S. Bhave*, T.D. Wakade* & A.R. Wakade. Dept. of Pharmacology, Wayne State Univ. School of Medicine, Detroit, MI 48201.

We have shown that Ca^{2+} influx into sympathetic neurons causes a greater increase in free Ca^{2+} concentration ($[Ca^{2+}]_i$) in the nucleus than in the cytosol (Przywara et al., FASEB J. 5:217, 1991). In the present work we examined the mechanism underlying this pattern of Ca^{2+} distribution. Ca^{2+} imaging revealed a similar Ca^{2+} distribution regardless of the stimulus used to elevate $[Ca^{2+}]_i$. The differential increase in nuclear $[Ca^{2+}]_i$ was apparent when Ca^{2+} entered neurons during depolarization by excess K^+ or electrical stimulation, during receptor activation by acetylcholine or by application of ionophore. The same pattern of Ca^{2+} distribution resulted from caffeine-induced Ca^{2+} release from internal stores. It is well known that Ba^{2+} passes through Ca^{2+} channels and may "substitute" for Ca^{2+} in stimulating transmitter release. When neurons were stimulated in Ca^{2+}-free medium containing 2.5 mM Ba^{2+} a significant increase in $[Ca^{2+}]_i$ occurred. The greatest $[Ca^{2+}]_i$ was in the nucleus. These results indicate that Ba^{2+} is able to induce Ca^{2+} release from internal sites. We conclude that the similar pattern of Ca^{2+} distribution produced by the various stimuli is due to Ca^{2+} induced Ca^{2+} release following the initial rise in $[Ca^{2+}]_i$.

606.2

PEROXIDE INDUCED DECREASE OF I_{Ca} IN LOBSTER NEURONS; REGULATION BY $[Ca]_i$? R.S. Hernandez, R.J. Lowy, D.A. Miller, D.R. Livengood. Physiology Department, AFRRI, Bethesda, MD 20889-5145.

Previously we reported that H_2O_2 blocked calcium currents (I_{Ca}) in neurons of the lobster cardiac ganglion. We proposed that an increase in $[Ca]_i$ could be responsible (Biop. J. 53: 555a, 1988). To test this hypothesis, FURA-2 (free acid pentapotassium salt) was pressure injected into Neurons #1 or #2 of the ganglion, which were ligated and bathed in control lobster saline at 18°C. Fluorescence changes were quantified using a low-light-level video microscope and image processing system. Perfusion with 3.5 mM H_2O_2 saline for 30 min. failed to alter the $[Ca]_i$ signal from control values in either soma or dendritic components of the neuron. The dye responded to 100 mM KCl saline exposure, with a large reversible increase in the $[Ca]_i$ signal. In vitro exposure of Fura-2 acid to hydroxyl free radical generating conditions failed to alter the amount or Kd of the dye. To determine if FURA-2 was chelating internal Ca and thereby preventing the Ca-dependent I_{Ca} block, FURA-2 was pressure injected into neurons and electrophysiological measurements taken using a 2-electrode voltage clamp. FURA-loaded cells were found to respond similarly to non-loaded cells except, the Ca-mediated outward K currents displayed a small time-dependent decrease in amplitude. This may have indicated the chelating of internal Ca by FURA-2. Thus the H_2O_2 induced block appeared to be independent of $[Ca]_i$. However, when cells were depolarized by clamping them to -20 mV for 20 min., the inward I_{Ca}, was also blocked. This electrical depolarization block mimicked the block caused by H_2O_2. Therefore one of two possibilities exists: 1) The H_2O_2-induced block is not calcium mediated and what we have described is an epi-phenomenon; both H_2O_2 and depolarization independently produce the same results. 2) or: calcium mediated calcium block occurs, but the change in $[Ca]_i$ that produced the block is so small and/or localized that it is not measured with FURA-2.

606.3

THAPSIGARGIN STIMULATES CALCIUM INFLUX IN NEURONS.
W.C. Moore*, H.M. Hargrove* and J. Patel. Department of Pharmacology, ICI Americas, Inc., Wilmington, DE 19897.

Using the neuronal tumor cell line SK-N-SH, we demonstrate that receptor-mediated activation of phosphoinositide hydrolysis not only causes the release of Ca^{++} from intracellular stores but also causes a concomitant influx of Ca^{++} from the extracellular space. Treatment of fura-2 loaded cells with 1μM thapsigargin (TG) in the presence of extracellular Ca^{++} causes a depletion of the inositol 1,4,5-trisphosphate-sensitive intracellular Ca^{++} stores and a sustained elevation of intracellular Ca^{++} concentration ($[Ca^{++}]_i$). In the absence of extracellular Ca^{++}, TG produces only a transient increase in $[Ca^{++}]_i$. These data suggest that TG activates both the release of intracellular Ca^{++} and the influx of extracellular Ca^{++}. This supports the proposal that the depletion of the inositol 1,4,5-trisphosphate-sensitive intracellular Ca^{++} pool initiates extracellular Ca^{++} influx.

606.4

ACTIVATION OF RYANODINE-SENSITIVE CALCIUM RELEASE BY THE IP_3-FORMING AGONIST HISTAMINE IN BOVINE ADRENAL CHROMAFFIN CELLS.
K. A. Stauderman and M. M. Murawsky. Marion Merrell Dow Research Institute, Cincinnati, OH 45215.

Histamine stimulation of bovine adrenal chromaffin cells leads to the release of Ca^{2+} from internal stores. Since histamine also stimulates the formation of inositol 1,4,5-trisphosphate (IP_3), Ca^{2+} release is believed to result from activation of IP_3 receptors located on IP_3-sensitive organelles. The possible involvement of additional Ca^{2+} releasing mechanisms on other organelles was suggested by the recent observation that caffeine-sensitive, IP_3-insensitive, Ca^{2+} pools are also discharged during histamine treatment. To see if histamine might be activating a caffeine-sensitive Ca^{2+}-induced Ca^{2+}-release (CICR) mechanism, we tested the effects of ryanodine, an inhibitor of CICR in muscle, on repetitive histamine- or caffeine-induced Ca^{2+} spikes in single cells loaded with fura-2. The presence of a ryanodine-sensitive mechanism was demonstrated by both use-dependent inhibition of repetitive 10 mM caffeine-induced Ca^{2+} spikes by 10 μM ryanodine, and high-affinity binding of [^{3}H]ryanodine to cell membranes (Kd 3.3 nM). Most importantly, repetitive 10 μM histamine-induced Ca^{2+} spikes were also inhibited by ryanodine in a use-dependent manner, implicating the involvement of CICR. These data are consistent with the suggestion that Ca^{2+} released from IP_3-sensitive stores serves to activate CICR from caffeine-sensitive stores via the ryanodine-receptor.

606.5

PHARMACOLOGY OF INOSITOL 1,4,5-TRISPHOSPHATE (IP_3) SENSITIVE CALCIUM INFLUX (ISCI) IN *XENOPUS* OOCYTES.
H. J. Taub*, S. C. Sealfon, S. Mundamattom*, B. Gillo. Fishberg Center in Neurobiology, Mount Sinai School of Medicine, New York, NY 10029.

Xenopus oocytes injected with IP_3 develop an increase in the plasmalemma permeability to calcium ions. Increases in $[Ca]_i$ were assayed via activation of the oocyte's native Ca-sensitive Cl channel. When oocytes were loaded with > 5pmol IP_3 and kept in Ca-free solution, the increase in Ca membrane permeability lasted for more than 1h. We studied the pharmacology of this response in order to explore the mechanism involved in ISCI. The ISCI was not sensitive to organic voltage-sensitive calcium channels (VSCC) modulators (Bay K, nifedipine, verapamil, MK 801) and exhibited low sensitivity to inorganic VSCC blockers (Cd, Ni, La,). ISCI was not affected by agents that modulate Na/Ca cotransport (amiloride, Na/Li substitution, Na/NMG substitution). Lowering the extracellular pH to 6.5 or applying the protonphore (FCCP) completely blocked the ISCI. Application of the Ca-ATPase blocker vanadate did not affect this current. However, addition of the H-ATPase blocker (NBD-Cl) potentiated this current and at high concentrations enhanced Ca entry by itself. The pharmacology may suggest that H/Ca exchange (possibly as part of the H-ATPase complex) contributes to ISCI.

606.6

EVALUATION OF VOLTAGE- AND Ca^{++}-DEPENDENT MECHANISMS IN THE INACTIVATION OF THE Ca^{++} CURRENT IN HELIX NEURONES.
I.M.Stanford* and J.E.Chad, Dept. of Physiology and Pharmacology, Univ.of Southampton, SO9 3TU, UK.

The inactivation kinetics of the whole cell Ca^{++} current measured in isolated Helix neurones has been widely interpreted as due to separate Ca^{++}- and voltage-dependent processes, however the relative contributions of these processes are less widely agreed. We have attempted to separate the two processes by manipulation of the intracellular buffering agents (10mM) and extracellular divalents (50mM). Experiments were conducted on isolated dialysed, whole cell patch clamped neurones, using conditions designed to isolate the divalent current through Ca^{++} channels. Inactivation produced by a 50ms prepulse 100ms before a test pulse generating maximum inward current, and inactivation within single 200ms pulses to different membrane potentials were measured. In the prepulse experiments, maximal inactivation was obtained with Ca^{++} as charge carrier and no added buffering (values given as mean ±SD, n>=5; 0.23 ±0.06), reduced inactivation was obtained with Ba^{++} and EGTA (0.1 ±0.03), and Ba^{++}/BAPTA further reducted inactivation. Even under these conditions, the inactivation was not monotonic with regard to prepulse voltage. The inactivations of currents during single 200ms test pulses were tested with a series of BAPTA analogues, showing a dependence related to the Ca^{++} K_D of the buffer. Dimethyl-BAPTA (K_D 40nM) permitted less inactivation (ICa^{++} 0.32 ±0.03; IBa^{++} 0.18 ±0.04) than was observed with 4,4' difluoro-BAPTA (K_D 2450nM; ICa^{++} 0.57 ±0.09; IBa^{++} 0.30 ±0.07), for both Ca^{++} and Ba^{++}. Thus either Ba^{++} can produce some divalent dependent inactivation itself, or acts via an elevation in intracellular Ca^{++}, due to contamination or displacement from buffers. Cautioning against the assumption that inactivation kinetics with Ba^{++} and less stringent buffering conditions reflect voltage-dependent inactivation alone.

Supported by MRC studentship to I.S. and SERC grant No.GR/E 6349.7.

606.7

GADOLINIUM SELECTIVELY BLOCKS Ca++ INFLUX THROUGH VOLTAGE-OPERATED CALCIUM CHANNELS IN CENTRAL NERVE ENDINGS.
L.M.T.Canzoniero, A.M.Rossi*, A.Fatatis, G.F.Di Renzo* and L.Annunziato# Sect. of Pharmacology Dept of Human Communcation Sciences 2nd Sch.Med. Univ. Naples "Federico II", Naples and Chieti#, ITALY

The pharmacological modulation of Ca++ entrance in cerebral nerve endings is still a matter of debate and there is a lack of suitable agents which potently and selectively block Voltage-Operated Ca++ Channels. Ca++ influx in Percoll-purified rat brain synaptosomes was investigated either through the measurement of cytosolic Ca++ levels, monitored by Fura-2 or by the evaluation of the "fast phase" of 55mM K+-elicited 45Ca++ influx. The trivalent rare earth lanthanide, Gadolinium (Gd+++) (1-1000uM) dose-dependently counteracted the increase of[Ca++]i induced by 35mM K+ . Gd+++ (1-30 uM) also inhibited the "fast phase" of 45Ca++ influx induced by 55mM K+ . Among the organic Ca++ entry blockers, dihydropiridines were completely ineffective, whereas the phenilalkylamine Verapamil (100 and 300uM) and the aminoglycoside Neomycin (300 and 1000uM) prevented [Ca++]i elevation and 45Ca++ "fast phase " influx only at elevated concentrations. These results suggest that Gd+++ is a suitable tool to selectively block Ca++ influx in cerebral nerve endings.(Supported by C.N.R. and M.U.R.S.T. 883416 Grants to L.A.)

606.8

ZINC (Zn^{2+}) BLOCKS VOLTAGE GATED CALCIUM CHANNELS IN CULTURED RAT DORSAL ROOT GANGLION CELLS.
D. Büsselberg, M.L. Evans, D.O. Carpenter, and H.L. Haas. Institute für Physiologie der Heinrich-Heine Universität Düsseldorf, D-4000 Düsseldorf, FRG, SmithKline Beecham, Harlow, Essex CM19 5AD, UK, and NYS Dept. of Health and School of Public Health, Albany, NY 12201-0509, USA.

Dorsal root ganglion cells (DRGs) exhibit three types of voltage-dependent calcium channels. We have cultured DRGs from 2 to 4 day old rat pups and obtained whole cell patch clamp recordings of calcium channel currents after 1 to 5 days in culture. These currents, carried by barium cations, were recorded in the following external solution: TEA Cl 135 mM, HEPES 10 mM, glucose 10 mM, $BaCl_2$ 10 mM, $MgCl_2$ 1 mM, TTX 0.002 mM. A cesium based internal solution containing 2 mM ATP was used in the recording pipette. The divalent cation zinc (Zn^{2+}) blocked sustained and transient voltage sensitive calcium channel currents of cultured rat dorsal root ganglion cells. The IC_{50} for inhibition of the total peak current evoked by a step depolarization from -80 mV to 0 mV for 100 ms was 35 μM. The current evoked by a voltage step from -80 mV to -30 mV (T-type current) was more sensitive (>80% block with 20 μM Zn^{2+}). During wash the effect was only partly reversible. Thus Zn^{2+} is a potent blocker of all three types of voltage dependent calcium currents in mammalian neurons, but shows some specificity for the T channel.

606.9

DIVALENT IONS FROM STAINLESS STEEL HYPODERMIC NEEDLES REDUCE NEURONAL CALCIUM CURRENTS.

S.C. Nam and P. E. Hockberger. Dept. of Physiology, Northwestern Univ. Medical School, Chicago, IL 60611.

Stainless steel hypodermic needles are widely used in both biomedical research and in patient care because they are purportedly non-toxic and corrosion-resistant. The most common type of stainless steel (#304) is formed primarily with Fe, Ni and Cr, although other elements may be present depending upon the manufacturer's raw materials and processing steps. We have compared nickel- and chrome-plated stainless steel needles from several manufacturers on their ability to affect voltage-dependent Ca currents in isolated chick (E13-E16) DRG neurons and rat (P7-P13) cerebellar Purkinje cells. Our protocol involved allowing external saline (in mM: choline-Cl, 120; $CaCl_2$, 5; $MgCl_2$, 2; HEPES, 10; pH 7.3) to sit in needles for various durations of time (20s-1hr), and then analyzing Ca currents during local application of the test salines. Salines exposed to needles with brass hubs, but not those with plastic hubs, for 20s reduced currents by 30±10%. Longer exposures resulted in proportionately greater reductions. These reductions were recorded immediately upon saline application and were completely reversible. Elemental analysis of the test salines using ICP-AE spectroscopy revealed that Cu and Zn (but not Cd, Cr, Co, Fe, Pb, Mn or Ni) were released from brazed needles. The amount of Cu+Zn released in 20s was estimated to be 10-30μM, depending upon the specific needle examined. Longer exposures produced higher concentrations. Application of 100μM $CuSO_4$ in external saline reduced Ca currents in both cell types by 65±15% (n=6), whereas 100μM $ZnSO_4$ reduced currents by 20±5% (n=6). These results indicate that Cu (and possibly Zn) ions released from stainless steel needles with brass hubs can reduce neuronal Ca currents.

This research was supported by NIH grants #NS-26915 and NS-17489. ICP-AE spectroscopy was perfomed by Saman Shafaie, Chemistry Dept., Northwestern University, Evanston, IL.

606.10

PURKINJE CELL CALCIUM CURRENTS: ARE THEY UNIQUE?

P.E. Hockberger and S.C. Nam. Dept. of Physiology, Northwestern Univ. Medical School, Chicago, IL 60611.

Voltage-dependent calcium currents are distinguished by differences in biophysical and pharmacological properties. At last year's meeting we reported that calcium currents in isolated rat (P7-P13) cerebellar Purkinje cells (PCs) were sensitive to dihydropyridines and Ω-conotoxin, as is the case for many other nerve cell types. Those results were obtained using salines exposed to stainless steel needles possessing brass hubs, and were consequently confounded by the presence of interfering divalent ions (cf. Nam and Hockberger, these abstracts). We have therefore reinvestigated the actions of dihydropyridines and Ω-conotoxin on these cells, as well as on isolated chick (E13-E16) DRG neurons. Using local application of drugs via micropipet, we have found that high-threshold currents in both cell types were insensitive to 10μM nifedipine (dissolved in ethanol or acetone), 10μM nimodipine (in ethanol or polyethyleneglycol), and 10μM BayK8644 (in ethanol). This was the case regardless of the holding potential (between -100 and -40mV), although current inactivation increased progressively at holding potentials positive to -80mV in both cell types. In contrast to the effects of dihydropyridines, 10μM Ω-conotoxin irreversibly reduced DRG calcium currents by 90±5% within seconds. PC currents, on the other hand, were reduced 20±5%, and this effect was reversible. The sensitivities to divalent ions and diacylglycerol analogs (e.g., OAG) also differed between cell types. PC currents were uniformly more sensitive to Ni, Cu, and Zn than DRG cells (cf. Nam and Hockberger, these abstracts). However, DRG currents were more sensitive to OAG being reduced 50±5% by 25μM while PC currents were unaffected by this concentration of drug. Thus, upon reevaluation, we have found several differences in the pharmacological properties of calcium currents in rat PCs and chick DRG cells. These results support the notion that calcium currents in PCs, as well as chick DRG cells, are different from dihydropyridine-sensitive cell types.

This research was supported by NIH grants #NS26915 & #NS17489.

606.11

LEAD IONS (Pb^{2+}) PERMEATE THROUGH CALCIUM CHANNELS.

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Neurotoxic properties of lead are associated with the entry and intracellular interaction of Pb^{2+} ions with Ca^{2+}-mediated cell functions. In this study we have employed Fura-2 to monitor Pb^{2+}entry into isolated, bovine chromaffin cells exposed to low (micromolar) concentrations of PB^{2+} in basal or depolarizing, high-K^+ media. In basal media, the accumulation of Pb^{2+} in chromaffin cells was a linear function of $[Pb^{2+}]_{out}$, indicating passive leakage. Exposure of cells to high K^+ stimulated the entry of Pb^{2+} from 2-8-fold as a function of $[K^+]_{out}$. The depolarization-stimulated Pb^{2+} entry was further enhanced by calcium channel agonist Bay K 8644 and blocked by channel antagonist nifedipine, suggesting the involvement of L-type channels. In contrast to fura-2-monitored $[Ca^{2+}]_{in}$ transients in K^+-stimulated cells, the increase in $[Pb^{2+}]_{in}$ was sustained over a period of minutes, suggesting lesser rate of channel inactivation and/or saturaton of lead-buffering capacity of the cell cytosol.

Supported by NIEHS.

606.12

PATTERNS OF SECRETION ELICITED BY CA^{2+} vs. BA^{2+} IN ISOLATED RAT NERVE TERMINALS.

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Peptide secretion from neurohypophysial terminals isolated from the rat neurohypophysis (NHP) is triggered by Ca^{2+}-entry through voltage-dependent Ca^{2+}-channels. Exocytosis may be measured with whole terminal patch clamp techniques as changes in membrane capacitance (C), presumably resulting from fusion of secretory vesicles with the plasma membrane. It has been shown previously that the magnitude of the C jumps associated with Ca^{2+}-entry are influenced by the pattern of stimulation used, with repetitive depolarization producing 'facilitation' (Lim *et al.* 1990, Nature **344**, 449).

In isolated NHP terminals, 5 step depolarizations (46ms duration, 280ms interval; 10mM $[Ca^{2+}]_o$) elicited large C jumps. Despite Ca^{2+} -channel inactivation, in 60% of these terminals (n=15) the amplitude of the C jumps initially increased for successive pulses, usually peaking at the third pulse (Fig. a). With 10mM $[Ba^{2+}]_o$, the inward Ca^{2+}-channel current was increased. A striking difference between Ca^{2+}-triggered and Ba^{2+} -triggered exocytosis was that much larger depolarizations were necessary for the latter and the C jumps were small, of fairly uniform amplitude, and did not show 'facilitation' (Fig. b). This data suggests that Ba^{2+} may support peptide secretion, although with lower affinity than Ca^{2+} , and does not induce 'facilitation'.

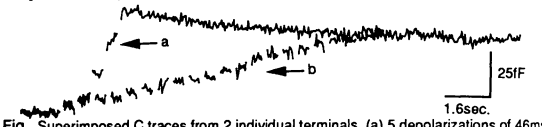

Fig. Superimposed C traces from 2 individual terminals, (a) 5 depolarizations of 46ms duration in 10mM $[Ca^{2+}]_o$, (b) 20 depolarizations of 72ms duration in 10mM $[Ba^{2+}]_o$.

606.13

CALCIUM MOBILIZATION AND TRANSMITTER RELEASE IN SYMPATHETIC NEURONAL GROWTH CONES.

P.S. Chowdhury, A.S. Bhave*, T.D. Wakade*, S.V. Bhave, D.A. Przywara, R. Skoff & A.R. Wakade. Dept. of Pharmacology, Wayne State Univ. School of Medicine, Detroit, MI 48201.

An equal number of stimulus pulses applied at increasing frequency is known to increase release of norepinephrine (NE) in neuroeffector organs. Kirpekar et al. (Naunyn-Schmiedeberg's Arch. Pharmac. 287:205, 1975) proposed that greater influx of Ca^{2+} may occur at high frequency. This idea was tested by measuring cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) in growth cones in cultured sympathetic neurons (SN). Release of [^{3}H]NE increased from $0.49x10^{-2}$ to $3.35x10^{-2}$ when 150 pulses were delivered at 1 and 10 Hz, respectively. The net increase (stimulated - basal) in $[Ca^{2+}]_i$ at 1 Hz was 230 nM and at 10 Hz 310 nM. The rate of rise in $[Ca^{2+}]_i$ at 10 Hz was much faster than at 1 Hz. Continuous stimulation of SN at 1 Hz for 3 min resulted in a sustained increase in $[Ca^{2+}]_i$ and [^{3}H]NE release. At 10 Hz there was a faster and greater sustained rise in $[Ca^{2+}]_i$ and [^{3}H]NE release. 55 mM K^+ caused a sharp rise in $[Ca^{2+}]_i$ and [^{3}H]NE release but both parameters rapidly declined during 3 min exposure to excess K^+. We conclude that net increase and rate of increase of $[Ca^{2+}]_i$ determine [^{3}H]NE release at different frequencies. We also show that sustained depolarization (excess K^+) inactivates Ca^{2+} entry and [^{3}H]NE release while high frequency stimulation maintains the release.

606.14

EFFECTS OF TEMPERATURE ON CALCIUM CURRENT OF BULLFROG SYMPATHETIC NEURONS.

E. van Lunteren, K.S. Elmslie and S.W. Jones. Departments of Medicine, Physiology and Biophysics, and Neurosciences, Case Western Reserve University, Cleveland, OH 44106.

The present study assessed the effects of temperature on the magnitude and kinetics of voltage-activated calcium current in dissociated frog sympathetic neurons. Calcium current increased with increasing temperature, peak current being ~1.5 to 2 fold greater at 30°C compared to 20°C. Estimates of the magnitude of the effects of temperature on peak current amplitude were complicated by the considerably faster run-down of current at high compared to low temperatures. Activation and deactivation kinetics became slightly faster over time during current run-down, but were substantially faster at high compared to low temperatures. The time constant of activation (over a range of -30 to 20 mV) was 4 to 7 times larger at 20°C compared to 30°C (eg. 4.18 ± 0.75 vs. 0.61 ± 0.10 msec at -10mV; mean ± SD; n=6), whereas the time constant of deactivation (over a range of -70 to -20 mV) was 1.5 to 4 times larger at 20°C compared to 30°C (eg. 1.42 ± 0.14 vs. 0.56 ± 0.07 msec at -40mV; n=5). Current inactivation was tested during a short depolarization to -10 mV following either a longer (0.2 to 2 second) prepulse of -10 mV or no prepulse. The reduction of current following the -10 mV prepulse compared to no prepulse was 1.8 to 2.3 fold greater at 30°C than at 20°C (eg. 47.3 ± 4.5 vs. 25.0 ± 8.4% reduction following a 2 second prepulse; n=6). Furthermore, the rate of inactivation became faster over time and with current run-down. These findings confirm the importance of temperature as a major determinant of the magnitude and kinetics of calcium currents.

Support: HL-42215 and NS-24471.

606.15

CALCIUM TRANSIENTS IN INDIVIDUAL SCHWANN CELLS UPON NERVE STIMULATION V. Lev-Ram and M. H. Ellisman. San Diego Microscopy and Imaging Resource, Department of Neurosciences, University of California, San Diego, La Jolla, Calif.92093-0608.

Our working hypothesis for nodal function incorporates the idea that the axon/glial cell ensemble plays an active role in the physiology of impulse conduction. This study was designed to expand on previous observations indicating active participation of the Schwann cell in the functioning of the node of Ranvier. In order to evaluate the potential role of Ca in nodal activation we developed a system for *in vitro* studies that combines simultaneous optical and electrophysiological recording of single fibers.

Frog sciatic nerve was dissected and loaded with the membrane permeable Ca indicator fluo3-AM. Action potentials of single fibers were recorded with a fine suction microelectrode. These active fibers were followed to observe healthy looking active nodes of Ranvier after they entered a bundle of surrounding fibers. The Ca transients in the ensheathing Schwann cell were then recorded using a laser-scanning confocal microscope system. The fluo3 staining was observed only in the Schwann cells and did not stain the axoplasm. This is probably due to physical barriers associated with the structure of the node of Ranvier in the PNS. Upon stimulation, an increase in intracellular [Ca]i concentration was observed in the paranodal region and other cytoplasmic pockets of the ensheathing Schwann cells. Calcium transients were observed in the single fibers monitored with the microelectrode as well as in Schwann cells of surrounding fibers. These other fibers were presumably also ensheathing electrically activated nerve fibers.

Fibers were also observed after the stimulation was halted. The [Ca]i continued to increase briefly after the cessation of long and rapid stimulation. After a recovery to near prestimulation levels, repeated stimulation of shorter duration produced a fast increase in [Ca]i, which decreased more rapidly after cessation stimulation. This observation is interpreted to indicate that the Schwann cells response was not damaged by the initial long periods of stimulation. The physiological basis for these Ca transients will be investigated further.

606.16

INVESTIGATION OF FAST EXTRACELLULAR CA^{2+} TRANSIENTS IN GUINEA-PIG HIPPOCAMPAL SLICES USING LOW IMPEDANCE ION SENSITIVE ELECTRODES. J.C. Behrends*, E. Ujec*‡ and G. ten Bruggencate. Dept. Physiol., Univ. of Munich, Germany and ‡ Dept. Physiol., Čechoslovak Acad. Sci., Prague, ČSFR. Spon: EBBS.

The measurement of changes in extracellular free $[Ca^{2+}]$ ($[Ca]_e$) using ion-sensitive microelectrodes (ISMs) has been useful in studying activity-related movements of Ca^{2+}-ions implicated in synaptic plasticity, epileptogenesis and excitotoxicity. Recordings with conventional ISMs, however, show very limited time resolution and signal to noise ratio due to high electrode resistance (1-50 GΩ). Using low impedance (100-500 MΩ) ISMs with a concentric, double barrelled ion-sensitive channel and an effective exchanger column length of 20-50 μm, we were able to record fast decreases in $[Ca]_e$ during single epileptiform interictal burst discharges ($\Delta[Ca]_e$=50-100 μM, time to peak=200-300 ms) in the guinea-pig hippocampus in vitro.

Further, these fast-responding ISMs enable repetitive (1/30 s) and stable recordings of $[Ca]_e$-decreases in response to short (0.5 s) ionophoretic application of glutamatergic agonists ($\Delta[Ca]_e$=100-400 μM), greatly facilitating pharmacological studies. N-methyl-D-aspartate- (NMDA-) receptor antagonists reduced glutamate-induced $[Ca]_e$-decreases in stratum radiatum of the CA1 subfield without affecting the associated field potentials, whereas perfusion with Mg^{2+}-free solution potentiated the $[Ca]_e$-signal. Ionophoresis of the non-NMDA-receptor agonist quisqualate produced smaller decreases in $[Ca]_e$ which on recovery showed a characteristic overshoot.

In summary, this is the first report of the use of fast-responding ISMs in brain slices. Using this technique, it is possible to pharmacologically determine the contribution of different glutamate receptor mechanisms and/or voltage-dependent Ca^{2+}-channels to Ca^{2+}-fluxes during neuronal activity.

606.17

EFFECTS OF ISOFLURANE ON CALCIUM CURRENTS RECORDED BY THE WHOLE-CELL PATCH CLAMP TECHNIQUE IN NEOCORTICAL SLICES. B. Hutcheon, E. Puil and P.B. Reiner. Dept. of Pharmacology and Therapeutics, Dept. of Anaesthesia, and Dept. of Psychiatry. Univ. of British Columbia. Vancouver, Canada. V6T 1Z3.

Rat sensory-motor cortical neurons were voltage-clamped using whole-cell patch clamp techniques in an *in vitro* slice preparation. Calcium currents were isolated by the extracellular application of 4-aminopyridine, tetraethylammonium, cesium, and tetrodotoxin. Patch pipettes contained 2 mM ATP; $[Ca^{++}]$ in the pipette was buffered to 10 nM. High-threshold (-30 to -10 mV) inward currents were evoked by depolarizing pulses from holding potentials near -80 and -40 mV. The evoked Ca^{++} currents were completely blocked by 3% isoflurane applied in the bathing solution. Lower concentrations of isoflurane reduced the peak amplitude, slowed the onset, and raised the threshold of activation of the Ca^{++} currents. The depressant effects were seen within 1-2 minutes after beginning isoflurane application (flow rate >2 ml/min), and recovered within 1-2 minutes. The present studies reveal that isoflurane may depress neuronal excitability by blocking a high-voltage activated Ca^{++} current.

606.18

CHRONIC DEPOLARIZATION ELEVATES INTRACELLULAR CALCIUM LEVELS IN CULTURED RAT MYENTERIC NEURONS. D.J. Fickbohm[1] and A.L. Willard[1,2]. Curric. in Neurobiology[1] and Dept. of Physiology[2], Univ. of North Carolina, Chapel Hill, NC 27599-7545.

We have been studying the effects of chronic depolarization on survival and development of rat myenteric neurons in cell culture. Previously, we found that growth in medium containing elevated potassium (25 mM), which depolarizes these neurons to a mean resting membrane potential of -40 mV, alters their development in several ways: it enhances survival, it increases neuronal size, and it suppresses voltage-dependent Ca currents. Here, we report that chronic depolarization causes persistent elevation of intracellular Ca ($[Ca]_i$) and that dihydropyridine (DHP) antagonists prevent this increase.

Myenteric neurons dissociated from intestines of 2-3 d old rat pups were grown in cell culture for 3-6 d in medium containing control (5 mM) or elevated (25 mM) KCl. $[Ca]_i$ was estimated by Indo-1 and fura-2. Although the 2 dyes gave different estimates of $[Ca]_i$, both methods revealed significantly higher $[Ca]_i$ in depolarized neurons. The DHPs nitrendipine and nifedipine (5 μM) prevented or reduced the effects of elevated potassium. The estimated values of $[Ca]_i$ depended critically on the method of calibration. "In situ" calibration (using ionophores) yielded values roughly half those estimated with "in vitro" calibrations, possibly due to vigorous intracellular Ca buffering mechanisms reducing the apparent R_{MAX}. With in vitro calibration, estimated $[Ca]_i$ was 337 ± 9 nM (Indo) or 187 ± 12 nM (fura) for depolarized neurons and 196 ± 5 nM (Indo) or 126 ± 9 nM (fura) for control neurons (all n's ≈ 100).

These results support the hypothesis that persistent elevation of $[Ca]_i$ is a mechanism by which chronic depolarization alters the development of myenteric neurons in culture.

Supported by NIH grants to ALW.

606.19

CHARACTERIZATION OF A PANCREATIC BETA CELL LINE FROM TRANSGENIC MICE. R.E. Hice, C. Katnik*, L. Philipson*, D.F. Steiner*, and D.J. Nelson*. Depts. of Medicine & Neurology, and the Howard Hughes Institute, University of Chicago. Chicago, IL 60637.

We have examined insulin secretion, voltage-gated ionic conductances, and cellular Ca^{2+} levels in βTC3 cells. βTC3 cells are a nonclonal cell line derived from insulinomas of mice carrying hybrid genes of an insulin promotor and SV 40 (T) antigen, described by Efrat, *et al.* (*PNAS* 85:9037,1988). Perifusion experiments have established that our cultures retain the ability to secrete insulin in response to 5 mM glucose (16-18 hr preincubation in 1 mM glucose). Under normal ionic conditions, voltage-dependent ionic conductances are predominated by outward K^+ currents (typically > 1 nA during depolarizations to 60 mV). A transient Na^+ current was also present, but was largely masked by the outward currents. Outward currents were substantially reduced, but not fully blocked when external Na^+ was replaced by TEA^+ (140 mM). Net inward Ca^{2+} currents were not observed at room temperature (ca. 22°C) with 140 TEA in the external solution but were observed in some cells under identical ionic conditions when the temperature was elevated to 30°C. However, the cells fire action potentials in response to glucose (10 mM) at room temperature. FURA-2 fluorescence (340:380 ratio) was used to monitor changes in cell Ca^{2+} levels in response to glucose. Glucose (20 mM) induced a biphasic change in cellular Ca^{2+} levels. Glucose rapidly (52 ± 10 sec, n=5 @ 27°C) caused a transient lowering (ca. 10% ratio decrease) of $[Ca^{2+}]_i$. The initial decrease in $[Ca^{2+}]_i$ was followed (253 ± 170 sec, n=5 @ 27°C) by a sustained increase comparable in magnitude to the increase in $[Ca^{2+}]_i$ elicited by 25 mM K^+ (ca. 2-fold increase in ratio 340:380). Both responses were also present at 22°C.

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606.20

PHARMACOLOGICAL CHARACTERIZATION OF K^+-INDUCED Ca^{2+} FLUX INTO RAT NEOCORTICAL PRISMS. J.J. Geer and D.J. Dooley. Parke-Davis Research Division, Warner-Lambert Company, Ann Arbor, MI 48105.

$^{45}Ca^{2+}$ flux into 100μm x 100μm prisms of adult rat neocortex was measured at 25°C in HEPES/Tris buffered HBSS (137mM Na^+, 3mM K^+, 1.25mM $Ca2^{2+}$) and also under conditions where K^+ was substituted for Na^+ up to 100mM K^+. After 30s incubation, elevated K^+ induced a 3-fold increase in Ca^{2+} flux over basal conditions which was half maximal at 25mM, and reached a plateau at 50mM K^+. The time course for 30mM K^+-stimulated flux showed a rapid rise in Ca^{2+} from 1-30s followed by a plateau between 30-100s. A 60s pre-depolarization in 30mM K^+ buffer containing zero Ca^{2+} did not affect the time course for Ca^{2+} flux, suggesting that the relevant Ca^{2+} channels are of the non-inactivating type.

K^+(30mM)-induced Ca^{2+} flux was unaffected by TTX(1μM), the NMDA antagonists CPP(100μM) and MK-801(10μM), and the non-NMDA antagonist NBQX(100μM). Divalent cations inhibited Ca^{2+} flux with a rank potency order of Cd^{2+} > Ni^{2+} > Co^{2+}. The following rank potency order, with IC_{50}s ranging from 20-500μM, was seen with various classes of Ca^{2+} channel antagonists: verapamil > nimodipine > nifedipine > dextromethorphan > diltiazem > neomycin. ω-Conotoxin GVIA(1μM) was inactive. These relatively high IC_{50} values and the inactivity of ω-conotoxin GVIA do not correlate well with classic Ca^{2+} channel pharmacology, suggesting the possibility of a novel Ca^{2+} channel subtype.

607.1

INTRACELLULAR CHANNELS: THE VOLTAGE-DEPENDENT ANION CHANNEL (VDAC) OF RAT BRAIN MITOCHONDRIA: CHARACTERIZATION AND IMMUNOHISTOCHEMICAL LOCALIZATION. M.W. McEnery, T.M. Dawson, A. Verma, and S.H. Snyder, Dept. of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, MD 21205

The voltage-dependent anion channel (VDAC) is a 32 kDa protein that has been localized to the outer membrane of mitochondria. VDAC has been shown by other investigators to function as: 1) an anion channel, 2) a non-specific pore through which many solutes may pass to gain assess to the mitochondrial inner membrane, and 3) an intracellular receptor for cytoplasmic kinases, such as hexokinase. Rat brain VDAC has been purified by conventional procedures and this protein cross-reacts with an anti-kidney VDAC antiserum. Immunohistochemically, VDAC is shown to be very heterogeneous. While the localization is primarily to glia, specific neurons were positively identified with the anti-VDAC antiserum. The glomerular layer of olfactory bulb, thalmus, caudate putamen, supraoptic nuclei, pontine nuclei, CA4 region of the hippocampus (glia and pyramidal cells), deep cerebellar nuclei, cell bodies and dendrites of Purkinje cells were the regions most intensely stained. These regions were also the most intensely stained with histochemical stains for mitochondrial enzymes: cytochrome oxidase, monoamine oxidase, and succinate dehydrogenase. VDAC localization is thus coincident with the regions of greatest mitochondrial density in rat brain. These results demonstrate heterogeneous mitochondrial populations in glia and neurons.

607.2

Secretory Vesicle-Specific Channels from the Neurohypophysis. K.E. Krebs, S.H. Goldberg*, and G. Ehrenstein*. Lab. of Biophysics, NINDS, National Institutes of Health, Bethesda, MD 20892.

Two distinct populations of secretory vesicle are present in nerve terminals in the bovine neurohypophysis: 100-300 nm diameter neurosecretory granules (NSG) that contain peptide neurohormones, and 40-60 nm microvesicles (MV) of unknown function that are biochemically related to small synaptic vesicles of presynaptic nerve terminals. We now report that these two classes of neurohypophysis vesicle contain distinct ion permeable channels. NSG and MV from bovine neurohypophysis were prepared by differential centrifugation, followed by separation on a 3000 Å controlled pore glass bead column, to yield highly purified vesicles on the basis of size. Purified NSG or MV were added to the *cis* side of painted lipid bilayers (80% PE/20% PC). Fusion of NSG with the bilayer resulted in the incorporation of a calcium-dependent (peak open probability of 80% at 300 nM Ca^{2+}), imperfectly selective chloride-permeable channel (>150 pS) that was blocked with 400 μM DIDS. In contrast, the fusion of MV with the bilayer resulted in the incorporation of a potassium-permeable channel (>100 pS) that appeared to be similar to channels identified in small synaptic vesicles purified from presynaptic nerve terminals of bovine brain.

607.3

MEMBRANE CAPACITANCE CHANGES ARE CORRELATED WITH $[CAMP]_I$ AND ACTIVATION OF CL^- CONDUCTANCE. **Sarah S. Garber, Cystic Fibrosis Res. Ctr., Neurobiology Res. Ctr., and Dept. Physiol. & Biophys., Univ. Alabama, Birmingham, Birmingham, AL 35294.**

I have used the whole-cell (w/c) patch clamp technique to simultaneously monitor changes membrane capacitance (C_m) and activation of Cl^- conductance (g_{Cl}) in the colonic cell line, T84. An EPC-9 amplifier was used to assess a possible relationship between vesicle fusion events stimulated by $[cAMP]_i$ and Cl^- conductance. C_m and R_{series} were determined by analyzing the RC decay of the membrane current in response to a series of voltage pulses. When recording in (mM) NaCl 155, $MgCl_2$ 2, $CaCl_2$ 1, Hepes 10 (bath) and Cs glutamate 130, $MgCl_2$ 2, Hepes 10, ATP 2, GTP 0.2 with $[Ca^{++}]_i$ buffered to $\leq 10^{-8}$ M with EGTA (pipet), 0/12 cells showed an increase in C_m, while g_{Cl} was activated in 9/12 cells. In contrast, when 100 μM $[cAMP]_i$ was included in the pipet solution, C_m increased in 5/8 cells and g_{Cl} was activated in 7/8 cells. Increases in C_m ranged from 0.5-4.0 pF, or ~10-20% of cell membrane area. No cells (n=6; 100 μM $[cAMP]_i$) showed a change in C_m when 50-100 μM DIDS was added to the bath to block Cl^- conductance. Recordings made in Na glutamate (bath) and K^+ or Cs glutamate 100 μM $[cAMP]_i$ (internal) also prevented increases in C_m (12/13 cells). These observations suggest that activation of g_{Cl} alone is insufficient to stimulate an increase in C_m, but is required for cAMP-dependent fusion of vesicles to the plasma membrane.

This work was supported by the CFF and DK 38518. I thank Dr. R.A. Frizzell for encouragement and support.

607.4

EFFECTS OF RECOMBINANT HUMAN TUMOR NECROSIS FACTORα ON CHLORIDE CHANNELS IN HUMAN NEUTROPHILS. M. A. Schumann and T. A. Raffin*. Division of Pulmonary and Critical Care Medicine, Stanford University School of Medicine, Stanford, CA 94305.

Tumor necrosis factor α is a cytokine with stimulatory effects on neutrophil functions such as phagocytosis, degranulation, and respiratory burst activity. To date, little is known about the intracellular pathways of cytokine signal transduction in neutrophils. By means of whole-cell, voltage-clamp recording we studied the effects of the recombinant human tumor necrosis factor α (rhTNFα) on macroscopic chloride currents in human neutrophils freshly isolated utilizing Ficoll-Hypaque density gradient centrifugation. To separate chloride currents, *N*-methyl-D-glucamine and choline were used as major cations and glutamate and chloride as major anions in pipette and bath solutions. Internal calcium ions were buffered at 10 nM. Bath application of the rhTNFα (0.01 μM), induced an increase (172.2±s.e.m 6.8%, n=9) in the amplitude of the outward chloride currents within 1 minute of exposure. Due to an ensuing desensitization, there was a time-dependent recovery toward the control level of current amplitude. Some cells did not respond (n=3) or responded with a minor increase in current amplitude (n=4), while others (n=2) exhibited a slight reduction in current amplitude. Phorbol 12-myristate 13-acetate (PMA; 0.1 μM) augmented the outward chloride currents within 1 minute (n=6). There was also an observed variability in response among cells. In the presence of PMA (0.1 μM), bath application of the rhTNFα (0.01 μM) did not result in an additive or a synergistic response of current enhancement. There was an insignificant increase (compared to control) in the amplitude of the current in the presence of both rhTNFα and PMA (n=14). This observation might be explained by a resultant cross desensitization between the two agents. These data suggest that protein kinase C activation may play a role in TNFα signal transduction in some human neutrophils.

607.5

Effects of monosialoganglioside GM1 on rat hippocampal CA1 pyramidal cells. P. Miu and K. Krnjević. Anaesthesia Research Dept., McGill University, Montréal, Québec, Canada, H3G 1Y6.

When recorded with CsCl-filled electrodes, and superfused with ACSF (at 33°) containing TTX (1 μM), TEA (10 mM), Cs (4 mM), and 4-AP (100 μM), pyramidal cells consistently responded to exogenous monosialoganglioside GM1 with an increase in the leak conductance (G_L) and an inward shift in the holding current (I_H) *(Agopyan et al, 1990, Soc. Neurosci. Abstr., 16:623)*.

Under identical condition (V_H = -40 mV) but recording with Cs acetate-filled electrodes, 4 out of 6 CA1 pyramidal cells responded to 1 μM GM1 application with a reduction in G_L by 7.9 ± 2.1 % (n = 5; $p < 0.025$, 1 tail t-test), and a slight outward shift in I_H (0.007 ± 0.007 nA; n = 7; N.S., 2 tail t-test). As observed previously, 1 μM GM1 reduced the high threshold calcium currents by 39.3 ± 6.5 % (n = 5; $p < 0.005$, 1 tail t-test). However, the GM1-induced reduction in the high threshold calcium currents could be reversed by 1 mM kynurenic acid.

In conclusion, the present findings suggest that a) GM1 may activate chloride conductance, and b) GM1 facilitates TTX-insensitive glutamate release as shown by the enhanced excitatory postsynaptic currents *(Miu and Krnjević, 1990, CJPP, 21:261)*. The potentiated glutamate release would increase the intraneuronal Ca^{2+} concentration, and thereby trigger Ca^{2+}-dependent Ca^{2+} inactivation *(Eckert and Chad, 1984, Prog. Biophys. Molec. Biol., 44:215)*, thus explaining the reduction in high threshold calcium currents.

This project was supported by MRC of Canada, and by Fidia.

607.6

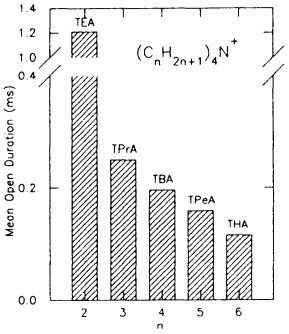
Ca SUPPRESSED NONGATED CATION CONDUCTANCE IN NEURONS. F.N. Quandt. Dept. of Physiology and Multiple Sclerosis Research Center. Rush University, Chicago, IL 60612

The properties of the resting conductance were studied using patch clamp techniques applied to N1E-115 neuroblastoma cells. Current across a patch of membrane was recorded in response to depolarization from a holding potential of -50 with successive 10 mV voltage steps through +50 mV. The current-voltage relationship exhibited outward rectification when measured without contributions from channels which generate current jumps due to opening and closing. In an excised membrane with NaCl, 3 mM Ca external solution, and Kglutamate EGTA internal solution, the slope conductance was 10 pS for a 10 mV step from -50 to -40 mV, 100 pS for a step between -10 to 0 mV, and 600 pS for a voltage step from +40 to +50 mV. The nonlinear current was not associated with the seal between the pipette glass and the membrane since outward rectification could be recorded from an attached patch when depolarized in the whole cell mode using an independent electrode. The Q10 for the slope conductance between 0 and +50 mV was found to be 6.0. The conductance pathway was permeable to K, Na and Cs, but not N-methyl-D-glucamine (NMG). The conductance was controlled by external Ca through a voltage dependent block, since the current amplitude was increased and the current-voltage relationship became linear as Ca was reduced below 3 mM. The reversal potential with internal K and external Na was not a function of external Ca. However, the reversal potential for inward rectification recorded in internal NMG and external Na became more positive potential when external Ca was reduced. Depolarization of the membrane in low Ca could be eliminated by substituting external Na with NMG. Internal Ba blocked the conductance and also caused a voltage and time dependent membrane depolarization. Supported by the National Multiple Sclerosis Society.

607.7

TETRAETHYLAMMONIUM (TEA) AND RELATED QUATERNARY AMMONIUM IONS BLOCK CHLORIDE CHANNELS IN CEREBRAL CORTICAL NEURONS. D.Y. Sanchez and A.L. Blatz, Physiology Department, UT Southwestern Medical Ctr., Dallas, TX 75235-9040

TEA and other symmetric quaternary ammonium ions (QAs) are commonly used to block potassium channels. We have found that QAs also block current through fast chloride channels in rat cerebral cortex. Symmetric QAs with alkyl chain lengths of 1 - 6 carbons were studied using excised, inside-out membrane patches with either external or internal blocker. Unlike TEA, which reduced single channel conductance, longer chain QAs reduced mean open duration with no effect on conductance. QAs blocked current through these channels in a voltage-dependent manner when applied externally. Internal block was less dependent on voltage. The Figure shows mean open duration with 0.5 mM external blocker (currents filtered at 3kHz, -3dB) and demonstrates that, similar to internal QA block of K channels, the larger blocking ions have higher affinity for the channel (lower K_D). Increased concentration of blocker decreases open duration suggesting that the blocker binds the channel's open state. Supported by NIH grants HL07360 & GM39731.



607.8

Expression of glycine receptor subunits in higher brain regions. J. Kuhse*, A. Kuryatov*, Y. Maulet*, M.L. Malosio*, H.Betz Max-Planck-Institut für Hirnforschung, Deutschordenstaße 46, D-6000 Frankfurt 1, Germany

The postsynaptic glycine receptor (GlyR) is a ligand-gated chloride channel protein which mediates inhibition of neuronal activity in the vertebrate central nervous system. Two different subunits of 48 kD (α) and 58 kD (ß) were identified that form a pentameric receptor. Immunological and molecular cloning data have disclosed heterogeneity of GlyR α subunits during development. In the CNS of rat, α1, α2, α2* and α3 subunit genes are expressed.

We have used molecular cloning of cDNA and genomic sequences to demonstrate the expression of alternative splicing products of the α2 transcript (α2 A and α2B). Furthermore, we have compared the expression of these mRNAs with that of other GlyR mRNAs in various regions of the rat CNS using *in situ* hybridisation techniques. The α2A, α2B and α2 * mRNAs are expressed in spinal cord and various brain regions and are down-regulated during development. In contrast, ß subunit mRNA is expressed throughout the CNS at very high levels, suggesting the existence of additional α subunit genes.

607.9

INCREASED CYTOPLASMIC CHLORIDE CAUSES GLYCINE-ACTIVATED CHLORIDE CHANNELS TO SHIFT TO LOWER PERMEABILITY OPEN STATES. A. I. McNiven* and A. R. Martin. Dept. of Physiology, Univ. of Colorado Sch. of Med., Denver, CO 80262.

Outside-out membrane patches were pulled from mouse spinal cord cells maintained in culture for 2 - 4 weeks. Single channel activity was produced by application of glycine to the outer surface of the patch at a concentration of 10 uM. The extracellular (bath) chloride concentration, $[Cl]_o$, was maintained at 157 mM. With a cytoplasmic (electrode) concentration, $[Cl]_i$, of 7 mM, channels activated by glycine had chord conductances at zero membrane potential of 26 pS, 19 pS and 13 pS. A few openings at 33 and 47 pS were also observed. The equivalent permeabilities and relative number of occurrences of the major open states were 14 (41%), 10 (23%) and 7 (20%) x 10^{-14} cm^3/sec, respectively. With increased chloride in the electrode ($[Cl]_i$ = 20 mM), similar permeability states were observed, but with different distributions: 14 (13%), 10 (54%) and 7 (23%) x 10^{-14} cm^3/sec. The remaining openings were to a still smaller permeability of 4 x 10^{-14} cm^3/sec (10%). Thus it appears that one effect of increased $[Cl]_i$ is to shift the preferred channel openings to lower permeability states. (Supported by N.I.H. Grant #NS09660.)

607.10

TETRAHYDROCANNABINOL RAISES BASAL AND GABA-MEDIATED SYNAPTONEUROSOME 36-CHLORIDE ION INFLUX. D.T.Shirac and B.E. Morton, Dept. Biochem-Biophys, Univ. Hawaii Sch. of Med., Honolulu, HI 96822.

(-)-Delta-9-tetrahydrocannabinol (THC) is the major psychoactive compound present in marijuana (Cannabis savita). In humans THC alters nausea, pain, anxiety, muscle tension, and memory. Many of these effects are similar to those produced by benzodiazepines, barbiturates, and alcohol which act on the chloride channel regulated by the gamma aminobutyric acid (GABA) type A receptor. Here, we examined the effect of THC on the GABA chloride channel complex.

Synaptoneurosomes were prepared from rat whole brain and 36-chloride ion (36-Cl) influx was assayed, using a slightly modified version of a standard procedure (Harris, R.A. and Allan, A.M., Science 228, 1108, 1985). Basal 36-Cl influx was enhanced 8-45% (average, 22%) when 0.1-1.0 mM THC was present in a colloidal suspension with 3%, w/v, polyvinyl-pyrrolidone-40.

When the effects of GABA on basal 36-Cl influx were examined, half maximal stimulation was found at 15 uM GABA with a maximum being reached by 100 uM at which point influx was at least doubled. In the presence of 1 mM THC, there was a leftward shift of GABA stimulation so that it was enhanced by at least 20% for both 3 and 10 uM GABA, falling off at 30 uM GABA, so that by the 100 uM maxima, no increase was produced. Generally similar results were observed with muscimol. These effects suggest THC released endogenous GABA.

600 uM Picrotoxin, or 100 uM of the GABA-A antagonist, bicuculline, reduced both GABA-muscimol and THC induced chloride increases to basal levels. 100 uM Baclofen or 100 uM 2-hydroxysaclofen, GABA-B ligands, were ineffective, as was 10 mM caffeine.

THC appears not to compete directly with the chloride channel complex receptors. It is proposed that THC binds the newly identified cannabinoid receptor, whose distribution parallels that of the GABA-A receptor, to somehow mobilize endogenous GABA. This opens the chloride channel to produce THC behavior.

607.11

DUAL EFFECT OF GABA ON NEUROSECRETORY CELLS OF CRAYFISH. García, U., *Onetti, C., Valdiosera, R., and Aréchiga H. Depto. de Fisiología, Biofísica y Neurociencias, CINVESTAV 07000 México, DF *CUIB Univ.de Colima, 2800 Colima,México.

GABA has been known for long as an inhibitory neurotrans mitter. There are evidences of its role as a transmitter in medulla terminalis X-organ cells or crustaceans. (Aréchi ga, H., García, U. and Martínez Millán, L. in Frontiers in Crustacean Neurobiology K. Wiese et al Eds. Birkhauser, 1990 pp 373).

In isolated eyestalks of adult crayfishes, the effect of GABA was tested while recording from X-organ cells either with microelectrodes or with whole-cell voltage clamp. Spontaneous activity of single X-organ cells varies from a silent mode with a -65 mV M.P. to a tonic or bursting activity at -45 -50 mV. Pulses of GABA resulted in depolarization and firing of spikes in hyperpolarized cells, and in a slow hyperpolarization and arrest of spontaneous firing in depolarized cells. These responses were blocked by picrotoxin (50 uM). In whole-cell voltage clamp, GABA responses were dependent on the chloride gradient. These results suggest that chloride equilibrium potential in X-organ neurons determines wheather GABA acts as excitatory or inhibitory transmitter.

607.12

SINGLE CHANNEL CURRENTS INDUCED BY PALYTOXIN IN NEUROBLASTOMA CELLS. S.Y. Kim*, C.H. Wu and L Beress*. Dept. of Pharmacology, Northwestern Univ. Med. Sch., Chicago, IL 60611.

Palytoxin is a potent marine toxin isolated from corals of the genus Palythoa. We have shown previously that palytoxin forms ouabain sensitive ion channels in frog red blood cells (Rana pipiens) and neuroblastoma cells (N1E-115). Palytoxin-induced channels had a single channel conductance of 10 pS, were permeable to small cations, did not inactivate, and were inhibited by ouabain. Antagonism by ouabain suggested that palytoxin acted through the Na,K-ATPase to form ion channels. At present, we are examining a substate of the palytoxin-induced channel in the neuroblastoma cell. Palytoxin-induced currents, from cell-attached patches, frequently stepped down to an amplitude about 30% of the full amplitude. In most cases, the channels initially opened to the full amplitude, then converted to the smaller amplitude for a short duration, then returned to the fully open state. Less commonly, channels opened directly to the substate from the closed state. The conductance of the substate at the resting potential was similar to that of the fully open state. The reversal potential of the substate was significantly more negative than that of the fully open state, indicating a different ion selectivity. These characteristics of the substate suggest the presence of complex dynamics within the palytoxin-induced channel.

607.13

PALYTOXIN ACTIONS ON LONGITUDINAL SMOOTH MUSCLE FROM GUINEA PIG ILEUM. R. E. Sheridan and S. S. Deshpande*. Neurotoxicology Branch, U. S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010.

Palytoxin (PTX) is a potent vasoconstrictor and cytotoxin isolated from marine coelenterates of the genus *Palythoa*. The precise mechanism of the toxin action is not clear but has been attributed to increased cation conductance in the plasma membrane of affected cells. We have investigated the actions of PTX on intact and dissociated longitudinal smooth muscle from guinea pig ileum.

PTX at 1 to 10 nM produced a rapid contraction in strips of longitudinal muscle. These contractions were, at most, about 50% of the maximal contracture produced by 45 mM K^+ in the same tissue. This initial peak contraction was followed over a period of minutes by a gradual relaxation of the tissue. Subsequent applications of similar toxin concentrations after this relaxation failed to elicit a further response, suggesting a form of desensitization. The contractions induced by PTX were dependent on external calcium and could be reversed by 1μM verapamil, a Ca^{++} channel antagonist.

In isolated muscles under whole cell patch clamp, PTX in the range of 1 to 10 nM induced a substantial (3 to 5 fold) increase in the resting conductance of the muscle fibers. The conductance increase was associated with a positive shift in the reversal potential and could be blocked by complete replacement of extracellular Na^+ with TEA^+, suggesting an increase in sodium and possibly potassium permeability. Like the contractile effects in muscle strips, this PTX-induced conductance was not relieved by extensive washing with toxin-free solutions. Further, even after washes with toxin-free TEA^+ solution that blocked the PTX effects, return to control saline revealed the PTX-induced conductance. At high doses, the conductance increase produced by PTX declined to a plateau, similar to the desensitization-like decrease in tension seen in muscle strips.

ION CHANNELS: MODULATION AND REGULATION IV

608.1

CO-FACTOR(S) FROM RAT BRAIN MODULATES THE GATING MODES OF SKELETAL MUSCLE (μ1) AND BRAIN (RIIa) VOLTAGE-SENSITIVE Na CHANNELS

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Recent studies (Zhou, J. *et al.*, Biophys. J. (1990) and manuscript submitted) demonstrate that Na channels expressed in *Xenopus* oocytes from skeletal muscle μ1 cRNA exhibit fast and slow inactivating current components; single channel analysis reveals that individual channels may interconvert between any of several gating modes with distinct activation, inactivation and slow-inactivation properties. Co-expression of μ1 α-subunits with mRNA from rat brain or skeletal muscle, but not liver, results in a single, rapidly inactivating current component, but single channel studies reveal individual channels displaying the same gating modes present with μ1 α-subunit alone. The factor evidently modulates the time spent in each mode. cRNA transcribed from a subfraction of a rat brain expression library (RTB1; Hollmann *et al.*, Nature, 1989), by itself, fails to elicit Na currents. Co-expression with μ1 cRNA, however, results in a shift to rapidly inactivating gating modes. Precisely similar modulation is observed with Na channels expressed from rat brain RIIa α-subunit cDNA, demonstrating the co-factor(s) to be capable of modulating various Na channel isoforms.

608.2

PANCURONIUM AFFECTS Na^+ CURRENT IN CHICK EMBRYO SENSORY NEURONS. V.Magnelli*, M.Nobile*, E.Maestrone*, and C.Usai. Ist. di Cibernetica e Biofisica CNR, Genova, Italy and Ospedale Civile di Sondrio, Italy.

The interaction of pancuronium with Na^+ currents was investigated, by using whole cell patch-clamp technique, in dorsal root ganglion sensory neurons of chick embryo. The above agent is a largely employed neuromuscular blocking drug. After Ca^{++} and K^+ currents suppression, external bath application of pancuronium 10^{-4} M to 10^{-3} M caused a reversible reduction of Na^+ current amplitude. At $5*10^{-3}$ M, steady-state inhibition was reached in 5 seconds and complete recovery occurred within 20 seconds after washing. The amplitude reduction appeared to be voltage and concentration dependent, whereas the time course of the activation and inactivation of the Na^+ current was unalterated by the drug. These data suggest that the well known mechanism of action of pancuronium (competitive inhibition of NachR), may be potentiated by the additional block of Na^+ conductance.

608.3

THE TIME COURSE OF THE LOSS OF A SODIUM CURRENT IN ASCIDIAN EGGS IS ASSOCIATED WITH CELL CYCLE EVENTS. J.L.Coombs* and W.J.Moody. University of Washington, Seattle, WA 98195.

The densities of many ion currents in eggs are strictly regulated over time in conjunction with specific developmental events. The eggs of the ascidian, Boltenia villosa, have a voltage-dependent, TTX-insensitive sodium current that is known to disappear after fertilization, becoming barely detectable by the two cell stage. The sodium current is responsible for the fast, rising phase of the long lasting action potential which these eggs can produce. There are other currents in these eggs whose densities in the membrane do not change, and a chloride current that appears at each meiotic and mitotic cell division once the egg has been fertilized. We studied the loss of the sodium current in these eggs over time and found that this current is largest in the immature oocyte (the growing oocyte that is arrested in the first meiotic metaphase) and begins its decline around the time of egg maturation (when the nuclear envelope breaks down and the egg is ready to be fertilized). This loss continues after maturation and during the two hours between fertilization and the first mitotic cleavage. However, the decline is arrested transiently during times when the chloride current appears.

608.4

TEMPERATURE DEPENDENCE OF LIMITATION OF SRF BY MK-801. A.W. Wamil and M.J. McLean. Dept. of Neurology, Vanderbilt Univ. Med. Ctr., Nashville, TN 37212

Anticonvulsant compounds, including MK-801, which protect against maximal electroshock seizures in animals limited sustained repetitive firing (SRF) of intracellularly recorded sodium-dependent action potentials cultured mammalian neurons. We studied the effect of temperature on the limitation by MK-801 of SRF of mouse spinal cord neurons in cell culture. Limitation occurred between 10^{-9} and 10^{-6} M (EC_{50} $1.5x10^{-7}$ M) at 37°C; between 10^{-8} and 10^{-3} M at 32°C (bimodal distribution); and, between $5x10^{-7}$ and 10^{-2} M at 23°C (EC_{50} about 10^{-6} M). Thus, temperature changes altered concentration-dependence of limitation of SRF and could explain the high concentrations of MK-801 needed to see effects on I_{Na} in patch clamp experiments.

608.5

TEMPERATURE DEPENDENCE OF MAGNETIC FIELD EFFECTS ON SUBTYPES OF SENSORY NEURONS IN VITRO. R.R. Holcomb, A.W. Wamil, J.D. Pickett* and M.J. McLean. Dept. of Neurology, Vanderbilt Univ. Med. Ctr., Nashville, TN 37212

Dorsal root ganglion neurons (DRGn) in cell culture were classified by waveform of intracellularly recorded action potentials (APs; SD: duration <2 msec; LD: duration >2 msec). Current pulses (1 msec; 1 Hz) elicited firing in neurons with SD or LD AP waveform repeatedly with rare failures. During exposure at 37°C to a static magnetic field produced by an array of four permanent magnets of alternating polarity, the number of stimuli failing to elicit APs increased significantly and APs of both types were completely blocked in many neurons. At 32°C, SD APs failed, but less frequently than at 37°C; LD APs were not affected. No effect of the field was seen on SD APs at 23°C. Modulation of magnetic field effects on functional subtypes of DRGn with temperature may have implications in the treatment of neuropathic pain.

608.6

ATP-DEPENDENT RUN-DOWN OF $GABA_A$ RECEPTOR FUNCTION DURING WHOLE-CELL RECORDING IS STIMULATED BY PENTOBARBITAL. M. Gyenes*, T.T. Gibbs and D.H. Farb. Department of Pharmacology and Experimental Therapeutics, Boston University School of Medicine, Boston, MA 02118.

We have recently shown, using whole cell recording from chick spinal cord neurons in primary monolayer cell culture, that the currents evoked by 30 μM GABA progressively run down by about 60% within 30 min. This is accompanied by an acceleration of desensitization. Surprisingly, the response to 3 μM GABA remains stable, even after run-down of the response to 30 μM GABA. Inclusion of ATP or ATPγS in the recording pipet reduces the extent of GABA-induced run-down.

In the presence of 100 μM pentobarbital, the response to GABA is potentiated such that the peak current evoked by 3 μM GABA is comparable to that elicited by 30 μM GABA in the absence of pentobarbital (2.5 nA). The pentobarbital-potentiated GABA response exhibits rapid run-down and acceleration of desensitization, as is seen with 30 μM, but not 3 μM, GABA in the absence of pentobarbital. In this case as well, ATPγS effectively prevents both the run-down and the acceleration of desensitization.

Run-down of the 30 μM GABA response and inhibition of run-down by ATPγS occur also in isolated outside-out patches demonstrating that these phenomena are not contingent upon the organization of the intact nerve cell. As in whole cell recordings, the 3 μM GABA responses remain stable.

608.7

INHIBITION OF HYPERPOLARIZATION-ACTIVATED CURRENT I_h IN SUBSTANTIA NIGRA COMPACTA NEURONS BY GABA, TAURINE AND GLYCINE. M.G.Lacey, M.A.Häusser* and W.H.Yung*. Dept. of Pharmacology, University of Birmingham, B15 2TT, UK, and *Laboratory of Physiology, University of Oxford, OX1 3PT, UK.

The time-dependent hyperpolarization-activated inward current I_h (or I_f, I_Q, I_{AR}) has been observed in a variety of cell types including neurones. Several recent reports have described effects on cell excitability through modulation of I_h by intracellular messengers, and also by activation of muscarinic and aminergic receptors.

Intracellular recordings were made from presumed dopaminergic substantia nigra compacta neurons in rat brain slices. I_h was studied using single electrode voltage clamp. The inhibitory amino acids GABA (0.3 mM), taurine (3 mM) and glycine (0.3 mM) all increased membrane Cl^- conductance and also markedly reduced the amplitude of I_h. These effects were mimicked by the $GABA_A$ receptor agonist muscimol, but not by $GABA_B$ agonist baclofen. The modulation of I_h by activation of neurotransmitter receptors thought to be ligand-gated Cl^- channels represents a novel mechanism for control of cell excitability.

Experiments investigating the mechanism of this effect will be described.

608.8

ZINC REDUCES THE TRANSIENT OUTWARD CURRENT IN IMMATURE RAT HIPPOCAMPAL NEURONS.
I. Spigelman & P. L. Carlen. Playfair Neuroscience Unit, Toronto Western hospital, Toronto, Canada.

Previous studies have shown that exogenous zinc application has predominantly excitatory effects on mammalian central neurons. The giant GABA-mediated depolarizing potentials in immature hippocampal CA3 neurons (Ben-Ari et al. J. Physiol. 416:303-325, 1989) were recently proposed to result from an inhibitory action of Zn^{2+} on the pre- and postsynaptic $GABA_B$-mediated receptors (Xie & Smart, Nature 349:521-524, 1991). We studied the effects of Zn^{2+} and the $GABA_B$ receptor agonist baclofen on the transient outward current (I_A) in immature rat hippocampal neurons. Whole-cell patch recordings from dentate granule or CA1 neurons in hippocampal slices from immature (7-13 day old) Wistar rats revealed that bath application of Zn^{2+} (200 μM) reversibly decreased the amplitude of I_A (32%, n=7) and its rate of decay (54%), while the latency to peak I_A activation was increased. Application of baclofen (200 μM) increased the I_A amplitude (n=3). Concomitant baclofen and Zn^{2+} applications that followed application of Zn^{2+} alone partly offset the Zn^{2+}-induced decreases in I_A amplitude (n=3). These results suggest that one possible consequence of endogenous Zn^{2+} release in the immature hippocampus is an increase in both pre- and postsynaptic excitability via actions on the I_A.

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608.9

EFFECT OF TEMPERATURE AND TIME ON THE AFFINITY OF AN ENDOGENOUS SUBSTANCE OF CENTRAL BENZODIAZEPINE RECEPTOR. H.L. Komiskey, J. Harper * , S.E. Demick * and A. Rahman *. College of Pharmacy, Xavier University of Louisiana, New Orleans, LA 70125.

An endogenous compound with high affinity for central benzodiazepine receptor has been extracted from rat brain tissue (Pharmacologist 30: 25, 1988). The endogenous compound was stored at 25°C, 0-4°C and -80°C for 48 hours in 70% methanol. The central benzodiazepine binding sites in homogenate of rat cerebral cortex were labeled with ^{3}H-flumazenil. Incubations were conducted at 0-4°C. Specific binding was measured as the difference between the ^{3}H-flumazenil bound in the absence and presence of 1uM clonazepam.

The affinity of the endogenous compound for the central benzodiazepine binding site, as well as its ultraviolet light absorbance decreased over time at each temperature. Storage of the compound at colder temperatures did not slow the apparent breakdown of the compound. In contrast, the benzodiazepines, diazepam and desmethyldiazepam were found to be more stable relative to the endogenous compound in 70% methanol. (Supported by NIH grant NS27265).

608.10

CHLORIDE CHANNEL REGULATION IN SKELETAL MUSCLE OF AGED RAT
D.Conte Camerino*, A.De Luca*, D.Tricarico* and S.H.Bryant Dept. Pharmacobiology,Faculty of Pharmacy,University of Bari,Bari,Italy* and Dept.Pharmacology and Cell Biophysics, University of Cincinnati,Cincinnati,OH 45267.

Skeletal muscle fibers of aged rats have a reduced resting chloride conductance(GCl)(De Luca et al.,Pflügers Arch,415,642,1990). Our recent data evidenced that protein kinase C(PKC) exterts an inhibitory control on chloride channel of adult rat and goat skeletal muscle fibers(Bryant and Conte Camerino,Pflügers Arch,417,605,1991; Tricarico et al.,Pflügers Arch, in press). The present study was aimed at clarifying if perturbation of PKC system can explain the GCl defect of aged rats. Measurements of GCl were made on extensor digitorum longus muscle fibers from 3-4 and 29 months old rats, "in vitro" at 30°C with standard two microelectrodes metodology. The PKC activator 4-B-phorbol-12,13-dibutyrate was more potent in blocking GCl in old (IC50=5nM) than in adult rats(IC50=50nM). PKC inhibitor staurosporine(1uM) did not restore the low GCl of aged rats but prevented the block of GCl by the phorbol ester.The present data suggest that an overactivity of PKC may play a role in the reduced GCl of aged rat skeletal muscle.
Supported by Italian CNR 90-1460, 90-1431 and MURST.

608.11

BETA-ENDORPHIN MODULATES A MECHANO-SENSORY CALCIUM CHANNEL IN *STENTOR*. M. J. Marino and D. C. Wood. *Dept.of Behavioral Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA 15260*

Mechanical stimulation opens a population of mechano-sensory calcium channels in the ciliate protozoan *Stentor*. The resulting depolarizing receptor potential can trigger an action potential and lead to a contraction of the organism. We have found that the peptide beta-endorphin (BEND) markedly reduces the probability that *Stentor* will contract to mechanical stimuli. This action of BEND is naloxone sensitive, and dose dependant (100 nM - 10 uM). The drug is most effective immediately after application, with the contraction probability returning to control values after two hours of continued exposure. The reduction in response probability is specific to mechanically induced contractions, with no change in probability of contraction produced by electric or photic stimulation. Electrophysiological studies have shown that BEND produces a reduction in mechano-sensory receptor potential amplitude without altering other passive or active membrane properties. On going voltage clamp studies are aimed at elucidating the nature of this channel modulation.

608.12

BINDING CHARACTERISTICS OF [^{3}H]RYANODINE TO RAT BRAIN CORTICAL MICROSOMES. I. Zimanyi and I.N. Pessah* Department of Veterinary Pharmacology and Toxicology, University of California, Davis, CA 95616.

Specific binding of [^{3}H]ryanodine to sucrose gradient purified rat brain cortical microsomes recently has been characterized pharmacologically (Zimanyi and Pessah, Brain Res. 1991). The association of 0.5 nM [^{3}H]ryanodine is complete within 80 min and remains stable for up to 5 hr. Dissociation of the [^{3}H]ryanodine-receptor equilibrium complex has been examined in two ways. Addition of 1000-fold unlabeled ryanodine results in biphasic dissociation curves best fit by a double exponential decay which yield two dissociation rate constants. The dissociation rate constants and the pseudo first order rate constant of the association yield two dissociation constants of 0.1 and 1.7 nM for the high- and low-affinity binding of ryanodine. Dissociation of [^{3}H]ryanodine by 100-fold dilution of the assay medium results in monophasic dissociation with halftime of 63 min and yields a calculated K_d of 0.5 nM. Pretreatment of the microsomes with 1 μM unlabeled ryanodine which was subsequently removed by sufficient washing and centrifugation steps reduced the available specific binding sites for [^{3}H]ryanodine by 50%. These results indicate an irreversible action of ryanodine on multiple binding sites in the brain. In general [^{3}H]ryanodine receptors found in brain have higher affinities than in those found in skeletal muscle SR. Supported by NIH grant ES05002 and BRSG 90-19 to INP.

608.13

ALTERATIONS IN CALCIUM AND POTASSIUM CHANNEL ANTAGONIST BINDING IN CARDIOMYOPATHIC HAMSTER TISSUE. J.A. MILLER, P.A. Chmielewski* and D. Rampe*, Marion Merrell Dow Research Institute, Cincinnati, OH, 45215.

The Syrian cardiomyopathic hamster (CMH) (BIO 14.6) develops cardiac hypertrophy and congestive heart failure, possibly due to cellular calcium overload. To characterize the pathophysiology involved in this animal model we have examined the density of L-type calcium and ATP-sensitive potassium channels in 30 and 180 day old hamster heart and brain using membrane receptor binding and quantitative autoradiography (QAR). In the heart, [^{3}H]PN200-110 binding to calcium channels was elevated 84% above controls in 30 day old CMH and 31% above controls in 180 day old CMH. In contrast, [^{3}H]glibenclamide ([^{3}H]GLI) binding to cardiac potassium channels was unaltered in 30 day old animals but was decreased to 64% of control levels in 180 day old CMH. In brain membranes there was no significant difference in the binding density of either ligand between groups or between ages. However, QAR revealed a 41% increase in [^{3}H]GLI binding in frontal cortex of 30 day old CMH versus the same age control. There was no difference in [^{3}H]GLI binding in frontoparietal or striate cortex or in cerebellum or brainstem. Further QAR analyses will include other brain and heart regions with both [^{3}H]PN and [^{3}H]GLI binding. These findings suggest distinct differences in regional levels of potassium and calcium channels in CMH that may be related to the progression of the disease.

608.14

BLOCKADE OF K^+ CHANNELS IN PC12 CELLS BY PROPOFOL. M.Nobile*, V.Magnelli*, E.Maestrone*, L.Spadavecchia*. Ist. Cibernetica e Biofisica CNR, Genova, Italy and Osp. Civ. Sondrio, Italy. (SPON: Europ. Neurosci. Ass.)

The general anaesthetic Propofol (PR) reduced reversibly K^+ currents in voltage clamped PC12 cells. In control conditions, current activation time course was fitted by 2 exponential terms, with n=2 and n=1 kinetics, whereas, when PR was applied extracellularly, the n=2 component only gave the best fit. PR $5*10^{-5}$ to 10^{-3}M decreased currents amplitude in a concentration dependent way, but independently of holding potential (V_H). K^+ currents block was neither frequency nor use dependent. A complex voltage-dependent block pattern was observed, when the above concentrations were employed. Single channel outside-out experiments (V_H= -50 mV) revealed 2 different K^+ channels, with unitary conductances of 10 and 22 pS. PR $5*10^{-5}$ M decreased the open probability of the bigger channel, but did not affect the smaller one. At higher concentrations channels were fully blocked. These results might suggest either different sensitivities or different blocking mechanisms for the two channels.

608.15

INTERACTIONS OF POLYAMINES WITH THE NICOTINIC ACETYLCHOLINE RECEPTOR IN RAT BRAIN. R.D. Kusztos, C.R. Mantione and E.D. London. Neuropharmacology Laboratory, NIDA Addiction Research Center, Baltimore, MD 21224

A previous report indicated the presence of specific recognition sites for polyamines on the nicotinic acetylcholine receptor (nACh-R) of *Torpedo* electic organ (Anis, N. et al., *J. Pharmacol. Exp. Ther.*, **254**:764, 1990). We, therefore, were interested in whether polyamines, which modulate activity of the N-methyl-D-aspartate receptor, also influence nACh-R in brain. In this regard, we tested the effects of various endogenous and synthetic polyamines on the binding of [^{3}H]methylcarbamylcholine and [^{3}H]mecamylamine ([^{3}H]MEC), which label the acetylcholine recognition site and the open cationic channel of the nACh-R (London, E.D. et al., *Soc. Neurosci. Abstr.*, **15**:64, 1989, Banerjee, S. et al., *Biochem. Pharmacol.*, **40**:2105, 1990), respectively. Although the polyamines had no effect on the binding of [^{3}H]methylcarbamylcholine, they inhibited the binding of [^{3}H]MEC at μmolar concentrations. The rank order of potencies of the compounds tested was as follows: spermine > spermidine > 1,10-diaminodecane > putrescine > diethylenetriamine. The sequence is similar to the rank order of these substances in inhibiting the binding of [^{3}H]spermidine, a radioligand for polyamine binding sites in brain (Mantione, C.R. et al., *Eur. J. Pharmacol.*, **180**:393, 1990). The present findings support the view that polyamines in brain may modulate the activity of nACh-R through recognition sites on the receptor.

608.16

QUINIDINE SULFATE ACTION ON GUINEA PIG SMOOTH MUSCLE. X. García, L. Herrera*, and E. Gijón. Dept. of Physiol. Sch. of Med. Universidad Nacional Autónoma de México. Ap. P. 70-250, México, D.F. 04510. MEXICO.

Previous studies in our laboratory have shown an anticholinergic effect of quinidine sulfate, an antiarrythmic compound, carachterized by producing a slight increase in tone, at low doses, and a decrease in tone and rhythm when applied at high doses on the isolated intestine of guinea pigs. Segments of intestine from 300 g male guinea pig were prepared for isometric recording. Quinidine inhibited the contraction elicited by acetylcholine. This effect can also be observed with histamine and barium chloride. These effects are calcium non-dependent as they can be observed in low calcium solutions. Our results support an anticholinergic, an antihistaminic, and an antibarium effect of quinidine, calcium non-dependent.

608.17

EVALUATION OF A MEMBRANE CONDUCTANCE FROM SPIKE TRAINS. Jon Berner and C.D. Woody. MRRC, UCLA Medical Center, Los Angeles CA 90024.

A method for detecting changes in one membrane conductance, the slow gK+(Ca), which does not require intracellular penetrations, could speed the study of associative learning (Alkon, 1979; Coulter et. al., 1989).

A single compartment, Hodgkin-Huxley model of a cortical pyramidal cell (containing features of gK+(A), gNa+, gNa+(P), gK+(DR), fgK+(Ca), and the slow gK+(Ca)) generated a spike train in response to random perturbation of membrane potential (c.f. Abeles, 1982) which was represented by a two dimensional plot of the interspike interval between successive spikes. A distinctive effect of a reduction in the maximal conductance of the slow gK+(Ca) on the geometry of the underlying spike train was revealed by subtractive techniques. This effect, a symmetric reduction in short-medium/medium-short intervals, was observed to be robust to changes in the variance and the mean of the stimulus and the maximal conductance of gK+(A).

Although comparison of these plots with other data from in-vivo preparations suggests that the baseline spike train geometry might need to be uniquely specified for a cell type of interest, these techniques might also be used to identify changes in other voltage-gated conductances.

ACETYLCHOLINE: SYNTHESIS AND DEGRADATION

609.1

REGULATION OF HIGH-AFFINITY CHOLINE TRANSPORT IN THE NEUROBLASTOMA CELL LINE LA-N-2. A. Lambros*, S. Goddard* and R.J. Rylett. Department of Pharmacology and Toxicology, University of Western Ontario, London, Ontario, Canada, N6A 5C1.

The sodium-dependent, high-affinity choline transport protein has not been purified or characterized biochemically and very little is known about its long-term regulation. We have observed that transfer of cholinergic neuroblastoma LA-N-2 cells into serum-free N2 (SF-N2) media results in enhanced high-affinity choline uptake (HACU) activity accompanied by proportional increases in acetylcholine (Ach) synthesis, but not choline acetyltransferase (ChAT) activity. Maximal increases in HACU (approximately 7-fold) were achieved after four days in SF-N2 media. Enhanced HACU was characterized by an increase in the maximum velocity of transport (Vmax) for the high-affinity carrier with no significant change in the affinity (Km) of the carrier for substrate. In the presence of cycloheximide or α-amanitin,increases in HACU activity were substantially reduced. This suggests that transfer of LA-N2 cells into SF-N2 media results in increased synthesis of mRNA transcript for the high-affinity carrier which is effectively translated into new transport protein. Our findings indicate that LA-N-2 may serve as an effective model for studying regulation of HACU in culture. (Supported by MRC Canada and The Upjohn Company).

609.2

ANALYSIS OF REGULATORY ELEMENTS IN THE 5' FLANKING REGION OF THE *DROSOPHILA* CHOLINE ACETYLTRANSFERASE GENE. K. Ikeda, T. Kitamoto* and P. M. Salvaterra. Division of Neurosciences, Beckman Research Institute of the City of Hope,1450 East Duarte Road, Duarte, CA 91010.

We have analyzed the 5' flanking region of the *Drosophila* choline acetyltransferase gene (ChAT, E.C.2.3.1.6) for the presence of cis-regulatory elements. DNA fragments containing various lengths of flanking DNA were fused to the *E. coli* lacZ gene used to transform *Drosophila*. The histochemical localization of β-galactosidase expression was used to evaluate the ability of different fragments to direct lacZ expression. A 7.4 kb flanking sequence was found to direct β-gal expression in specific somal and synaptic layers of the optic lobe and other cephalic structures. The pattern of β-gal staining corresponded closely to the distribution of endogenous ChAT protein. In contrast, a construct containing only the proximal 3.3 kb of 5' flanking DNA resulted in a qualitatively and quantitatively different expression pattern. In general, a more restricted pattern of expression was observed which appeared to be a subset of the structures seen with the 7.4 kb construct. Further deletion to the proximal 1.2 kb of flanking DNA led to an even more restricted pattern. Our results indicate that both qualitative and quantitative regulatory elements are present in the 5' flanking DNA and these elements distinguish subsets of cholinergic neurons. We have also fused the 5' flanking DNA to wild type ChAT cDNA, and used these constructs to transform *Drosophila* stocks carrying a temperature sensitive ChAT allele. The 7.4 kb-cDNA transformants had higher levels of wild type ChAT activity than either the 3.3 or 1.2 kb constructs. All three constructs were able to rescue mutant flies from paralytic and lethal phenotypes.

609.3

ALTERNATIVE SPLICING OF CHOLINE ACETYLTRANSFERASE TRANSCRIPTS IN THE NEMATODE *C. elegans*. J. B. Rand, A. Alfonso*, K. Grundahl*, J. R. McManus*, and J. M. Asbury*, Program in Molecular and Cell Biology, Oklahoma Medical Research Foundation, Oklahoma City, OK 73104.

We have previously cloned and analyzed the *cha-1* locus from the soil nematode *Caenorhabditis elegans* (Alfonso et al., Soc.Neurosci.Abstr. **16**:199). *cha-1* is the structural gene for choline acetyltransferase (ChAT). Genetic evidence indicates that *cha-1* interacts with the closely linked *unc-17* locus (Rand and Russell, Genetics **106**:227; Rand, Genetics **122**:73). *unc-17* mutants have normal ChAT activity, but are resistant to AChE inhibitors. This suggests a probable cholinergic function for the *unc-17* gene product.

We have identified and characterized two classes of cDNA which appear to arise through alternative splicing and which correspond to *cha-1* and *unc-17* transcripts, respectively. The coding sequence of the *cha-1* transcript is disrupted by mutations in the *cha-1* gene. Sequence comparison with ChAT from other species confirms that *cha-1* encodes ChAT. Transcription of both transcripts is from right to left on the genetic map, and both share a common 5' untranslated exon at the right end of the region. The *cha-1* transcript then has a very long (~5kb) intron. The *unc-17* transcript has a much shorter intron, and the entire *unc-17* coding sequence lies within the long *cha-1* intron. The two cDNAs have no coding sequence in common.

The open reading frame of the *unc-17* cDNA gives a deduced 58 kd protein with no significant homology to any known protein. The precise nature and function of the *unc-17*-encoded protein are unclear.

Supported by grants from NIGMS and NSF.

609.4

THE EFFECT OF CHLORIDE IONS ON CHOLINERGIC FUNCTION IN RAT HIPPOCAMPAL SYNAPTOSOMES. B.M. Schmidt and R.J. Rylett. Dept. Physiology, University of Western Ontario, London, Canada. N6A 5C1.

Choline acetyltransferase [ChAT; EC 2.3.1.6] catalyses the biosynthesis of acetylcholine (ACh) in cholinergic nerve terminals. Little is known about possible short-term regulation of this enzyme which exists in both cytosolic (cChAT) and membrane-associated (mChAT) pools. Previously, it was shown that rat brain ChAT is regulated *in vitro* by anion concentration, with chloride ions being the most effective (Rossier, J.Neurochem, 29:1007, 1977). These studies were done with the enzyme following extraction from tissue, and it is not yet known whether the two distinct fractions of ChAT in intact tissue are regulated similarly. Using rat hippocampal synaptosomes as a model, we observed that partial replacement of extracellular chloride ions (131 mM reduced to 48 mM) with the largely impermeant anion isethionate selectively reduced the activity of mChAT (61.7±1.9% control, n=7) following 30 min incubation. There was no change in cChAT activity. Further reduction of extracellular chloride to 6mM decreased mChAT to a similar extent (47.4±11.4% control, n=7). High affinity choline uptake (HACU) and concomitant ACh synthesis were also depressed in 6 mM chloride (50.7±7.2 and 43.0±2.7% control, respectively, n=7). To determine whether chloride ions were specifically required for maintaining normal cholinergic function, equimolar concentrations of bromide or iodide ions were also substituted for chloride in the incubation medium. Bromide ions substituted well for chloride in maintaining mChAT activity, HACU, and ACh synthesis (143.8±17.1, 113.1±12.0, and 103.1±16.9% control, respectively, n=6). Results obtained with iodide ions paralled those of isethionate. It appears that of the two distinct ChAT pools in rat hippocampal synaptosomes, only mChAT activity was sensitive to extracellular anion substitution. (Supported by M.R.C. of Canada).

609.5

MICRODIALYSIS REVEALS REGIONAL DIFFERENCES IN RESPONSE TO CHOLINESTERASE INHIBITION BY HEPTYL-PHYSOSTIGMINE.

E. Messamore, U. Warpman*, N. Ogane, and E. Giacobini, Dept. Pharmacol., SIU Sch. Med., Springfield, IL 62794.

Heptyl-physostigmine (HEP) is a long-acting physostigmine (PHY) derivative which inhibits acetylcholinesterase (AChE) and elevates acetylcholine (ACh) concentration in homogenates of the cerebral cortex and striatum (De Sarno et al, 1989). This study examined the effect of systemically administered HEP on extracellular ACh using a microdialysis technique which did not incorporate additional AChE inhibitors in the probe perfusate. Basal ACh concentration in the cortical dialysate of conscious rats was 5.7 ± 0.3 nM which increased by 200% after 1 mg/kg (i.p.) and by 1200% after 5 mg/kg (i.p.) HEP. The observed HEP effects were not altered by the presence of atropine (0.1 μM) in the dialysis probe perfusate. In contrast to the increases noted in cerebral cortex, microdialysis in the striatum did not reveal increased extracellular ACh in response to either dose of HEP. When the dialysis probe contained PHY (10μM), the 5 mg/kg HEP injection decreased extracellular ACh by 20%, despite an overall 60% increase in ACh content of the striatal homogenate. These results do not support a necessary correlation between the ACh content in brain homogenate and the extracellular space. They further suggest marked regional variation in the response to cholinesterase inhibition.

Reference: De Sarno et al, Neurochem. Res. 14(10:971-977, 1989.

609.6

DO CHOLINESTERASE INHIBITORS PREFERENTIALLY INHIBIT CERTAIN BRAIN MOLECULAR FORMS? N. Ogane, E. Messamore and E. Giacobini, Dept. Pharmacol., Southern IL Univ. Sch. Med., Springfield, IL

Aqueous-soluble and detergent-soluble acetylcholinesterase (AChE) molecular forms were separated from rat brain by sucrose density sedimentation. The bulk AChE corresponds to globular tetrameric (G_4) and monomeric (G_1) forms. The effect of eight AChE inhibitors (AChEIs) was studied on separated AChE molecular forms. Five drugs, physostigmine (PHY), echothiophate (ECH), BW284C51 (BW), tetrahydroaminoacridine (THA), and metrifonate (MTF), inhibited both forms of aqueous- and detergent-soluble AChE with similar potency. However, heptyl-physostigmine (HEP) and di-isopropylfluorophosphate (DFP) were more selective for the G_1 forms than G_4 forms in aqueous-soluble extract. Neostigmine (NEO) showed slightly but significantly higher inhibition of G_1 form in both aqueous- and detergent-soluble extracts. These results suggest allosteric effects of AChEIs on globular forms which involve structure-dependent affinities. (Supported by Snow Brand Milk Products Co., Ltd. Japan)

Specificity	HEP	PHY	NEO	ECH	BW	DFP	THA	MTF
$G_1^A > G_4^A$	*	-	*	-	-	*	-	-
$G_1^D.T > G_4^D.T$	-	-	*	-	-	-	-	-
$G_1^A > G_1^A.T$	*	-	-	-	-	-	-	-
$G_4^A > G_4^A.T$	*	-	-	-	-	-	-	-

* $p < 0.05$, significant difference by 2 x 3 ANOVA. - = no difference. A: Aqueous-soluble form. D: Detergent-soluble form. T: Triton X-100 complex

609.7

SARIN-INDUCED NEUROPATHOLOGY IN RATS - A COMPARATIVE STUDY WITH OTHER CHOLINESTERASE INHIBITORS. T. Kadar*, G. Cohen*, R. Sahar*, D. Alkalai*, L. Raveh* and S. Shapira. Dept Pharmacol, IIBR, Ness-Ziona IL 70450, ISRAEL.

Sarin, a highly toxic organophosphate administered at near 1LD50 dose causes persistent brain lesions, the mechanism of which is not fully understood. The present study was designed to evaluate the immediate and long-term neuropathology of a single 1LD50 dose of sarin, and to elucidate the role of excessive acetylcholine in creating these neuronal lesions, by comparing them to other cholinesterase (ChE) inhibitors. Rats surviving 1LD50 dose of sarin, soman or DFP were sacrificed at 1, 7, 30 and 90 days after administration, and their brains were histologically evaluated. While brains of all soman-injected rats were severely damaged, only 70% of the sarin and none of the DFP injected rats were affected. Although the general location of brain neuropathology following sarin resemble that of soman, sarin induced lesions were variable and uniquely affected nerve fibers whereas soman initially damaged only cell-bodies. The different patterns of brain lesions of soman, sarin and DFP indicated a distinctive mode of action for each of them, not mediated solely by ChE inhibition.

609.8

IN VIVO AND IN VITRO INTERACTION OF CARBAMATES WITH QUATERNARY OXIMES G. Cohen*, E. Cohen*# and G. Amitai, IIBR, Ness Ziona 70450, and #Agricul. Faculty The Hebrew Univ., Rehovot 76100, Israel.

The antidotal treatment employed in the case of poisoning by AChE inhibitors usually consists of a combination of atropine and a quaternary oxime (e.g. toxogonin or 2-PAM). However, in the case of carbaryl poisoning toxogonin increases its toxicity by 3.5 fold. AB-8 and AB-13 are quaternary oximes previously shown to be potent antidotes against poisoning by various AChE inhibitors. In the case of carbaryl poisoning in mice, no survival was obtained with either AB-13 (50 mg/kg, i.v.) or toxogonin (12.6 mg/kg, i.v.) in combination with atropine (0.2 mg/kg, i.m.). However, AB-8 (50 mg/kg) protected 80% and 60% of the animals against 3 and 4xLD50 of carbaryl, respectively. In vitro reactivation studies with purified eel AChE showed that toxogonin and AB-13 are 1500 and 500 fold more active than AB-8, respectively. It is, therefore, suggested that oximes which are potent AChE reactivators should be excluded from the antidotal treatment of carba-ryl poisoning. In addition, the mechanism of action of AB-8 may not be solely related to either AChE reactivation or anticholinergic activity.

609.9

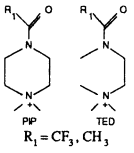
BOTULINUM NEUROTOXIN TYPE A BREAKS DOWN AT pH 5.0 AT THE TWO ASP-PRO BONDS OF THE HEAVY CHAIN. B. R. DasGupta and W. Tepp*. Food Research Institute, University of Wisconsin, Madison, WI 53706.

The neurotoxin (NT) is made of L-chain (residue 1-447) and H-chain (residue 448-1295) linked by an interchain disulfide (J.Biol. Chem. 265, 9153). The NT binds to the cholinergic presynaptic membrane and is internalized by endocytosis. A segment of the N-terminal half of the H chain is thought to form channels in the vesicular membrane after the vesicles become acidic. L-chain or L-chain and a part of H-chain is presumed to escape from the endosome through these channels into the cytosol where the L-chain inhibits neurotransmitter release [J.Physiol. (Paris) 84, 180, 220, 237]. We had reported that the NT breaks down at pH 5, 25°C in 5 min at the Asp 906-Pro 907 bond. We have now exposed the isolated H-chain to pH 5.0 at 25° for 5 min. The 40 and 20 kDa fragments, also found earlier from the NT, gave the N-terminal sequences (Edman degradation): Pro.Ile.Asp.Lys.Asn.Gln.Ile.Gln.Leu.Phe- and Pro.Asn.Lys.Tyr.Val.Asp.Val.Asn.Asn.Val-, respectively. The only two Asp-Pro bonds in the H-chain, residue #906-907 and 1117-1118, are therefore acid labile. Cleavages of the H chain at these two sites may allow segments of the NT containing the L-chain (residue 1-906 or 1-1117) to escape the endosome leaving the rest of the molecule presumed to have the receptor binding site anchored to the receptor. Funded by NIH; NS17742.

610.1

EFFECTS OF SOLUTION STRUCTURE AND DYNAMICS ON THE FUNCTION OF A SERIES OF NICOTINIC CHOLINERGIC AGONISTS. K.A. McGroddy, A.A. Carter, M.M. Tubbert & R.E. Oswald*. Dept. Pharmacology, Cornell Univ., Ithaca, NY 14853.

The binding of a ligand to its receptor, which leads to receptor activation, is dependent upon the structure and dynamics of the agonist both in solution and in the binding site. We have synthesized two classes of chemically similar cholinergic ligands based on the parent compounds shown below in order to determine the contributions of solution structure and dynamics to the ability of these compounds to bind to and activate nicotinic acetylcholine recptors (nAChRs).



The structural and dynamic variations of these compounds have been compared experimentally and theoretically. 1D and 2D ^1H and ^{19}F NMR studies have shown that the TED compounds exist in two stable solution structures of different energy, with a high barrier to interconversion. Only the higher energy conformer appears to achieve the correct conformation for binding to the receptor. The more stable TED structure differs significantly from the rigid PIP structure and this can account for a large portion of the difference in binding affinities between the two compounds. Molecular dynamics simulations have been been carried out using the CHARMm program. The systems have consisted of one ligand in a box of TIP3P water with periodic boundary conditions. Trajectories of each of these compounds have been simulated and analyzed in order to study the effect of solvation on the structures and dynamics of these molecules and to determine the relative contributions of enthalpy and entropy to the interaction of these drugs with nAChRs. These studies indicate that the solution structure and dynamics of a compound can make an important contribution to the binding affinity of a ligand to its receptor.

610.2

DO NONCOMPETITIVE INHIBITORS BIND TO SUBSITES WITHIN NICOTINIC ACETYLCHOLINE RECEPTOR CHANNELS? J.M. Herz and E. Schmid*. Institute for Neuroscience and Cell Research Inst., Univ. of Texas at Austin, Austin, TX 78713.

We have examined the interactions of noncompetitive inhibitor ligands (NCIs) with the allosterically-coupled NCI site on the AChR. To determine whether NCIs bind to a single site or spatially distinct subsites within the channel, effects of a series of NCIs on ethidium binding were analyzed. In the presence of 100 μM carbamylcholine, all of the following NCIs completely displaced ethidium with the indicated K_ds: chlorpromazine (0.15μM), proadifen (0.14μM), meproadifen (0.24μM), PCP(0.52μM), TPMP(0.95μM), QX-314 (20.1μM) and QX-222(494μM). Indirect Hill coefficients for all NCIs were 1.0. Analysis of ethidium's excited state lifetime in the presence of competing NCIs showed that >90% of the total amplitude of the double-exponential decay process was characterized by a short lifetime component of 1.6 ns. This lifetime is attributed to ethidium in buffer indicating that ternary complexes, in which ethidium and another NCI are simultaneously bound, do not form (Herz, et. al., JBC 262, 7238, 1987). Dissociation rates induced by addition of PCP, TPMP and meproadifen were similar, but distinct from chlorpromazine and QX-222. Ethidium association rates in the presence of competing NCIs have been measured. These data will be discussed in terms of competitive mechanisms and subsite models. Supported by AHA Texas Affiliate 89G-401.

610.3

BENZOQUINONIUM(BZQ) INTERACTIONS WITH PERIPHERAL NICOTINIC ACETYLCHOLINE RECEPTORS (AChR): ELECTROPHYSIOLOGICAL AND BIOCHEMICAL STUDIES. T. Tano[1,2], A. Maelicke[3]*, R.S. Aronstam[4] and E.X. Albuquerque[1,2]. [1]Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. of Med., Baltimore, MD 21201; [2]Lab. Mol. Pharmacol.II, IBCCF, 21944; [3]Inst. Physiol. Chem., Johannes-Gutenberg Univ. of Med. Sch., Duesbergweg, D-6500 Mainz, FRG; [4]Dept. Pharmacol. Toxicol., Med. Coll. Georgia, Augusta, GA 30912.

BZQ, a synthetic neuromuscular blocking agent (*J. Am. Chem. Soc.*, 72: 2661, 1950), has been proposed to block the physostigmine (PHY) agonistic action on the AChR complex from *Torpedo* (Okonjo et al.; *in press*). In this work, further characterization of the BZQ effects at the AChR was done by electrophysiological studies in *Rana pipiens* muscles and ligand-binding assays in *Torpedo* membranes. Voltage-clamp was performed on sartorius muscle-sciatic nerve preparation, and ACh-activated single channel currents were recorded from interosseal fibers using cell-attached modality. At the neuromuscular junction, BZQ (10 and 30 μM) depressed endplate currents (EPC) peak amplitude and shortened the τ_{EPC} in a voltage- and concentration-dependent manner. In addition, analysis of ACh-activated single channel currents showed that BZQ (3 and 10 μM) decreased the mean open (τ_o) and burst (τ_b) times. At -140 mV, 3 and 10 μM BZQ decreased both τ_o and τ_b by 44% and 80% of control values, respectively. In agreement with the EPC data, these effects were also enhanced at hyperpolarized potentials. At 1 μM BZQ, [^{3}H]histrionicotoxin binding was enhanced by 30% in the absence of agonist, however it was reduced at higher concentrations. ^{125}I-α-bungarotoxin binding was also reduced at higher concentrations of BZQ. These results suggest that BZQ has complex effects at the AChR: binding studies suggest that BZQ at concentrations < 1 μM might activate channel openings; however, binding and electrophysiological data show that higher concentrations of BZQ block the nicotinic channel in the open conformation. Support: U.S. Army Med. Res. Dev. Com. Contr. DAMD-17-88-C-8119 & NIH #P50-MH44211.

610.4

EXPRESSION OF ALPHA-BUNGAROTOXIN-INSENSITIVE FUNCTIONAL NICOTINIC ACH RECEPTORS IN *XENOPUS* OOCYTES. V. M. Gehle and K. Sumikawa. Dept. of Psychobiology, Univ. Calif., Irvine, CA 92717.

A conserved feature of many ligand-gated ion channels, including the muscle and neuronal ACh receptors, GABA receptor, and glycine receptor, is a pair of cysteine residues separated by 13 amino acids in the extracellular portion of the protein. In the *Torpedo* AChR α subunit, this pair has been demonstrated to form a disulfide bond. This disulfide bond may be important for receptor function because removal of the two cysteines from the AChR α subunit was demonstrated to affect the ligand binding and ACh response of the AChR (Mishina et al., 1985, Nature 313: 364-369). That report indicated the cysteines are critical for AChR function, but did not precisely define the role they perform. We created the same mutant α subunits, having one or the other of the cysteines replaced by serine, for additional detailed studies. Both of the mutant α subunits assembled with β and γ subunits when expressed in *Xenopus* oocytes. The mutant α subunits also assembled with δ subunits, but with greatly decreased efficiencies. Additionally, electrophysiological studies of these oocytes using voltage-clamp techniques documented small responses to ACh. These responses could not be blocked by application of α-BuTX. Thus, formation of a disulfide bond between Cys128 and Cys142 of the *Torpedo* AChR α subunit is necessary for α-BuTX binding, but not ACh binding and ion channel function.

610.5

EFFECT OF SUBSTITUTION OF *TORPEDO* ACETYLCHOLINE RECEPTOR (AChR) α-SUBUNIT RESIDUES WITH SNAKE RESIDUES ON BINDING OF α-BUNGAROTOXIN (α-Btx). D. L. Donnelly-Roberts*, V. Chaturvedi*, and T. L. Lentz. Department of Cell Biology, Yale University School of Medicine, New Haven, CT 06510.

A fusion protein consisting of the Trp E protein fused to residues 166-211 of the AChR α-subunit was produced in *E. coli*. A cDNA clone encoding *Torpedo* AChR α-subunit (gift of Toni Claudio) was digested with restriction enzymes to yield a 137 base pair fragment encoding residues 166-211. This fragment was fused to the 3' terminus of the *E. coli* TrpE gene using a pATH10 expression vector. Whereas *Torpedo* AChR binds α-Btx with high affinity, AChR from snakes are toxin-resistant (Burden *et al.*, PNAS 72, 3245, 1975). Three residues in the *Torpedo* sequence were mutated to residues present in snakes (Neumann *et al.*, PNAS 86:7255, 1989) in order to assess the role of these residues in binding. The 137 base pair *Torpedo* fragment was subcloned in an M13 vector and mutagenesis performed by means of oligonucleotide-directed mutagenesis. The following mutations were produced in the *Torpedo* sequence: W184F, K185W, and W187S. The affinities of the fusion proteins for ^{125}I-α-Btx were compared. Competition with unlabeled α-Btx yielded IC_{50} values of 5.5×10^{-8}M and 1.6×10^{-5}M for the *Torpedo* and mutated proteins respectively. Equilibrium saturation binding experiments yielded K_Ds of 6.0×10^{-8}M for the *Torpedo* sequence and 1.7×10^{-5}M for the mutated sequence. Conversion of three *Torpedo* residues to snake residues causes a 500-fold decrease in affinity of the peptides for α-Btx. Therefore, these results suggest that one or more of the residues at positions 184, 185, and 187 of the AChR α-subunit plays an important role in the binding of α-Btx. Supported by NIH grant NS 21896.

610.6

A MUTANT GAMMA SUBUNIT OF *TORPEDO* ACHR CAUSES DEGRADATION OF CO-EXPRESSED NORMAL SUBUNITS. K. Sumikawa and V. M. Gehle. Dept. of Psychobiology, Univ. Calif., Irvine, CA 92717.

Using site-directed mutagenesis, we changed the asparagine residue at the conserved N-glycosylation site (position 141) on the γ subunit of the *Torpedo* acetylcholine receptor (AChR) to aspartic acid. When these mutant subunits were expressed in *Xenopus* oocytes along with non-mutant α, β, and δ subunits (mγ-AChR), very little ^{125}I-α-bungarotoxin (α-BuTX) binding activity was seen, but, interestingly, α-BuTX binding was easily detected in *Xenopus* oocytes expressing only α, β, and δ subunits. When ^{35}S-methionine labeled mutant and normal subunits were precipitated with AChR antibodies and analyzed on SDS gels, we could detect neither the mutant γ subunit nor most of the co-expressed other normal subunits. Since the incomplete receptor consisting of only the α, β, and δ subunits could be precipitated easily from other oocytes, the mutant γ subunit was likely to be responsible for the apparent absence of the co-expressed non-mutant subunits. Oocytes expressing the α, β, and δ subunits produced larger membrane currents in response to ACh than those elicited in oocytes expressing the mγ-AChR. This apparent mγ-AChR response may have been due to a low-level expression of AChRs consisting of only the α, β, and δ subunits and not AChRs containing the mutant γ subunit. These results suggest that the mutant γ subunit assembled with the other normal subunits, leading to the subsequent degradation of all the subunits.

610.7

MODULATION OF NICOTINIC ACETYLCHOLINE RECEPTOR ION CHANNEL FUNCTION BY THE M4 TRANSMEMBRANE DOMAINS. L. Li*, Y-H. Lee*, and M. G. McNamee. Dept. of Biochem. & Biophys., Univ. of California, Davis, CA 95616.

The role of M4 transmembrane helices in nicotinic acetylcholine receptor (AChR) channel function was investigated by studying the functional consequences of the site-specific mutations of cysteine residues in the M4 helices of *Torpedo californica* AChR α and γ subunits using *Xenopus laevis* oocytes injected with *in vitro* synthesized AChR RNA transcripts. The mutation of αCys418 to Trp increased the AChR *normalized* channel activity (measured as the ACh-induced conductance per femtomole of surface α-bungarotoxin (α-BGT) binding sites) by more than 40 fold. In contrast, the mutation of γCys451 to either Ser or Trp decreased the normalized channel activity by about 50%. The surface ^{125}I-α-BGT binding assay and the Western blot analysis of the oocyte membrane preparation revealed that these mutants had the same expression level as the wild type receptor. These mutant AChRs displayed a linear current-voltage relationship with a reversal potential of around 0 mV similar to that of the wild type receptor, suggesting that these mutations did not alter the selective permeability of the AChR channel for cations. Moreover, these mutations did not change other AChR functional properties such as agonist binding ability, the slow phase of desensitization, and boockade by competitive and noncompetitive antagonists. These results suggest that M4 transmembrane domains can modulate AChR ion channel function, possibly by altering channel gating. (Supported by USPHS Grant NS 22941).

610.8

LOCALIZATION OF ACETYLCHOLINE RECEPTORS SUBUNITS MRNA IN CULTURED MYOTUBES. X.SU*,S.Berman,and S.Bursztajn. Mailman Research Center, Harvard Medical School, Boston, MA 02178

One of the hallmarks of neuromuscular synapse formation is the accumulation of the acetylcholine receptors (AChRs). The AChRs are composed of five different subunits, two of which are identical. Our previous studies have shown that the levels of AChRs α mRNA vary greatly from one nucleus to another in cultured non-innervated myotubes. In the present study we have addressed the question whether the other subunits also exhibit a heterogenous distribution of mRNA. For this purpose we have prepared intron/exon, or intron probes. Since the γ and δ subunits have a high degree of homology to one another, we have used computer analysis to obtain least homologous DNA sequences. We isolated specific DNA regions by the polymerase chain reaction (PCR) and ligated the synthesized DNA into Bluescript plasmid (SKII+). The ligated DNA was amplified by PCR in the presence of 5′ primer and the universal primer to produce extended DNA sequences containing a multicloning site and universal primer region at a defined end for use in further labelling and cloning procedures. We made 35S labeled probes via a modified previously described primer extension method, checked probe specificity by dot blot assay, and hybridized the myotubes with 35S labeled AChR α, γ, and δ subunits. Using nuclear track emulsion autoradiography and combined dark field/fluorescent microscopy we observed a heterogenous distribution of silver grains over nuclei of the same myotube for the AChR α and δ subunits. The γ subunit is being analyzed. Our results show differential activation of myotube nuclei for the AChR subunits. (Supported by NIH and MDA grants).

610.9

MUSCLE ACTIVITY SUPPRESSES EXPRESSION OF EMBRYONIC-TYPE NICOTINIC ACETYLCHOLINE RECEPTOR (nAChR) GENES VIA A CYCLIC AMP SECOND MESSENGER SYSTEM. K. G. Chahine*, J. Staple, L. Vanover, W. Walke* and D. Goldman. Mental Health Research Institute and the Department of Biological Chemistry, University of Michigan, Ann Arbor, MI 48109.

During development of the neuromuscular junction there is a switch in expression of nAChR genes from an embryonic- ($\alpha_2\beta\gamma\delta$) to an adult-type ($\alpha_2\beta\varepsilon\delta$). Embryonic-type genes are expressed throughout the muscle fiber prior to innervation. However, after innervation, extrajunctional expression of these genes is suppressed by nerve induced muscle activity. We investigated the mechanism by which this repression occurs by using a rat primary muscle culture system that responded to muscle activity (induced by stimulation with extracellular electrodes) in a similar manner as observed in vivo. We found that increasing intracellular cAMP reversed the effects of muscle activity on embryonic-type nAChR gene expression. Genes not regulated by muscle activity, such as those encoding the nAChR ε-subunit and creatine kinase, were not induced by increasing cAMP. Consistent with these results was the finding that muscle inactivity, induced in vitro by tetrodotoxin or in vivo by muscle denervation, caused an increase in cAMP and protein kinase A activity prior to inducing nAChR gene expression. In order to define the cis-acting DNA sequences conferring electrical sensitivity to the embryonic-type nAChR genes we have cloned the rat δ-subunit gene promoter and have used it to drive expression of the luciferase structural gene in transfected cultured muscle cells. These experiments have identified a 950 bp fragment that confers electrical regulation on expression of the luciferase reporter gene. Deletion analysis, band shift assays, and DNA footprinting are being used to characterize the cis-acting element(s) conferring electrical sensitivity to this promoter.

610.10

STABILIZATION OF ACETYLCHOLINE RECEPTORS IN ANEURAL RAT MUSCLE CULTURES BY CYCLIC AMP ANALOGUES J.P.O'Malley*, M.L. Shelanski & L.L.Rubin, Department of Pathology, Columbia Univ., New York, NY 10032.

The stabilization of AChRs at the neuromuscular junction (nmj) occurs at birth when the receptor's half life increases from the fetal value of approximately 1 day to the mature value of 7-10 days. Denervation has been shown to partially reverse AChR stabilization and results in the expression of two populations of AChR, one with a rapid turnover of 1 day (R_F AChRs) and another with a slower turnover of approximately 4 days (R_S AChRs). Recently it has been shown that agents which elevate cAMP can stabilize the R_S AChRs at the nmj of denervated muscle to a level similar to that of adult-type AChRs found in normal muscle ($t_{1/2}$~7 days). We wanted to determine if these agents were also able to stabilize AChRs in aneural muscle cell cultures, which normally only express fetal-type AChRs with a half-life of 1 day.

We report that cells treated for 4 days with 250 μM 8-(4-chlorophenylthio)-cAMP, 1 mM Dibutyryl-cAMP or 10 μM forskolin express two populations of AChR, one with a half-life of 1 day and another with a half life of approximately 12 days and which constitute approximately 75 % and 25 % of the total receptor population respectively. However if the period of treatment was shortened to less than 4 days the stable AChR population was not seen.

It would be interesting to know if the receptors which have a half-life of 12 days share other properties, such as channel kinetics, with adult-type AChRs in normal muscle and if they are thus physiologically equivalent to the stable AChRs of adult muscle.

610.11

TRANSCRIPTIONALLY-MEDIATED REGULATION OF NICOTINIC ACETYLCHOLINE RECEPTOR EXPRESSION IN HUMAN CLONAL CELLS. Ronald J. Lukas and Linda Lucero. Division of Neurobiology, Barrow Neurol. Institute, Phoenix, AZ 85013.

Chronic treatment of TE671/RD human cells with phorbol 12-myristate-13-acetate (PMA) or with nicotinic agonists induces upregulation of muscle-type nicotinic acetylcholine receptors (nAChR). Only the PMA effect is blocked by cycloheximide or the topoisomerase II inhibitor, novobiocin, suggesting a requirement for protein and RNA synthesis. Northern blot analysis of mRNA in PMA-treated cells (but not in control cells or in cells treated with nicotinic ligands or dibutyryl cyclic AMP) indicates that there are coordinate and sustained (for at least 5 days) increases in levels of transcripts corresponding to human muscle-type nAChR alphal, betal, gamma and delta subunit genes within 6 hours of drug application. Treatment with sodium butyrate, which induces nAChR downregulation alone or in the presence of PMA, induces a transient (at 6 and 24 hours) decrease in nAChR gene transcript levels that is followed by a return to control levels at 3 days of drug exposure. Other studies done using cells of the SH-SY5Y human neuroblastoma clonal line indicate that chronic PMA treatment (but not treatment with nicotinic ligands, nerve growth factor, or dibutyryl cyclic AMP) also induces increases in neuronal nAChR alpha3, beta2 and beta4 subunit mRNA levels. Thus, chronic PMA treatment can activate transcription of muscle-type and neuronal nAChR genes.

610.12

UP REGULATION OF ACETYLCHOLINE RECEPTORS IN THERMAL INJURY J.M.Ward and J.A.J.Martyn*. Dept. Anaesthesia, Harvard Medical School and Anesthesia Services of Massachusetts General Hospital and Shriners Burns Institute, 51, Blossom Street, Boston MA 02114.

Severe burn injury results in dramatic changes in human pathophysiology which can result in many pharmacotherapeutic difficulties. Important sequelae in muscle include weakness and respiratory muscle failure and an altered response of peripheral nicotinic acetylcholine receptors (nAChR) to neuromuscular relaxant type drugs used clinically.

In thermally injured rats using a single, saturating concentration of [125I] alpha-bungarotoxin (a-BGT), we have demonstrated an increase of a-BGT binding to nAChR extracted from gastrocnemius muscle with respect to control (P=0.05 ANOVA oneway). This result has been confirmed by Scatchard analysis which also revealed that the Kd in the thermally injured animals was lowered.

We propose that the altered responses to neuromuscular relaxant type drugs are due, in part, to a decrease in affinity which promotes up-regulation of the nAChR.

610.13

INNERVATION OF MYOTUBES REGULATES TYROSINE PHOSPHORYLATION OF THE NICOTINIC ACETYLCHOLINE RECEPTOR. Z. Qu and R.L. Huganir, Dept. of Neuroscience, Howard Hughes Medical Inst., The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205

The nicotinic acetylcholine receptor (nAChR) is highly phosphorylated on tyrosine residues in vivo. Previous studies have shown that denervation of muscle decreases tyrosine phosphorylation of the AchR suggesting that innervation of muscle regulates phosphorylation of the AChR. To examine more directly the role of innervation in the stimulation of tyrosine phosphorylation of the AChR, we have used neuron-muscle cell cultures. Immunocytochemical staining with anti-phosphotyrosine antibodies show that AChR clusters at synapses between nerve and muscle contain high levels of phosphotyrosine. In addition, neuronal innervation of muscle cell cultures increased tyrosine phosphorylation of the AChR as analyzed by immunoblot techniques with anti-phosphotyrosine antibodies or by directly examining the ^{32}P-incorporation into the AchR. Recent results have suggested that the neuronal extracellular matrix protein, agrin, which is thought to be released from the neuron and induce clustering of the AChR, may be the factor from the nerve that regulates tyrosine phosphorylation. These results suggest that tyrosine phosphorylation may mediate nerve induced clustering of the AChR at the neuromuscular junction.

610.14

EXPRESSION OF THE NEURONAL AND A NOVEL FORM OF FYN PROTEIN TYROSINE KINASE IN TORPEDO CALIFORNICA ELECTRIC ORGAN S.L. Swope, K.R. Wagner, E. Moritz*, and R.L. Huganir, Dept. of Neuroscience, Howard Hughes Medical Inst., The Johns Hopkins Univ. Sch. of Med., Baltimore, Md. 21205

Phosphorylation of the nicotinic acetylcholine receptor (AchR), by protein kinase A, protein kinase C, and an unidentified protein tyrosine kinase(s) appears to regulate the rate of desensitization as well as clustering of the AchR at the neuromuscular junction. The intent of the present study is to identify, by molecular cloning, tyrosine kinase(s) involved in the phosphorylation and modulation of the AchR. Oligonucleotides based on conserved subdomains of the tyrosine kinase src were used in the polymerase chain reaction (PCR) with cDNA prepared from Torpedo californica electric organ, a tissue highly enriched in AchR. PCR products which encoded tyrosine kinase fragments were generated. The cloned fragments were used to screen a λgt10 library prepared from electric organ. Two distinct clones, Torpedo Tyrosine Kinase (TTK) I and II, were identified and shown by Northern analysis to be highly expressed in electric organ. The nucleotide sequence of TTKI encoded a 61.2KD protein which was most homologous to the neuronal form of FYN tyrosine kinase whereas TTKII encoded a 60.8KD novel form of FYN. The role of TTKI and/or TTKII in modulating the AchR is under investigation.

610.15

DEPHOSPHORYLATION OF NICOTINIC ACETYLCHOLINE RECEPTOR: PURIFICATION AND CHARACTERIZATION OF THE PROTEIN TYROSINE PHOSPHATASE. L. Mei and R.L. Huganir. Dept. of Neuroscience, Howard Hughes Medical Inst., The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

Tyrosine phosphorylation has been shown to regulate the functional properties of the nicotinic acetylcholine receptor (nAChR). The level of tyrosine phosphorylation of nAChR is regulated through a balance between tyrosine phosphorylation by protein kinases and phosphotyrosyl dephosphorylation by protein phosphatases. We have purified and characterized a protein tyrosine phosphatase that dephosphorylates tyrosine-phosphorylated nAChR from Torpedo electroplax. The [^{32}P]-labeled tyrosine phosphorylated nAChR was used as a substrate to monitor the enzyme activity during purification. The protein tyrosine phosphatase activity was purified using three consecutive cation-exchanger columns (phosphocellulose, S Sepharose Fast Flow, BioRex 70), followed by two affinity matrices (p-aminobenzylphosphonic acid-agarose and thiophosphotyrosyl nAChR-Sepharose 4B). The enzyme activity was purified to homogeneity, with an overall purification of 25,000-fold and a yield of 20 %. The purified enzyme had an apparent molecular weight of 43 kDa on SDS polyacrylamid gels and migrated as a monomer during Superose 12 chromatography. It had a neutral pH optimum and a specific activity of 18 μmol/mg protein/min., with a Km of 4.7 μM for tyrosine phosphorylated nAChR. The phosphatase was specific for tyrosine phosphorylated nAChR; it showed no activity towards the nAChR phosphorylated on serine residues by cAMP-dependent protein kinase. The tyrosine phosphatase had unique sensitivities to inhibitors and metal ions, suggesting that it may be a novel protein tyrosine phosphatase that dephosphorylates the nAChR and other neurotransmitter receptors. We have recently obtained protein sequence of tryptic peptides of the purified phosphatase and are currently attempting to isolate cDNA clones of the protein tyrosine phosphatase.

610.16

STAUROSPORINE INHIBITION OF RECOVERY FROM DESENSITIZATION IS REVERSED BY CALCIUM AT SNAKE TWITCH FIBER ENDPLATES. J.C. Hardwick and R.L. Parsons. Dept. of Anat. & Neuro., Univ. of Vermont, Burlington, VT 05405. We showed previously, at snake nicotinic acetylcholine receptors, that the recovery of receptor sensitivity to agonist following desensitization was inhibited by pretreatment with the protein kinase inhibitor, staurosporine (Hardwick & Parsons (1990) Neurosci Abstr 16:205). These findings suggested that recovery of the desensitized receptor involved protein phosphorylation. We demonstrate here that elevation of the external calcium concentration during agonist application, which results in an increase in calcium influx through agonist-activated endplate channels, reversed the inhibition of the extent of recovery produced by staurosporine. Garter snake *(Thamnophis)* twitch fibers were maintained in an isotonic potassium propionate solution and individual endplates voltage clamped to +30 mV. Desensitization was produced by microperfusion with 540 μM carbachol containing either 1 mM or 10 mM Ca^{++} for 2-3 minutes. Recovery was determined by measuring the reappearance of spontaneous miniature endplate currents (MEPCs) during the wash period. Elevation of the external calcium concentration during the carbachol application completely antagonized the inhibition of recovery produced by pretreatment with 0.5 μM staurosporine. Elevation of external calcium in the absence of agonist increased the frequency of MEPCs but did not alter their amplitude or decay time course in either control or staurosporine treated fibers. Elevation of external calcium also increased the rate of desensitization onset of the initial carbachol-induced current in both control and staurosporine-treated fibers. However, the subsequent acceleration of desensitization seen with a second application of carbachol was less in staurosporine-treated fibers as compared to controls. (Supported by NS 08580 (JCH), NS 25973 (RLP) and an MDA grant (RLP)).

ACETYLCHOLINE RECEPTORS: MUSCARINIC IV

611.1

ISOLATION AND CHARACTERISATION OF GENOMIC ELEMENTS REGULATING THE EXPRESSION OF THE CHOLINERGIC MUSCARINIC m4 GENE. I.C.Wood*, C.E.Stansfeld and N.Buckley*. Dept Pharmacology, University College London, London WC1E 6BT.

There are five genes (m1-m5) encoding cholinergic muscarinic receptors. Each of the receptors has a characteristic pharmacological profile and the mRNA coding for each receptor has a unique distribution within the mammalian brain. To investigate genomic cis elements responsible for this transcriptional control, genomic clones, which contain the coding region for the m4 gene, have been isolated from a rat cosmid library. As transcriptional regulatory elements are usually located 5' to the transcription initiation site, work has concentrated on one particular clone which contains approximately 25kb of upstream sequence from the coding region.

Since a full length cDNA clone for the m4 gene is unavailable to aid in the location of the transcription initiation site within the genomic clone, primer extension and PCR techniques have been used in an attempt to identify this sequence in the clone. Complementary to this, DNA fragments upstream from the coding region have been subcloned into a CAT reporter plasmid, ptkAGPT, for transfection into cell lines. These lines include those that express endogenous m4 receptors, *e.g.* NG108-15, PC12 and those that do not express m4 receptors *e.g.* CHO. The use of these cell lines should identify those promoter elements which are m4 specific.

611.2

REGULATION OF CARDIAC mAChR EXPRESSION AND FUNCTION BY INSULIN. M.E. Morton and N.M. Nathanson. Department of Pharmacology, University of Washington School of Medicine, Seattle, WA 98195.

Muscarinic acetylcholine receptors (mAChRs) coupled to guanine nucleotide regulatory-binding proteins (G-proteins) are part of an important signal transduction pathway in cardiac tissue. Alterations in the number and/or function of cardiac mAChR have been observed in various animal models of diabetes, suggesting that insulin may regulate mAChR in the heart. Here we demonstrate that insulin regulates muscarinic acetylcholine receptor (mAChR) number, function, and mRNA levels in embryonic chick heart cells cultured in serum-free, defined medium. In embryonic chick heart cultures, insulin regulates expression of mAChR protein and mRNA in a concentration dependent manner. Increasing concentrations of insulin [0.01 nM-2000 nM] caused a 60% decrease in the number of mAChR binding sites. Similar changes in mRNA levels of both cm2 and cm4 were also measured by solution hybridization analysis, although cm2 was more strongly regulated. Insulin-like growth factor type I mimics the effects of insulin on mAChR protein expression in these cultures. Carbachol-mediated inhibition of forskolin-stimulated cAMP accumulation in these cultures was measured in order to determine if the change in mAChR binding sites was accompanied by a change in functional responsiveness. Chick cardiomyocytes cultured in the presence of higher concentrations of insulin (therefore expressing lower numbers of mAChR), are less responsive to carbachol than cells cultured in low concentrations of insulin. These data demonstrate that insulin directly influences cardiac mAChR function, protein and mRNA expression and suggests that regulation of mAChR expression and function may contribute to the cardiomyopathies associated with diabetes.

611.3

DEVELOPMENT OF THE MUSCARINIC ACETYLCHOLINE RECEPTOR IN THE CHICK RETINA. M.P. Lambert, S. Aguilar, M-F. Aguilar, J. Sullivan*, J. Bergin*, and W. L. Klein. Northwestern University, Institute for Neuroscience, Evanston, IL 60025.

In an effort to understand the developmental control of cell specific surface proteins in the CNS, our laboratory has utilized the muscarinic acetylcholine receptor (mAChR) as a model system. Previous work has shown that there are two molecular weight forms of the mAChR in the chick retina, the ratio of which changes as a function of embryonic development (Large, et al.,1985, PNAS, 82:8785). To determine the source of these two forms, we began to probe the chick CNS with a 516 bp fragment of the rat m1 mAChR gene (generously provided by T. Bonner). The fragment recognizes muscarinic sequences in the chick as indicated by hybridization with several bands of an Eco I and Bam HI digest of chick genomic DNA. Screening of an E13 chick brain cDNA library (expressed in lambda gt11) with the m1 probe yielded several clones. We selected one to sequence, based on its hybridization at high stringency and size of 2.3 kb. This 2.3 kb fragment was subcloned into pGEM 7Z and partially sequenced using the method of nested deletions supplemented with synthetic oligonucleotide primers. It was found to be 98% identical in the open reading frame to the sequence of the muscarinic gene found in chick heart (cm4, Tietje, et al., 1990, J.Biol. Chem. 265:2828). The sequence of the 3' untranslated end is presently being evaluated. The 2.3 kb fragment was used to probe the developmental appearance and control of the mAChR message in retina. Preliminary data indicate that the one species of mRNA which hybridized with the probe at high stringency varies with developmental age, attaining a peak near E15, the time of maximum synaptogenesis. Furthermore, in cultured cells, a 4 hour exposure to muscarinic agonist results in an increase of the mAChR message, indicating a coupling between receptor activity and gene regulation.

611.4

Development of muscarinic receptor proteins in the forebrain of the Balb/CByJ mouse. E. Potter, A. Levey, C.A. Kitt, D. Price, J.T. Coyle and C.F. Hohmann. Depts. of Psychiatry, Neuropathology and Neuroscience, The Johns Hopkins University, Baltimore, Maryland.

Five different muscarinic cholinergic receptor genes are expressed in the CNS and at least 3 of these can be immunocytochemically detected in the forebrain of the adult rat. Here we show the ontogenetic time course and distribution pattern of these three subtypes m1, m2 and m4 in the mouse.

Antisera were raised against fusion proteins specific for each receptor subtype and purified by affinity chromatography. Immunohistochemistry was performed in brains aged postnatal day [PND] 5, 7/8, 14, 21, 30 and adult (2-4month). Immunoreactivity for m1,m2 and m4 was diffusely distributed in the neuropil at PND5 and 7 and only thereafter became associated with neuronal somata in some areas. The neuropil distribution pattern of all three antibodies changed with maturation, particularly in neocortex, hippocampus, striatum and thalamus. In addition, localization of m1 and m4 to fiber tracts, such as the corpus callosum, fimbria fornix and descending cortico-bulbar systems abated following the first postnatal week. Patterns of m1 and m4 immunoreactivity were similar but not identical. At all ages m2 displayed a distribution pattern virtually complimentary to both m1 and m4. Immunostaining intensity for all antisera appeared to increase postnatally until the end of the first month and thereafter declined.

Ontogenetic pattern changes, for all muscarinic receptor subtypes, appeared closely linked to the maturational schedule of each brain area. Thus the present results are compatible with a role of cholinergic receptors in the regulation of forebrain development.

611.5

ONTOGENIC PROFILE OF ACETYLCHOLINE MUSCARINIC RECEPTORS QUANTIFIED BY PCR ANALYSIS. U di Porzio, F Morelli*, N J Buckley*$ and M C Steel*$. International Institute of Genetics and Biophysics C.N.R. Naples, Italy and $National Institute for Medical Research Mill Hill, London, U.K.

Recent molecular cloning studies have demonstrated the existence of five acetylcholine muscarinic receptor genes, differentially distributed and pharmacologically distinct. We examined the ontogenic expression of m_1-m_5 receptor gene by the Polymerase Chain Reaction (PCR) method. RNA from foetal and newborn rat brains, from selected brain regions, or from primary cultures generated from the various CNS areas was amplified by the PCR using specific sets of primers designated to recognize each individual receptor subtype. Cycle conditions were optimized to permit coamplification of receptor reverse transcribed RNA and that of hypoxanthine phosphoribosyltransferase which was included as a constitutively expressed internal standard. The signals were quntifyed by densitometric analysis using a^{32}p-dATP. This technique revealed that each receptor subtype displays a unique pattern of expression during ontogeny: m_1, m_3 and m_5 are apparently expessed aroud the time of birth. m_2 and m_4 can be detected as early as E13. In vitro they also show caracteristic expression profiles, which might be modulated by different culture conditions. This suggests a controlled regulation of muscarinic receptor gene expression. There may be common regulatory mechanisms for the members within the two groups, the nature of which requires to be examined further.

611.6

POSTNATAL EXPRESSION OF MUSCARINIC RECEPTORS IN RAT SEPTUM AND HIPPOCAMPUS. J.B.Suszkiw and J.L.Tomsig
Dept. Physiol. and Biophys. Univ. of Cincinnati Sch. of Med. Cincinnati, OH 45267.

Since it has been reported that the septo-hippocampal projection neurons are sensitive to atropine, the objective of this study was to determine whether or not the expression of muscarinic receptors correlates with functional maturation of the septo-hippocampal pathway i.e., the development of hippocampal theta activity. In rat, the hippocampal theta activity appears at postnatal day 10 and attains adult characteristics within two weeks thereafter.

[^{3}H]QNB binding was measured in homogenates of rat septa and hippocampi isolated from postnatal day 1 through 31 (PN1-PN31) and adult (3 months) animals. In adult animals, the concentrations of high affinity (K_D @ 50 nM) QNB binding sites were similar in septal (551±99 fmol/mg P) and hippocampal (487±34 fmol/mg P) homogenates. The QNB binding capacity of PN1 septum was @ 20% of adult and reached maximal levels between PN14 and PN21. QNB binding in PN1 hippocampus was < 10% of adult, increased to 75-85% between PN14-PN21, and attained adult levels by PN31.

These results suggest that postnatal expression of muscarinic receptors in both septum and hippocampus may be an important parameter in the development of atropine-sensitive, hippocampal theta activity. Supported by BRSG S07RR05408-29 and University Research Council.

611.7

DIFFERENTIAL REGULATION OF m1-m5 MUSCARINE ACETYLCHOLINE RECEPTOR GENE EXPRESSION IN THE RAT CNS DURING ONTOGENY. S. Vincent, L. Tsiokas and M. Watson. Department of Pharmacology, UMDNJ-NJ Med. Sch., Newark, NJ 07103-2714.

Data from expression cloning studies reveal five muscarinic receptor (mAChR) subtypes. We studied postnatal ontogeny in brain sections from rats at postnatal days 1,7,14,21,28,35 and adult (3mo) to map development using adjacent 10um sections for quantitative ligand autoradiography and in situ hybridization histochemistry (ISHH). LiCl was used in cerebral cortical slices (350x350um) to study inositol phospholipid (IP) hydrolysis by prelabeling with myo[-2-3H]inositol (0.5uM) in Krebs PO4 buffer (95%O_2,5%CO_2). [3H](-)quinuclidinylbenzilate, a very specific nonsubtype selective antagonist, [3H]cismethyl-dioxolane, a label for the super high affinity agonist state, [3H]pirenzepine, an M1 antagonist, [3H]AF-DX 384, an M2 antagonist and [3H]hemicholinium-3, a Na-dependent high affinity choline uptake inhibitor, were each used as has been previously described to obtain quantitative autoradiograms. Quantitative ISHH data was obtained via 3'-end labeled oligonucleotide probes (^{35}S-dATP;>1,000 Ci/mmol) complementary to 4-48 or 4-51 base sequences of m1-m5 mAChR after verifying specificity by Northern blot analysis. Slices were incubated (25°C,24hr), washed (Tm= 55°C;0.5xSSC), dried, apposed (~4wk;0-4°C) to Hyperfilm-ßmax and quantified via DUMAS densitometer. Differential progressive increases occur during ontogeny. (MH-43024).

611.8

ALTERATIONS IN CORTICAL MUSCARINIC RECEPTOR SUBTYPE mRNAs FOLLOWING KAINIC ACID LESION OF RAT NUCLEUS BASALIS MAGNOCELLULARIS. Z. Zang and Ian Creese. Center for Molecular & Behavioral Neuroscience, Rugters, The State University of New Jersey, Newark, NJ 07102.

The cholinergic deficit seen in Alzheimer's disease (AD) is associated with cells loss in the nucleus basalis magnocellularis (nBM). Previous studies have shown that a major portion of the cholinergic projection to the cerebral cortex is derived from the nBM. This cholinergic input can be destroyed in rat by kainic acid microinjection into the nBM. The present studies examined the expression of muscarinic receptor subtype (m1, m2, m3, m4 and m5) mRNAs by solution hybridization/RNase protection analysis in rat frontal cortex following unilateral kainic acid lesion of the nBM. The absolute quantification of muscarinic receptor mRNAs in frontal cortex of rats indicated high levels of m1 mRNA (3.31 ± 0.28 pg/per µg total RNA), while the levels of m3 or m4 mRNAs were relatively lower (m3: 0.93 ± 0.13 pg/per µg total RNA; m4: 1.09 ± 0.10 pg/per µg total RNA). Almost no m2 or m5 mRNA was detected. 1 week or 3 weeks following unilateral injection (1.5 µl) of kainic acid (3 µg), we found that the m1 receptor mRNA on the nBM-lesioned side, in comparison to the unlesioned side, was significantly decreased (1 week -24.4%, 3 weeks -26.3%). The level of m4 receptor mRNA was decreased by 28.2% at 1 week, and by 18.2% at 3 weeks post lesion. However, the m3 receptor mRNA was not significantly affected by lesion. Elevation of m1 receptor mRNA has been reported in Alzheimer's brain. These results suggest that other pathogenic mechanisms are involved in AD, in addition to the cholinergic denervation, that regulate muscarinic receptor gene expression.

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611.9

CHRONIC SCOPOLAMINE ADMINISTRATION DIFFERENTIALLY ALTERS REGULATION OF m1-m5 CNS MUSCARINE ACETYLCHOLINE RECEPTOR GENE EXPRESSION IN AGED BUT NOT YOUNG RATS. M. Watson, S. Vincent, E.A. Tolomeo and L. Tsiokas. Dept. of Pharmacol. UMDNJ- N.J. Medical School, Newark, NJ 07103-2714.

Prolonged drug treatment (Rx) may up- or down-regulate CNS muscarine acetylcholine receptor (mAChR) subtype densities. Using previously described methods, quantitative autoradiograms were obtained for the nonsubtype selective antagonist [^{3}H](-)quinuclidinylbenzilate, an agonist to bind the super high affinity state, [^{3}H](+)cismethyldioxolane, an M1 antagonist [^{3}H]pirenzepine, an M2 antagonist, [^{3}H]AF-DX 116, and an inhibitor of Na+-dependent high affinity choline uptake, [^{3}H]hemicholinium-3, with adjacent 10uM CNS sections. Quantitative in situ hybridization histochemistry (ISSH) was done via oligonucleotide probes complementary to 4-48/4-51 base sequences of m1-m5 mRNA by ^{35}S-dATP 3'-end labeling (s.a.>2.4x10^{9}dpm/ug) via terminal deoxynucleotidyltransferase. Slices were hybridized (36h;25°C), washed (Tm=55°C;0.5xSSC), dried and Hyperfilm-ßmax-apposed. 14d scopolamine Rx (10mg/kg/d;ip) induced no affinity changes but raised mAChR subtype densities. Levels of m1-m5 mRNA expression were similar. Up-regulation may not be regulated at the gene transcription level. Yet aged (30mo) Rx rats had high (p<.05) m1 mRNA & low mAChRs in some areas. One may speculate this reflects failed efforts to up-regulate mAChRs to necessary levels, and may be linked to lower cognition with aging. MH-43024

611.10

REGULATION OF m1-m5 MUSCARINE ACETYLCHOLINE RECEPTOR GENE EXPRESSION IN ALZHEIMER'S DISEASED BRAINS AND AGE-MATCHED POST-MORTEM CONTROLS. X. Ming, L. Tsiokas, S. Vincent, E.A. Tolomeo, Z. Jelisijevic, Y. Robitaille#, R. Quirion# and M. Watson. Dept. of Pharmacol., UMDNJ-N.J. Med. Sch., Newark, NJ 07103-2714 & #Dept. of Psychiat., McGill U. & Douglas Hos. Res. Ctr., Verdun, Quebec, Canada.

Binding, biochemical and autoradiographic data from post-mortem brain tissue of Alzheimer's Diseased (AD) and control (C) patients suggest changes occur in muscarinic acetylcholine receptor (mAChR) subtypes seen on both pre- and post-synaptic neurons. [3H](-)quinuclidinylbenzilate, [3H](+)cismethyldioxolane, [3H]pirenzepine, [3H]AF-DX 116 and [3H]hemicholinium-3 autoradiograms and in situ hybridization histochemistry (ISHH) data was quantified as described previously. No Kd changes are seen. [3H]HC-3 density in AD hippocampal formation is ~53% of C, consistent w/ low CAT activity. Other ligands show changes. ISHH via 5 human 3'-end labeled (^{35}S-dATP;SA~0.8mCi/ug) oligodeoxyribonucleotide probes complementary to 3 regions of 4-48/4-49 base sequences of m1-m5 mAChR is done after verifying specificity by Northern blots. Slices are incubated (36h;25°C), washed (Tm=46-53°C;.5xSSC) dried & apposed to Hyperfilm-ßmax (1w;3°C). Low levels of hippocampal m3 expression (p<0.05) and higher m5 mRNA transcript levels in the temporal cortex (p<0.05) are seen in AD. No other alterations are significant. Lost or altered abilities to modulate mAChR gene expression may occur in AD.(MH-43024)

611.11

MEASUREMENTS OF BRAIN MUSCARINIC RECEPTOR mRNA LEVELS IN ALZHEIMER'S DISEASE BY DNA-EXCESS SOLUTION HYBRIDIZATION. S.-Z. Wang*, S. Zhu*, D.C. Mash and E.E. El-Fakahany. Department of Pharmacology and Toxicology, University of Maryland School of Pharmacy, Baltimore, MD 21201 and Department of Neurology, University of Miami School of Medicine, Miami, FL 33136.

The levels of mRNA encoding various subtypes of muscarinic cholinergic receptors (m1-m4) were measured using the quantitative and sensitive technique of DNA-excess solution hybridization. Single stranded hybridization probes corresponded to segments of the third cytoplasmic loop of the muscarinic receptor sequence which is unique to each receptor subtype. The probes were also chosen to exhibit a minimal degree of self complementarity. Measurements were performed in brain regions obtained postmortem from Alzheimer's disease patients and age-matched controls as follows: frontal cortex (m1, m3 and m4); temporal cortex (m1 and m4); occipital cortex (m1, m3 and m4); hippocampus (m1); nucleus basalis (m2) and brainstem (m2). Data were expressed as amol mRNA/100 µg of total RNA. Our results indicate that there was generally no significant change in the level of the message encoding the various muscarinic receptor subtypes in the different brain regions in Alzheimer's disease, although some trends for a decrease were noted in certain cases. The only exception was a statistically significant decrease in the concentration of mRNA coding for m1 receptors in the occipital cortex. These data suggest that the spared neurons in Alzheimer's disease mostly contain a normal level of muscarinic receptor messages.

611.12

CHOLINERGIC RECEPTOR CHANGES IN AN ANIMAL MODEL OF ALZHEIMER'S DISEASE. B. Thorne and P.E. Potter, Dept. Anesthesiology, Albert Einstein College of Medicine, Montefiore Medical Center, Bronx NY 10467.

The effects of cholinergic agonists on the evoked release of ^{3}H-acetylcholine (ACh) were studied in male Sprague-Dawley rats in which hippocampal cholinergic terminals were lesioned with the neurotoxin AF64A (ethylcholine mustard aziridium, 2 nmoles/ventricle). AF64A treatment causes reductions in cholinergic markers similar to those in Alzheimer's disease. Two weeks after AF64A infusion, choline acetyltransferase activity was decreased by more than 40%. ACh release was evoked from superfused hippocampal slices by electrical stimulation (1 or 2 Hz, 2 min). In control tissues, ACh release was enhanced by nicotine (EC_{50} 40 μM). In slices from AF64A treated rats, the dose response curve for nicotine was shifted to the left (EC_{50} 3.5 μM). The effects of nicotine were blocked by the antagonist dihydro-ß-erythroidine (10μM). In contrast, the dose response curve for inhibition of ACh release by the muscarinic agonist oxotremorine, was shifted significantly to the right in slices from AF64A-treated rats compared to controls. These results suggest that functional changes have occurred in hippocampal ACh receptors as a result of lesioning cholinergic inputs, with an increase in nicotinic receptor number or sensitivity, and a decrease in muscarinic receptor number or responsiveness.

611.13

QUANTITATIVE DISTRIBUTIONS OF MUSCARINIC RECEPTOR SUBTYPE m1-m5 mRNAS IN LONG SLEEP AND SHORT SLEEP MURINE BRAINS. L. Tsiokas, S. Vincent, J.J. McArdle and M. Watson. Dept. of Pharmacology, UMDNJ-N.J. Med. Sch., Newark, NJ 07103.

Molecular cloning of five distinct genes encoding each muscarine acetylcholine receptor (mAChR) subtype from rat tissue implies the rat synthetic oligodeoxyribonucleotide probes may specifically identify mouse mRNA analogs by in situ hybridization histochemistry (ISHH) and/or Northern blot (NB) analysis in long- and short-sleep (LS,SS) mice that are differentially sensitive to the hypnotic effects of ethanol. Our murine data show m1 mRNA predominates in cerebral cortical structures and is found in hippocampal, striatal and other regions of LS and SS mice. Murine m2 mRNA is found in extremely low levels in the murine CNS. The m3, m4 and m5 mRNAs were localized to hippocampus, cerebellum and brainstem in high abundance and m4 mRNA is also seen in significant levels in the caudate-putamen. Quantitative autoradiograms obtained via ISHH revealed no significant differences in relative CNS levels of m1, m4 and m5 mRNAs in LS vs SS. Unlike the strong signals seen in areas probed for m5 mRNA via ISHH, poly(A+)RNA NB of identical tissues show little, if any, detectable signal, even after unduly long exposures. Yet, ISHH shows m3 mRNA levels are lower (p<0.05) in hippocampal areas of SS. NB also show higher mRNA levels which imply higher levels of expression of a murine m3 mAChR gene in the hippocampus, as compared to other CNS or cardiac regions. (MH-43024).

611.14

BINDING CHARACTERISTICS OF PONTINE AND CORTICAL MUSCARINIC CHOLINERGIC RECEPTORS. H.A. Baghdoyan. Department of Anesthesia, Penn State Univ., College of Medicine, Hershey, PA 17033.

Microinjection studies using intact animals have demonstrated that muscarinic cholinergic receptors (MCR) in the medial pontine reticular formation are important for generating rapid eye movement (REM) sleep, and our recent studies have shown that cholinoceptive forebrain regions can influence pontine REM sleep generating mechanisms (Soc. Neurosci. Abs., 16:1056,1990). In order to pharmacologically characterize MCR in regions of cat brain important for controlling sleep and wakefulness, we have adapted a MCR binding assay for cat tissue (Sleep Res., 20:54, 1991). To date, non-linear least squares analyses of [^{3}H]QNB equilibrium binding to tissue homogenates from 3 cats revealed dissociation constants for pons and cortex of 96.2 pM and 52.9 pM, respectively, and number of binding sites (in fmol/mg protein) for pons and cortex of 506 and 1551, respectively. Thus, in comparison to cortex, MCR in the pons have a lower affinity for [^{3}H]QNB, and the pons contains fewer [^{3}H]QNB binding sites than the cortex. Competition binding assays will be used to identify and characterize subtypes of MCR in regions of cat brain known to be important in generating specific sleep states. *Supported by The Department of Anesthesia and MH45361.*

611.15

DEVELOPMENT OF SUBTYPE-SELECTIVE ANTISERA FOR THE m1-m5 MUSCARINIC CHOLINERGIC RECEPTORS. M. Li*, S.J. Wall, R.P. Yasuda, W. Ciesla*, and B.B. Wolfe. Dept. of Pharmacology, U. Pennsylvania, Philadelphia, PA and Georgetown U., Washington, D.C.

The third intracellular (i3) loop of each of the subtypes of muscarinic receptor is unique and was chosen to be used as an antigen to raise antisera selective for each of the subtypes. The cDNAs encoding most of the i3 loops of the m1, m2, m4, and m5 receptors were subcloned into expression vectors (either pRIT or pET). The areas close to the putative membrane-spanning regions 5 and 6 were avoided as these areas do not have the uniqueness found in the remainder of the i3 loop. The plasmids were sequenced to verify orientation and protein was produced by transforming *E. Coli.* Proteins were purified by a variety of methods (affinity chromatography, isoelectric focusing, size exclusion chromatography) to obtain full-length or nearly full-length proteins representing the i3 loop of a given subtype of muscarinic receptor. These proteins were injected into rabbits and sera were tested by immunoprecipitating solubilized receptors from cells transfected with the cDNA encoding a single muscarinic receptor subtype. Following 2 to 5 boosts with antigen, the antisera were able to quantitatively immunoprecipitate the expected subtype with no precipitation of any of the other subtypes. Additionally, for the m3 receptor, a short (18 amino acid) synthetic peptide corresponding to the sequence of the C-terminus was coupled to large carrier proteins and utilized as an antigen. Antisera obtained quantitatively precipitated m3 receptors with no precipitation of any other subtype of muscarinic receptor. Supported by GM31155 and NS26934, PDF and ADRDA.

611.16

EXAMINATION OF SUBTYPES OF MUSCARINIC CHOLINERGIC RECEPTORS USING SUBTYPE-SELECTIVE ANTISERA. R.P. Yasuda, S.J. Wall, M. Li*, C. Adams, S.J. Boyson, and B.B. Wolfe. Dept. of Pharmacology, Georgetown U., Washington, D.C., Dept. Pharmacology, U. Pennsylvania, Philadelphia, PA, Dept. Neurology, U. Colorado, Denver, CO.

Using a set of subtype-selective antisera we have generated against each of the five cloned muscarinic receptor subtypes (Li *et al. accompanying abstract*), the density and distribution of each of the subtypes has been determined in a number of tissues and following a number of experimental paradigms. Thus, the densities of each of the receptors in 7 areas (cortex, hippocampus, striatum, olfactory tubercule, thalamus/hypothalamus, pons/medulla, and cerebellum) of rat brain and 18 areas of human brain have been determined. Likewise, the densities of the subtypes were determined in several peripheral tissues of the rat (lung, ileum, bladder, parotid) as well as in several widely used clonal cell lines (SK-N-SH, 132-1N1). Additionally, the effects on receptor densities of chronic administration of the nonselective muscarinic receptor antagonist, atropine, were determined in rat cerebral cortex/dorsal hippocampus. Also, the ontogenetic profiles of each subtype were determined in rat forebrain. Furthermore, the effects of aging on the density of muscarinic receptor subtypes in the rat hippocampus were studied. It will be useful to compare these results to those obtained previously using ligand binding techniques. Supported by GM31155 and NS26934, PDF, and ADRDA.

611.17

ALTERATIONS IN CHOLINERGIC RECEPTORS FOLLOWING DEAFFERENTATION OF THE HIPPOCAMPUS AND CINGULATE CORTEX. L.F. Kromer, L.A. Pabreza*, S.J. Wall, K.J. Kellar and B.B. Wolfe. Dept. of Anat. & Cell Biol. and Dept. of Pharmacol., Georgetown Univ., Washington, DC 20007

In the present experiments, afferent input to the hippocampus (HPC) and cingulate cortex (CC) of adult female rats was removed unilaterally by an aspiration lesion of the rostral supracallosal stria/cingulum bundle and fimbria/fornix. Ten days after the lesion cholinergic receptors were examined in the HPC and CC. Nicotinic receptors were measured using ^{3}H-cytisine binding at near saturating concentrations (3.7nM). No significant difference in nicotinic binding between control and lesioned sides was found in the HPC, suggesting that few, if any, of these receptors are localized on cholinergic terminals. In contrast, nicotinic sites in the CC were significantly decreased (28%; $p<.001$) on the lesioned side. This decrease could reflect a loss of receptors on cholinergic or non-cholinergic afferents, such as dopaminergic axons. Total muscarinic receptors in the HPC (measured using 1nM ^{3}H-QNB) were increased slightly (19%; $p<.05$) on the lesioned side. More interestingly, measurements of each receptor subtype (m_1-m_5) using selective antisera revealed marked increases in m_3 (81%; $p<.01$) and m_4 (31%; $p<.05$) receptors and a decrease (27%; $p<.01$) in m_2 receptors. The direction of these changes may indicate the pre- or post-synaptic localization of these receptor subtypes.

611.18

INTERACTIONS OF HALOPERIDOL WITH MUSCARINIC RECEPTORS. RJ Smyth†, GR Luthin‡, TJ Shickley†. †Department of Pharmacology, Philadelphia College of Pharmacy & Science, Phila. PA 19104. ‡Department of Physiology & Biophysics, Hahnemann University, Phila. PA 19102.

Chronic administration of the antipsychotic drug haloperidol is often accompanied by extrapyramidal motor disturbances and dyskinesias. Other researchers have demonstrated that chronic haloperidol treatment causes an up-regulation of muscarinic cholinergic receptors. Comparison of primary amino acid sequences reveals a high degree of homology between anti-haloperidol monoclonal antibodies, and putative transmembrane domains of dopaminergic and muscarinic receptors. Our studies of secondary structure of these membrane bound proteins also predict structural similarity between dopaminergic and muscarinic receptors.

We therefore hypothesized the presence of a specific haloperidol binding site on muscarinic receptors, capable of interfering with agonist and antagonist binding, which may contribute to the muscarinic effects observed following haloperidol treatment.

Radioligand binding to muscarinic receptors was examined using [^{3}H]-oxotremorine-M for high affinity agonist binding and [^{3}H]-N-methyl-scopolamine for antagonist binding. Haloperidol inhibited both agonist and antagonist binding in rat cerebral cortex, and heart membranes with IC_{50} values in the μM range. Binding was unaffected by addition of GTP. Inhibition occurred over approximately a ten-fold concentration gradient, indicating possible positive cooperativity. A concentration-dependent effect of haloperidol on association kinetics was also observed. Together these data suggest that haloperidol inhibition of muscarinic ligand binding may not be explained by simple competitive interactions. Supported by USPHS Grant NS-26040 to TJS and by NS-23006 and a Scottish Rite Schizophrenia Grant to GRL.

611.19

MORPHOLOGY AND MUSCARINIC RECEPTOR DENSITY IN THE RAT BRAIN FOLLOWING EXPOSURE TO HYPOBARIC HYPOXIA

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The histological and histochemical consequences of hypobaric hypoxia were studied in five rats exposed for 16 hours to an altitude of 5500 m (18,000 ft) and in four sea level control animals. When hematoxylin and eosin stained sections from altitude-exposed rats were compared to sections obtained from control animals, no morphological differences were observed. Even the CA1 layer of the hippocampus and the pyriform cortex, which are known to be especially vulnerable to hypoxia, appeared normal. Using in vitro receptor autoradiography, changes in cholinergic muscarinic receptor density were examined. Frozen sections from five additional rats were cut at the level of the hippocampus after exposure to hypoxia or control conditions. These sections were incubated in [^{3}H]pirenzipine to label M_1 receptors or [^{3}H]AF-DX 116 to label M_2 receptors, and receptor densities were measured using a computerized image analysis system. Both M_1 and M_2 receptor densities were somewhat greater in the altitude-exposed animals in most brain areas assessed, especially in the deeper layers of the frontoparietal cortex. It appears that exposure to an altitude of 5500 m (18,000 ft) for 16 hours is not sufficient to produce significant structural damage or significant changes in muscarinic receptor density in the rat brain. Of course, other histological methods may revel changes in brain structure not readily apparent with the methods we employed.

612.1

HIGH pH INCREASES THE POTENCY OF PHOSPHONATE SUBSTITUTED COMPETITIVE ANTAGONISTS AT NMDA RECEPTORS

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Structure-activity relationships have shown that ω-phosphono-amino acids are more potent competitive antagonists at the NMDA receptor than ω-substituted analogs with carboxylate or tetrazole groups. The phosphonate group can have a maximum of two negative charges while carboxylate and tetrazole analogs have only one negative charge, suggesting that the higher potency of ω-phosphonate compounds could result from increased electrostatic interaction between these ligands and the NMDA receptor. Because the pK_a of one phosphonate oxygen in these compounds is ≈ 7.8 (Chenard et al. 1990 J. Med. Chem. 33:1077), the proportion of ionic species is strongly influenced by slight changes in pH in the physiological range.

Dose-inhibition analysis of NMDA activated currents for two ω-phosphonate antagonists, D-AP7 and its piperidine analog, LY 257883, indicated a > 3-fold increase in equilibrium potency between experiments on voltage clamped mouse hippocampal neurons conducted at pH 7.3 and pH 8.2. For the same pH range, the potency of the ω-carboxylate analog of LY 257883 increased only 1.4-fold. Analysis of the kinetics of block of NMDA activated currents in response to rapid concentration jumps of LY 257883, revealed a 79% increase in its association rate at the higher pH but also a 14% decrease in its dissociation rate. No such changes in rate constants were observed for the ω-carboxylate analog, LY 221501. The increase in the potency and concentration dependent association rate of LY 257883 is also consistent with an increase in concentration of the doubly charged antagonist species at higher pH.

Compound	pH	k_{on} ($\mu M^{-1}s^{-1}$)	k_{off} (s^{-1})	K_i (μM)
LY 257883	7.3	3.36 ± 0.99	0.83 ± 0.24	0.45 ± 0.05
LY 257883	8.2	5.99 ± 2.64	0.71 ± 0.20	0.14 ± 0.01
LY 221501	7.3	7.30 ± 2.58	3.45 ± 0.26	1.34 ± 0.15
LY 221501	8.2	7.06 ± 1.94	3.45 ± 0.27	0.92 ± 0.10

M.B. is supported by the National Research Council.

612.2

MECHANISM OF pH ANTAGONISM ON NMDA-INDUCED MEMBRANE CURRENTS IN ISOLATED CATFISH CONE HORIZONTAL CELLS.

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We have observed that the NMDA-induced membrane currents vary as a function of extracellular pH over the range of 5.5 to 9.5 with lower pH (pH < 7.4) decreasing current (Society for Neuroscience, 1990). The inhibitory effect of lower pH could be attributed to the effects of H^+ on various factors underlying the NMDA response. H^+ could affect the binding of NMDA with its receptor, block the NMDA-gated channel in a voltage dependent manner or alter an aspect of channel function such as channel kinetics, gating or conductance.

Concentration-response curves measured at pH 6.5 and 7.4 revealed a non-competitive type of inhibition indicating that H^+ does not interfere with agonist binding. Current-voltage curves measured at these pH's showed a uniform inhibition over the voltage range indicating that H^+ probably does not penetrate sufficiently far into the channel to feel the membrane field. To discriminate between an effect of H^+ on channel gating vs channel kinetics or conductance we are using fluctuation analysis to measure channel open time and single channel conductance. Supported by Grant NEI EY-01897.

612.3

EFFECTS OF EXOGENOUS GLUTAMINE (GLN) ON CA1 FIELD POTENTIALS AND SPONTANEOUS ACTIVITY IN RAT HIPPOCAMPUS IN VITRO.

W.D. YONEKAWA, I.M. KAPETANOVIC, AND H.J. KUPFERBERG*. Epilepsy Branch, NINDS, NIH, Bethesda, MD 20892.

GLN, normally present in brain extracellular fluid and CSF in the range of 0.2 to 0.5 mM, plays an important role in energy and neurotransmitter metabolism, but is not included in ACSF used for in vitro slice studies. This study examined the effects of GLN on the latency to onset (LAT) and amplitude (AMP) of extracellular CA1 population spikes (PS) and on spontaneous activity both in normal and in 9.4 mM K^+ ACSF. Rat slices were equilibrated for 60' in a submerged slice chamber either with or without 0.5 mM GLN in the ACSF. The slices were monitored for 15' and then the media switched to normal or 9.4 mM K^+ ACSF with or without 0.5 mM GLN. When 0 mM GLN + 9.4 mM K^+ was added to slices equilibrated in normal ACSF, an immediate increase in AMP and a decrease in LAT was seen in 4 of 5 slices. Full rhythmic bursting developed in 3 of the 5 slices while small infrequent bursts occurred in 1 other slice. When 0.5 mM GLN was added along with 9.4 mM K^+, 5 of 5 slices gave an increase in LAT while 2 of 5 had an increase in AMP. All 5 of these slices developed bursting, but in 4 of the 5 slices the bursts were small and short-acting. We found that GLN alone had no effect on the PS or on spontaneous activity. Adding 9.4 mM K^+ when 0.5 mM GLN was present throughout the entire run, led to an increase in AMP in 5 of 6 slices with no change in LAT in 4 of the 6 slices. Only 1 of the 6 showed full bursting, 2 of 6 had small and infrequent bursts, and 3 of 6 had no bursts at all. These data indicate that while 0.5 mM GLN appeared to decreased the onset and severity of K^+-induced spontaneous bursting activity, the effect on the evoked population spike was slight and limited to a small attenuating effect on LAT.

612.4

ISOPROTERENOL INHIBITS NMDA-INDUCED CURRENTS IN XENOPUS OOCYTES.

A. Omerovic* and S.R. Kelso. Dept. of Biological Sciences, Univ. of Illinois at Chicago, Chicago, IL 60680, USA.

The modulatory action of beta-1 adrenergic agonist on NMDA receptors was studied in Xenopus Leavis oocytes injected with total rat brain mRNA.Using two electrode voltage-clamp techniques, changes in NMDA currents were measured during bath application of different concentrations of isoproterenol. After incubation in isoproterenol (100 to 1000uM) for 1-30 minutes, the NMDA current was not significantly changed. However, in the presence of isoproterenol (200 uM) the NMDA current was significantly and reversibly reduced to 54.3±3.8 % of control (n=11).The minimal inhibition (10%) was obtained with 1uM; the maximal (96%) with 2mM (IC_{50} = 200 uM). The Beta-1 adrenergic antagonist, acebutolol (100-200uM) did not block the isoproterenol effect. A dose-response curve for NMDA in the presence of 200 uM of isoproterenol showed a noncompetitive antagonism, in which maximal response was decreased without change of EC_{50} for NMDA. The modulatory action of isoproterenol was selective for NMDA subtype of glutamate receptors. Kainate, AMPA and t-ACPD responses were not changed significantly in 5 cells tested. Isoproterenol inhibition of NMDA current was not voltage dependent; NMDA conductance was reduced and reversal potential was not changed by isoproterenol (5 cells). A similar reduction (to 71.4±2.7 % of control) was seen in 6 cells perfused with saline in which Ca^{2+} was replaced by Ba^{2+} in order to eliminate the contribution of Ca^{2+} dependent Cl channel to NMDA current. The inhibitory effect of isoproterenol on NMDA current was mimicked by norepinephrine; bath application of 100uM and 1mM of NE reversibly reduced the NMDA current to 44.1±2.9% and 13.6±0.9% of control, respectively.This inhibition was not blocked by acebutolol. The results suggest direct inhibitory effect of isoproterenol on NMDA receptors in total rat brain injected oocytes. Supported by NIH grant NS24591 and Klingenstein Fund.

612.5

EFFECTS OF GLYCINE AND STRYCHNINE ON RESPONSES OF RAT SPINAL NEURONS TO NMDA.

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The strychnine-insensitive increase in NMDA-activated channel opening by glycine (Gly) is distinct from its classical inhibition of synaptic transmission. It has been postulated that extracellular Gly saturates its binding sites on the NMDA receptors but we and others have found effects of exogenously administered Gly *in vivo*. We examined the effects of NMDA, Gly and strychnine on nociceptive dorsal horn neurons of the rat spinal cord to test the hypothesis that neurons responsive to NMDA are facilitated by Gly acting at a strychnine-insensitive Gly receptor. Compound recording and iontophoresis electrodes were used to record single unit extracellular activity from the lumbar spinal cord of anesthetized rats and to apply drugs iontophoretically. Computerized instument control and data acquisition were performed using an NB-MIO-16 multifunction board programmed in LabVIEW 2. Application of NMDA by iontophoresis produced excitation in all 26 neurons tested. Gly significantly inhibited the NMDA-evoked increase in the neuronal firing rate (71±3%). Strychnine potentiated the NMDA-induced excitation (140±3%), perhaps because of the presence of endogenous glycine. When Gly was co-ejected with strychnine, the number of action potentials evoked by NMDA was further elevated (170±9%). These data provide evidence *in vivo* for the unsaturated nature of the Gly binding site of the NMDA receptor complex. *(Supported by USPHS grants DA04090 and DA04274).*

612.6

SINGLE CHANNEL PROPERTIES OF THE NMDA ION CHANNEL WITH DIFFERENT AGONISTS.

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The glutamate receptor subtype, N-methyl-D-aspartate (NMDA), is activated by a variety of ligands. We have used the cell-attached patch-clamp technique, with agonist included in the patch pipette, to study the single channel properties of the NMDA channel, with different ligands. The compounds used included the D and L forms of N-methyl-aspartate (NMA) and the D and L forms of homocysteic acid. All of the ligands activated the high conductance (45 pS) state of the NMDA ion channel. In nominally free Mg^{2+} solution, the mean open times of the NMDA channel were decreased with patch hyperpolarization. At the cell resting potential, the mean open times were longer for NMDA compared with NMLA and were longer for L-homocysteic acid compared with D-homocysteic acid. The frequency of channel openings, but not the mean open times, were dependent on the concentration of ligand. When the pipette concentration of L-homocysteic acid was increased from 25 μM to 50 μM, the frequency of channel events was increased two-fold. Measurements of the single channel properties of the NMDA channel should be useful in the pharmacological characterization of agonist actions at the NMDA receptor.

612.7

DITHIOTHREITOL POTENTIATES N-METHYL-D-ASPARTATE STIMULATED NEUROTRANSMITTER RELEASE FROM RAT BRAIN SLICES

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Rat brain slices released tritiated or endogenous neurotransmitters during a two minute stimulation with N-methyl-D-aspartate (NMDA). The dose response curve for NMDA was dramatically shifted to the left and upwards after treatment of slices with dithiothreitol (DTT; 0.1-5 mM), a sulfhydral reducing agent. Following DTT treatment, cortical slices released approximately the same amount of ^{3}H-NE with 10 uM NMDA (about 5%) as control slices did with 500 uM NMDA. In addition, DTT treatment more than doubled the NMDA stimulated release of ^{3}H-NE from cortical and hippocampal slices and increased endogenous dopamine release from striatal slices by 7-10 fold. This effect was not due to an increase in basal non-stimulated release or to a non-specific effect on depolarization as DTT did not enhance KCl induced release. The effects of DTT were fully reversed by treating the slices with the sulfhydral oxidizing agent di-thio-bis-nitrobenzoic acid (0.5 mM) prior to NMDA stimulation. DTT did not significantly alter the ability of magnesium (1.3 mM) or the polyamine antagonist arcaine to inhibit NMDA stimulated release. In contrast, DTT treatment significantly attenuated the antagonist effects of the competitive glycine antagonist, 7-chloro-kynurenic acid, and the competitive NMDA antagonist, amino-phosphono-valeric acid (AP-5). These results suggest that oxidation and reduction of disulfide bonds located within the NMDA receptor complex might regulate the activation of the NMDA receptor *in vivo*. Supported by NIAAA AA08089 and a grant from the Alcoholic Beverage Medical Research Foundation.

612.8

DEVELOPMENTAL CHANGES IN THE EFFECT OF MAGNESIUM ON [^{3}H]TCP BINDING TO RAT HIPPOCAMPAL MEMBRANES.

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Mg^{2+} less potently blocks the NMDA-evoked depolarization of CA1 hippocampal pyramidal cells in 10-15 day old rats compared with adults. To investigate the cellular and molecular basis for this developmental change, we studied the effects of Mg^{2+} on the binding of [^{3}H]TCP to hippocampal membranes. The initial rate of binding reflects access of the radioligand to its binding site within the NMDA receptor channel. The rate is influenced by the state of the receptor and its membrane environment, but not by membrane potential, intracellular constituents or the presence of spare receptors. The initial rate of [^{3}H]TCP binding in the presence of saturating NMDA and glycine was greater in membranes from 10-15 day old rats. Mg^{2+} increased this rate at both ages, but to a lesser degree at 10-15 d. NMDA enhanced the initial rate of [^{3}H]TCP binding with similar potency at both ages. Low concentrations of Mg^{2+} increased the maximal effect of NMDA but to a lesser degree at 10-15 d. No age-related changes were observed in the equilibrium binding of [^{3}H]TCP, the effects of Mg^{2+} alone on equilibrium binding or the biphasic concentration-response curve for the effect of Mg^{2+} on the initial rate of [^{3}H]TCP binding. The weaker effects of Mg^{2+} on some aspects of [^{3}H]TCP binding during development may be related to its lower potency as an NMDA receptor antagonist in physiological studies. (Supported by NIH grant NS 16064.)

612.9

The distribution of spinal cord N-methyl-D-aspartate receptors is developmentally regulated.

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We have studied the molecular development of spinal motor neurons by examining the regulation of the cell surface proteoglycan recognized by monoclonal antibody Cat-301 (*J. Neurosci.***8**:2350). Cat-301 expression depends upon the coordinated activity of spinal segmental and descending afferents during a critical period in early postnatal life. This activity-dependent development is mediated through activation of the N-methyl-D-aspartate (NMDA) receptor: blockade of the NMDA receptor in early life inhibits the expression of the Cat-301 proteoglycan on motor neurons in a stereospecific and dose-dependent manner (*Science* **250**:294). The site of action of NMDA receptor antagonists at the spinal segmental level is not clear because the distribution of spinal NMDA receptors during postnatal development is not known.

We have examined the distribution of spinal cord NMDA receptors during development by employing quantitative receptor autoradiography. We studied NMDA-displacable 3H-Glutamate binding. All studies were carried out in the presence of CNQX and Kainate to block binding to non-NMDA glutamate receptors. In the adult spinal cord, NMDA receptors are concentrated in the substantia gelatinosa (SG) with very little binding elsewhere in spinal grey matter. In marked contrast, in P7 rat spinal cord, NMDA receptors are evenly distributed through out the grey matter. The neonatal ventral horn 3H-Glutamate binding to the NMDA receptor has approximately the same affinity and density as in the adult spinal cord. Between P7 and P28 the distribution of NMDA receptors gradually adopts the adult pattern. These changes cannot be accounted for by changes in spinal cord myelination over this period. These results demonstrate that the NMDA receptor-mediated development of motor neurons could occur through activation NMDA receptors in the ventral horn of the spinal cord.

612.10

DEVELOPMENTAL CHANGES IN THE NMDA RECEPTOR POPULATIONS OF THE RAT HIPPOCAMPAL SLICE.

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Information continues to accumulate indicating the existence of heterogeneity in the NMDA preferring excitatory amino acid population. We have previously reported observations of developmental changes in the characteristics of the NMDA receptor-channel complex in the CA3 infrapyramidal zone (CA3/IPZ) of *stratum oriens* in the hippocampal slice. In recent studies NMDA evoked responses have been recorded in both the CA1 and CA3 regions of the rat hippocampal slice. Agonist was iontophoretically applied in the IPZ region in CA3 and 100-150 μm away from the center of the cell body layer in *stratum radiatum* of CA1 (CA1/SR). The response to agonist application was recorded using whole-cell patch and field techniques. Recordings were obtained in mature (PND>35) and immature (PND 10-15) hippocampal slices treated with bath application of tetrodotoxin. The apparent potency of the competitive NMDA antagonist D(-)2-amino-5-phosphonovaleric acid (D(-)AP5) was measured in both regions during the different developmental periods. The effect of a series of increasing, bath applied, antagonist concentrations was assessed using the local negative extracellular field potentials produced by iontophoretic agonist application. Inhibition curves were then produced for the two regions during each age window. These relationships were then used to estimate the concentrations of D(-)AP5 needed to produce a 50% inhibition in each case. The antagonist was more potent in both regions of the immature hippocampal slice than in the older tissue. Within each age group the potency did not seem to differ significantly between regions. The 50% inhibition concentration in immature hippocampal CA1/SR and CA3/IPZ was approximately 4μM while the mature regions required approximately 11μM D(-)AP5. These differences in antagonist potency are further indications of developmental changes in the hippocampal NMDA receptor population. Supported by grants NS23071 to RJB and NS18309 to JWS from NIDS-NIH.

612.11

MODULATION OF THE DEVELOPING NMDA RECEPTOR-CHANNEL COMPLEX BY PHENCYCLIDINE IN RAT BRAIN.

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The NMDA receptor-channel complex has been shown to be involved in the activity-dependent synaptic plasticity in developing animal. The present study was directed at determining the effects of chronic postnatal phencyclidine (PCP) exposure on [^{3}H]MK-801 binding properties. We have earlier shown that [^{3}H]MK-801 binding can be used as a marker for NMDA channel activity. Rat pups were daily injected (i.p.) with PCP (5 mg/kg) or saline beginning on postnatal day 5 till day 15. Animals were sacrificed on postnatal day 21, six days after the last injection. [^{3}H]MK-801 binding was measured in well-washed experimental and control rat forebrain CSMs, both in the virtual absence of any excitatory amino acids and under various degrees of channel activation i.e. in the presence of L-glutamate and/or glycine. In the absence of any exogenous L-glutamate and/or glycine, data from equilibrium saturation experiments indicate that PCP treatment produced a decrease in the density of high-affinity [^{3}H]MK-801 binding sites. When binding was carried out in the presence of L-glutamate alone or in the added presence of glycine there was no difference in [^{3}H]MK-801 binding between PCP- and saline-treated brains. The affinities of [^{3}H]MK-801 binding did not differ under any condition. These data suggest that chronic PCP exposure during development can alter NMDA receptor regulation.

612.12

REGIONALLY DISTINCT NMDA RECEPTOR SUBTYPES DISTINGUISHED BY [^{3}H]MK-801 BINDING.

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The existence of NMDA receptor subtypes has been proposed, but has yet to be verified with detailed pharmacological analysis. We performed *in vitro* quantitative [^{3}H]MK-801 autoradiography in rat brain, under baseline and maximally stimulated conditions, to assess potential NMDA receptor subtypes. Displacements of [^{3}H]MK-801 binding were examined with the NMDA antagonist, 3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP) and the strychnine-insensitive glycine binding site antagonist, 7-chlorokynurenic acid (7-ClKyn).

Under control conditions, several brain areas were differentially sensitive to CPP displacement of [^{3}H]MK-801 binding. CPP inhibited [^{3}H]MK-801 binding in outer cortex (OC) and inner cortex (IC) with Ki values of 0.32-0.48 μM, while in medial striatum (MS), lateral striatum (LS), CA1 and dentate gyrus (DG) of hippocampus Ki values were 1.1-1.5 μM. In medial thalamus (MT) and lateral thalamus (LT) the Ki was 0.78 μM (the groups differed significantly, $P < 0.0001$, Fisher PLSD). In the presence of glutamate (3 μM), the Ki values between regions maintained a similar relationship with the exception of the Ki for OC which increased 2.5 times more than other areas.

7-ClKyn inhibition of [^{3}H]MK-801 binding also distinguished regionally distinct subtypes which differed from CPP displacements. Under control conditions, 7-ClKyn inhibited [^{3}H]MK-801 binding in OC, IC, MS and LS with Ki values of 6.3-8.6 μM, while in CA1, DG, LT and MT, Ki values were 11.4-13.6 μM (the groups differed significantly, $P < 0.0001$, Fisher PLSD). In the presence of glycine (1 μM), the Ki values shifted similarly, with the exception of OC which increased 1.5 times more than other areas.

These data support the existence of NMDA receptor subtypes that can be distinguished pharmacologically using [^{3}H]MK-801 binding. By analogy to the $GABA_A$ receptor, one would predict the existence of regional differences in subunit composition of the NMDA receptor. Supported by USPHS NS19613, AG08671.

612.13

AGE-DEPENDENT INHIBITION BY LEAD OF NMDA-INDUCED CURRENT IN CULTURED HIPPOCAMPAL NEURONS. H. Ujihara and E.X. Albuquerque. Dept. Pharmacol. & Exp. Ther., Univ. of Maryland, Sch. of Med., Baltimore, MD 21201.

The N-methyl-D-aspartate(NMDA)-evoked current recorded at early stages of development from hippocampal neurons kept in culture was very sensitive to Pb^{2+}, but after the second week of culture the sensitivity to this cation decreased significantly. The whole-cell current was recorded according to the standard patch-clamp technique. NMDA (5-100 μM) and glycine (0.5-10 μM) were delivered to the neuron under whole-cell clamp condition via a U-tube located 50-100 μm away. $PbCl_2$ at concentrations varying from 1 to 100 μM was applied in the extracellular perfusion medium. The perfusion medium contained (mM), NaCl 165, KCl 5, $CaCl_2$ 2, HEPES 5, D-glucose 10 and TTX 0.0003 (pH 7.3, 340 mOsm), and the micropipette solution contained (mM), CsCl 80, CsF 80, CsEGTA 10 and HEPES 10 (pH 7.3, 330 mOsm). The patch micropipette resistance was 2-4 MΩ. The hippocampal culture was obtained from 16-18 day fetal rats and the cells were used 3 to 28 days after plating. Pb^{2+}-induced inhibition of NMDA-current was concentration-dependent at the range over 1-10 μM, although this effect was reduced at higher doses. The inhibition was most prominent during the first week of cell culture, and reduced along with maturation. The current-voltage relationship disclosed a voltage-independent inhibition of NMDA current by Pb^{2+}. Analysis of the relationship between NMDA concentration and Pb^{2+}-induced inhibition demonstrated that Pb^{2+} was a non-competitive antagonist of the NMDA-site of the receptor-channel complex. The same analysis for the glycine-site showed a non-linear relationship between glycine concentration and Pb^{2+}-induced inhibition, and suggested heterogeneity of glycine-sites. The present results show 1) the NMDA receptor may undergo some molecular conformational adjustment during maturation, and as a result, Pb^{2+} sensitivity is markedly decreased, 2) the Pb^{2+}-site is located outside of the electrical field of the channel, and 3) there may be at least two types of glycine-site which differ from each other in their sensitivity to Pb^{2+}. (Supported by U.S. Army Med. Res. & Devel. Comm. Contr. DAMD-17-88C-8119)

612.14

Inhibition of the NMDA-Activated Ion Current by Ethanol in Rat Hippocampal Neurons Does Not Involve the Glycine or Proton Modulatory Sites. Robert W. Peoples and Forrest F. Weight. Section of Electrophysiology, National Institute on Alcohol Abuse and Alcoholism, Rockville, MD 20852.

Previous studies in this and other laboratories have shown that physiologically relevant concentrations of ethanol inhibit responses mediated by N-methyl-D-aspartate (NMDA) receptors in mammalian neurons. We have investigated whether ethanol interacts with the glycine or proton modulatory sites of the NMDA receptor/ion channel complex in voltage-clamped rat hippocampal neurons in culture. As shown previously, the NMDA-activated current in these cells was increased by glycine and inhibited by ethanol. Concentration-response data for glycine (0.1-100 μM) enhancement of NMDA-activated current indicated that ethanol, 50 mM, decreased the maximal response to glycine without significantly altering its EC_{50}. In addition, the percent inhibition by ethanol did not vary significantly with glycine concentration. Similarly, elevating extracellular pH enhanced NMDA-activated current in these cells, but did not alter the percent inhibition by ethanol. Thus, ethanol appears to inhibit NMDA-activated current in hippocampal neurons at a locus different from the glycine or proton modulatory sites. (This work was supported in part by a National Research Council-NIH Research Associateship to R.W.P.).

612.15

ETHANOL INHIBITION OF THE NMDA-STIMULATED INCREASE IN INTRACELLULAR CALCIUM IN HIPPOCAMPAL MICROSACS: EFFECT OF TEMPERATURE, MG, GLYCINE AND MK-801. Laura C. Daniell. Dept. of Pharmacol. & Toxicol., Medical College of GA., Augusta, GA. 30912-2300.

The effect of N-methyl-D-aspartate (NMDA) on the intracellular free calcium concentration (Ca_i) was determined in microsacs (a cell-free membrane vesicle preparation) isolated from mouse hippocampus loaded with the fluorescent indicator, Indo 1. NMDA increased Ca_i in hippocampal microsacs with an EC_{50} of 30 μM. Ethanol inhibited NMDA-stimulated Ca_i increases in a noncompetitive manner with an IC_{50} of 67 mM. NMDA responses were markedly temperature-sensitive, but lowered temperature (from 32 to 22°C) did not alter the relative degree of ethanol inhibition. Preincubation with ethanol also did not alter the degree of ethanol inhibition. However, ethanol inhibition was reduced, in a concentration dependent manner, by glycine, Mg and MK-801. These data show that a variety of allosteric modulators of the NMDA receptor can reverse ethanol inhibition of NMDA-mediated calcium flux. This work was supported by the Alcoholic Beverage Medical Research Foundation and NIAAA (AA01866).

612.16

NMDA RECEPTOR-MEDIATED CONTRIBUTION TO HIPPOCAMPAL DENTATE GRANULE CELL POPULATION EPSPs *in vivo*: INTERACTION BETWEEN STIMULUS INTENSITY AND GABAergic INHIBITION. Thomas A. Blanpied and Theodore W. Berger. *Departments of Behavioral Neuroscience and Psychiatry, University of Pittsburgh, Pittsburgh., PA 15260*

We have measured an NMDA receptor-mediated component of the hippocampal dentate granule cell population synaptic response *in vivo* to low frequency (<0.2 Hz) stimulation of the perforant path, and investigated the extent to which that component is dependent upon the stimulus intensity and the activation of local $GABA_A$ receptors. Extracellular recordings were obtained from the dentate molecular layer in anesthetized rabbits, and the perforant path was stimulated electrically using two intensities: below the threshold required to elicit a population spike ("low"), or 2-3 times the population spike threshold intensity ("high"). CNQX (50μM), APV (100μM), bicuculline (100μM), or saline were applied by pressure microinfusion from a multibarrelled pipette located 200-400μm from the recording site.

APV applied in the presence of saline alone did not reliably reduce the area of the EPSP evoked by either low or high intensity stimulation. However, the evoked EPSP contained an NMDA receptor-mediated component which was revealed in the presence of CNQX (50 μM; N=7): APV reduced the area of the CNQX-resistant EPSP on average by an amount corresponding to 12% of the pre-drug baseline in response to low intensity stimulation, and 27% of baseline in response to high intensity stimulation. In other preparations (N=6), bicuculline was infused in a concentration sufficient to eliminate paired impulse inhibition of the granule cell population spike. In 4 of these cases, a larger NMDA-mediated component of the EPSP was observed: APV reduced the area of the EPSP evoked using low intensity stimulation by 22%, and using high intensity stimulation by 40% of the respective pre-drug baselines. These data indicate that local inhibitory mechanisms *in vivo* interact with convergent excitatory afferents to determine the nonlinear intensity-response properties of the NMDA receptor channel. (Supported by an NSF Graduate Fellowship to TAB, as well as by ONR, AFOSR, MH45156, and MH00343.)

612.17

A MATHEMATICAL MODEL FOR THE DYNAMICS OF THE NMDA RECEPTOR-CHANNEL. Gilbert A. Chauvet, Nathaniel N. Urban*, and Theodore W. Berger. *Depts. of Behavioral Neuroscience and Psychiatry*, University of Pittsburgh, Pgh, PA 15260.

The kinetics of the NMDA receptor channel complex have yet to be fully characterized. While the kinetics of many non-zero conductance states can be directly observed, experimental determination of the kinetics of electrophysiologically invisible zero conductance states is much more difficult. Kinetic parameters of zero conductance states may be fixed through the use of computer simulations.

A kinetic model of the NMDA receptor channel complex is proposed which describes the actions of glutamate, glycine and magnesium at independent sites. The model assumes the existence of three distinct channel states: ground, open and desensitized. Extensions of the model to include additional ligands and channel states are also described. The case in which multiple glutamate and glycine sites are assumed is compared to the single site model. Experiments involving glycine-indenpendent desensitization, paired impulse activation and fast concentration stepping are simulated. Kinetic parameters including binding constants and state transition rates were modified to achieve the best fit to the experimental data. Thus, kinetic parameters which are presently experimentally inaccessible were constrained. Simulation results support the hypothesis that glycine-independent desensitization is mediated by the affect of glutamate binding on the duration of a desensitized state.

The formalism and notation used in this model is generalized and proposed as a general mathematical formalism for the description of receptor channel kinetics. The sensitivity of the system to changes in kinetic parameters is described. The mathematical analysis of the system, including the determination of steady states and stability are shown to be generally applicable to all kinetic models to which this formalism can be applied.

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612.18

CONTRIBUTIONS OF NMDA AND NON-NMDA RECEPTORS TO VISUALLY EVOKED POTENTIALS IN ALBINO RATS. D.F. Sisson, J. Siegel, B. Cox and S.J. Grant. School of Life and Health Sciences and Department of Psychology, University of Delaware, Newark, DE 19716.

Excitatory amino acids (EAA) are the putative transmitter of neurons that project from LGN to visual cortex (OC1). Because early components of visually evoked potentials (VEPs) are indicative of geniculo-cortical activity, the effects of 2-amino-5-phosphonopentanoic acid (AP5), an NMDA receptor antagonist, and 6-cyano-7-nitroquinoloxaline-2,3-dione (CNQX), a non-NMDA receptor antagonist, on flash VEPs from albino rats anesthetized with chloral hydrate were evaluated.

Drugs were superfused over left OC1 using a cortical cup. VEPs were recorded from this cortical surface and from the unoperated, right, cortex simultaneously.

CNQX decreased amplitudes of VEP components N_1 and N_2 in a dose-dependent manner; neither P_1 nor P_2 were affected. This effect was reversed after 30 min of CSF wash. AP5 at concentrations up to 200 μM had no effect on VEP amplitude.

CNQX has an effect similar to kynurenic acid, a non-specific, EAA antagonist. These results suggest that non-NMDA receptors generate epsp's in response to visually driven input into primary visual cortex, and that these epsp's are reflected in N_1. P_1 is either presynaptic or reflects a fast, non-EAA, visual pathway.

This work was supported in part by ARO Contract DAAL 0388K0043.

612.19

EFFECTS OF DEXTROMETHORPHAN AND CARBETAPENTANE ON THE NMDA RECEPTOR-CHANNEL IN SINGLE CHANNEL RECORDINGS. J.M. Wright and L.M. Nowak, Dept. of Pharmacology, Cornell Univ., Ithaca, NY 14853.

Dextromethorphan (DM) and carbetapentane (CBP) have anticonvulsant actions (Tortella & Musacchio, *Brain Res.* 383:314-318, 1986) DM, but not CBP, protects against NMDA induced mortality in mice (Leander, *Epilepsy. Res.* 4:28-33, 1989). We examined the actions of these drugs on single NMDA channels in preliminary experiments (DM: N=7, CBP: N=3) with excised outside-out patches from mouse cortical neurons in culture. In 10 μM NMDA, 1 μM DM reduced the average current through the patch 86% at -60 mV but only 45% at +40 mV. In 1 μM DM with 2 μM NMDA at -60 mV, current was reduced 56%. Responses stabilized within 3 min of DM application; full recovery was seen after removing the drug. A new closed time (1.1 mS) was introduced at -60 mV and burst length was reduced 15%. The reduction in total current by DM was due primarily to a decrease in open time per burst; a reduction in the number of bursts also occurred. In contrast to the inhibition seen with DM, 10 μM CBP applied at -60 mV in 2 μM NMDA introduced a new closed time of 3 mS but did not reduce total current through the patch. (Supported by NIH NS24467)

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613.1

ETHANOL INHIBITION OF N-METHYL-D-ASPARTATE (NMDA) RECEPTOR FUNCTION: SELECTIVE INTERACTIONS WITH THE GLYCINE RECOGNITION SITE. G. C. Yeh, D. W. Bonhaus, J.O. McNamara. Duke & V.A. Medical Center. Durham. N.C.

Ethanol inhibits N-methyl-D-aspartate (NMDA) receptor-mediated neurotransmission. To elucidate the mechanism of this inhibition, we examined effects of ethanol on the binding of ligands to the NMDA and glycine recognition sites of the NMDA receptor ([^{3}H]L-glutamate and [^{3}H]glycine respectively) and on the agonist-dependent binding of an NMDA channel blocker ([^{3}H]TCP). Ethanol decreased [^{3}H]glycine binding to a strychnine-insensitive glycine recognition site and inhibited the glycine-dependent binding of [^{3}H]TCP. By contrast ethanol had no effect on NMDA-sensitive [^{3}H]L-glutamate binding or on the potency of glutamate's stimulation of [^{3}H]TCP binding. Ethanol reduced [^{3}H]glycine binding by decreasing the maximum number of detectable sites (without altering binding affinity) and similarly reduced glycine-stimulated [^{3}H]TCP binding by reducing the maximum rate of glycine-stimulated binding (without modifying glycine's EC_{50} or steady state levels). Ethanol inhibited [^{3}H]TCP association and [^{3}H]glycine binding with similar potency (479 and 581 mM respectively). These data suggest that ethanol inhibits NMDA receptor function by modifying the glycine binding site so as to prevent ligand binding. This action of ethanol is similar to that of zinc. Thus regulation of the glycine binding site is a mechanism common to structurally diverse allosteric regulators of the NMDA receptor.

613.2

EFFECTS OF ETHANOL ON GLUTAMATE/GLYCINE ACTIVATION OF [^{3}H] TCP BINDING: A MODEL FOR THE NMDA RECEPTOR-ION CHANNEL. M.L. Michaelis, D. Joseph and E.K. Michaelis. Department of Pharmacology and Toxicology. University of Kansas, Lawrence, KS 66045.

Work from our laboratories back to 1978, has implicated brain glutamate receptors in the CNS actions of ethanol (ETOH) (e.g., Biochem. Pharm. 1978, 27:1685). Recent observations in several laboratories that the NMDA subtype of glutamate receptors is unusually sensitive to ETOH at physiologically relevant concentrations led us to use a model of NMDA channel activation, i.e., glutamate/glycine activation of [^{3}H] TCP binding, in studies designed to explore the site on the channel complex with which ETOH interacts in brain membranes. When both glutamate and glycine were present at 100nm, ETOH inhibited [^{3}H] TCP binding marginally (~12%) at concentrations up to 200 mM. When the effects of 100mM ETOH were examined with varying concentrations of each agonist alone, ETOH had little or no effect on glutamate stimulation of the [^{3}H] TCP binding but did inhibit activation by glycine. Dose response curves for ETOH measured at 120nM glycine alone revealed an IC_{50} of ~5mM ETOH and 90% inhibition of the glycine stimulated response by 10mM ETOH. Experiments to determine whether glycine could overcome the effects of ETOH indicated that, when ETOH was present at 2.5mM, its inhibitory effects could be overcome by glycine concentrations greater than 10uM. In agreement with studies involving ^{45}Ca influx, it appears that the site through which glycine activates TCP binding is likely to be the major site with which ETOH interacts. Studies are underway with the purified NMDA receptor complex reconstituted in liposomes (Ly and Michaelis, Biochemistry, in press) to determine the effects of ETOH on the isolated protein complex which does exhibit pronounced glutamate/glycine activation of TCP binding. [NIAAA grant #AA04732, DAAL03-88-0017 from ARO, and the Ctr. for Biomed. Res., Univ. of Kansas.]

613.3

GLYCINERGIC MODULATION OF [^{3}H]MK-801 BINDING TO THE NMDA RECEPTOR: EFFECT OF TEMPERATURE. J.C. Marvizon, P. Skolnick and I.A. Paul. Lab. Neuroscience, NIDDK/NIH, Bethesda, MD. 20892.

[^{3}H]MK-801 binding to an extensively washed rat forebrain P_2 fraction was measured at 4, 25 and 37 °C. At 25 °C, glycine (G) produced a monophasic enhancement of binding with an EC_{50} of 65 nM. D-serine (DS) and the putative partial agonists 1-aminocyclopropanecarboxylic acid (ACPC) and D-cycloserine (DCS) produced an enhancement (EC_{50}s 101, 88, 3000 nM) followed by an inhibition (IC_{50}s > 1 mM). This biphasic pattern was observed for all four compounds when the incubation temperature was reduced to 4 °C. At 37 °C G, ACPC, and DS elicited a biphasic enhancement of [^{3}H]MK-801 binding with EC_{50} values of 65, 31 and, 209 nM and > 1 mM. In contrast, DCS produced a monophasic inhibition of binding (IC_{50} > 1 mM). These data demonstrate that modulation of the NMDA receptor via the glycine binding site is temperature-dependent. Further, this evidence suggests that variations in incubation temperature may be employed to differentiate ligand subclasses at the strychnine-insensitive glycine receptor.

613.4

THE AMPA/QUISQUALATE RECEPTOR IS A SUBSTRATE FOR CALPAIN. P. Vanderklish*, M. Kessler, R. Hall*, B.A. Bahr, K. Sumikawa[1], and G. Lynch, Center for the Neurobiology of Learning and Memory and [1]Dept. of Psychobiology, University of California, Irvine, CA 92717.

The subclass of glutamate receptors selective for AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid) generates the largest component of postsynaptic responses evoked at glutamatergic synapses and recent data by Staubli *et al.* (*Psychobio.* 18:377, 1990) have identified the conductance of AMPA receptors as the variable of expression in LTP, a lasting enhancement of synaptic strength triggered by postsynaptic Ca^{2+}-influx. It is of interest, then, to elucidate the mechanisms regulating these receptors, especially those which are activated by Ca^{2+}. We have found the AMPA receptor to be a good substrate for the micromolar-requiring form of the Ca^{2+}-activated neutral proteases (calpains). *In vitro* digestion of synaptic plasma membrane proteins (prepared from telencephalon) with 1μg calpain I per mg membrane protein (in 20 mM HEPES/Tris, 35°C, 0.5 mM $CaCl_2$, pH 7.4) resulted in the cleavage of the AMPA receptor and a concomitant loss in specific [^{3}H]AMPA binding. As evidenced by the use of a polyclonal antibody specific for Glu-R1 (one of the subtypes of the AMPA receptor, cloned by Hollmann *et al.*, *Nature* 342:643, 1989), this cleavage is characterized by the generation of a 95 kDa intermediate from the parent 102 kDa band within 30 seconds, followed by a gradual and almost complete loss of immunoreactivity on immunoblots. A similar cleavage profile was observed when hippocampal slices were depolarized in the presence of NMDA. The specificity of the anti-Glu-R1 antibody was confirmed by *i*) the comigration of the antigen with a protein which had been photo-affinity labeled with [^{3}H]CNQX (an AMPA receptor antagonist), *ii*) a loss of labeling at 102 kDa on immunoblots when the antibody was preabsorbed with the immunogen, and *iii*) comparable increases in specific [^{3}H]AMPA binding and immunolabeling following successive chromatographic steps. Determining whether this cleavage represents a way to change receptor properties or merely a mechanism of turnover will require functional studies of partially cleaved AMPA receptors. (Supported by AFOSR #89-0383.)

613.5

SUBSTANCE P AND POLYAMINES MODULATE THE NMDA RECEPTOR OPERATED ION CHANNEL BY SIMILAR MECHANISMS.

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Substance P (SP) has been shown to enhance NMDA receptor elicited responses in spinal neuron preparations. We hypothesized that SP and the polyamines spermine and spermidine, potentiate glutamate by similar mechanisms. SP 1-11, 1-7 and C-terminal SP fragments 7-11, 8-11 and 9-11 were tested for possible interaction with the NMDA receptor operated ion channel. SP 8-11 was the most efficacious fragments (EC_{50} 60-200 μM), increasing [^{3}H]MK801 binding 900% in well washed membranes and 20% in the presence of maximal glutamate and glycine concentrations, 100 and 10 μM respectively. SP fragments did not have affinity for or affect the kinetics of either [^{3}H]glycine or [^{3}H]CGS19755 binding. The enhancement of [^{3}H]MK801 binding was eliminated by glutamate or glycine receptor antagonists as well as putative polyamine antagonists putrescine, cadaverine and arcaine. SP 8-11 increased the association rate of [^{3}H]MK801 without affecting dissociation or number of binding sites. This is consistent with enhanced channel accessibility to [^{3}H]MK801. These results suggest that SP fragments modulate the NMDA operated channel by a mechanism similar to polyamines.

613.6

EFFECTS OF STRESS AND CORTICOSTERONE ON THE BINDING PROPERTIES OF GLUTAMATE RECEPTORS.

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The hippocampus is particularly vulnerable to the deleterious consequences of both acute and chronic stress. Since glutamate is the main excitatory neurotransmitter in the hippocampus, we investigated whether either the NMDA or AMPA subtypes of glutamate hippocampal receptors were modified in response to stress and corticosteroid administration by quantitative ligand binding autoradiography using ^{3}H-TCP and ^{3}H- AMPA as ligands for the NMDA and AMPA receptors, respectively.

Male Long -Evans rats were exposed for 1 hour to restraint plus 60, 1 mA, 1 sec tailshocks. Naive controls received no handling at all and another group was injected with corticosterone (1mg s.c.). While no change was observed in the binding properties of the ^{3}H-TCP under any condition, ^{3}H-AMPA binding was significantly decreased in several areas of the hippocampus of corticosterone-injected rats relative to naive controls. Restraint plus tailshock, however increased AMPA binding, even though corticosteroid levels were high. These results indicate that glucocoticoids may down regulate the AMPA receptor , but that some forms of stress trigger additional mechanisms that override the effects of glucocorticoids.

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613.7

CHRONIC NEONATAL MK-801 TREATMENT RESULTS IN LONG-LASTING CHANGES IN NMDA RECEPTOR SENSITIVITY AND INCREASED SEIZURE SUSCEPTIBILITY

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Persistent effects of neonatal interference with NMDA receptors were investigated by measuring 1) NMDA receptor sensitivity to its agonist and antagonists and 2) susceptibility to "kindling" evoked seizures. Rats were neonatally treated with MK-801 (two s.c. injections per day, 0.25mg/kg, on postnatal days (PND) 8-19) and micro-iontophoretically tested for NMDA receptor sensitivity in the hippocampal CA1 pyramidal cells at PND 70-100. In a dose-dependent fashion NMDA evoked responses were more severely suppressed by APV, but not by MK-801 in the MK-801 treated group. Furthermore, hippocampal kindling in adulthood showed an increase in the electrical seizure duration during the first kindling stage in the MK-801 group. These results suggest that neonatal interference with MK-801 leads to a long-lasting change in NMDA receptor sensitivity to APV, which in turn could accompany the observed increase in seizure susceptibility.

613.8

CHOLERA AND PERTUSSIS TOXIN SENSITIVE G-PROTEIN ON NMDA/PCP RECEPTOR ION CHANNEL COMPLEX. T.Hori[1*], T.Yamamoto[1], H.Yamamoto[1], K. Hatta[1], T. Moroji[1] and K. Yoshikawa[2]. Dept. of Psychopharmacology[1] and Molecular biology[2], Psychiatric Research Institute of Tokyo, Tokyo 156, JAPAN.

We previously reported that there may exist two Mg^{2+} sites on the N-methyl-D-aspartate (NMDA)/phencyclidine (PCP) receptor ion channel complex; the high affinity Mg^{2+} (≤300 uM), which stimulates and low affinity Mg^{2+} (≥1 mM) site, which inhibits [^{3}H]TCP binding. We also showed that GppNHp decreased the affinity of Mg^{2+}-stimulated [^{3}H]TCP binding. In the present study, we investigated a possible interaction of the GTP binding site and high affinity Mg^{2+} site or glycine site.

The binding of [^{3}H]TCP to rat cerebral cortices was determined as described before (Neurosci Lett 119; 9-11, 1990). GDPβS, GTP, GTPγS, GppNHp and GDP potently inhibited Mg^{2+}-stimulated [^{3}H]TCP binding (by more than 50 %), whereas ATP, CTP, GMP, cGMP, ITP and guanosine exhibited no significant inhibition. GppNHp reduced Mg^{2+}-stimulated binding by only 16 % in cholera toxin (CTX)-, but not pertussis toxin (PTX)-, treated membranes. On the other hand, GppNHp inhibited the glycine-stimulated [^{3}H]TCP binding to CTX- and PTX-pretreated membranes by 31 % and 20 %, respectively. The present findings indicate that the high affinity Mg^{2+} site may be coupled to CTX sensitive G-protein (Gs) and glycine site may be coupled to both CTX and PTX sensitive G-protein (Gs and Gi/o).

613.9

DIFFERENTIAL EFFECT OF SUBCHRONIC COMPETITIVE AND NON COMPETITIVE NMDA RECEPTOR ANTAGONISTS ON HABITUATION IN RATS. M.P.Pellicano*, A.Cerbone and A.G.Sadile. (SPON: European Brain and Behaviour Society). Dip. Fisiologia Umana e Funzioni Biologiche Integrate "F.Bottazzi", Univ. Napoli, I.

Non contingent multiple injections of N-methyl-D-aspartate (NMDA) receptor antagonists were used to test the behavioral response on habituation to novelty. Adult male Sprague-Dawley rats were given non competitive (Ketamine: 6 mg/Kg; MK-801: 0.1mg/Kg) or competitive (CPP: 0.1mg/Kg) NMDA receptor antagonists, or drug vehicle intraperitoneally twice a day for 7 days. They were all tested in a Làt-maze 12 hr after the last injection. Additional injections were given 12, 24 and 36 hr after testing. Retention was run 48 hr later. Habituation of activity and defecation score was measured by the between-test decrement in corner-crossings and rearings (with prevailing cognitive and noncognitive meaning, respectively) and of fecal boli (non cognitive). Subchronic blockade of NMDA receptors by low doses of competitive antagonists had no effect on habituation of horizontal and vertical activity. In contrast, non competitive antagonists impaired habituation of horizontal and vertical activity during the entire test, but only MK-801 did it significantly ($p < 0.025$). Thus, allosteric and isosteric blockade of high-affinity NMDA receptors differentially affect the processing of cognitive and noncognitive behavioral components, as suggested earlier (Behav.Brain Res., 39:187, 1990). **(Supported by CNR/MURST 40% grants).**

613.10

CHLORIDE TRANSPORT BLOCKERS PREVENT NMDA RECEPTOR-CHANNEL COMPLEX ACTIVATION

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Activation of the N-methyl-D-aspartate (NMDA) receptor-channel complex has been implicated in a number of physiological and pathological phenomena. In particular, it mostly mediates the neurotoxic effect of the neurotransmitter glutamate. The NMDA complex contains several distinct binding sites targets for regulation by endogenous as well as exogenous compounds. In cultured spinal cord neurons we found that blockers of chloride transport (furosemide, a widely used loop diuretic, and the related compounds piretanide and bumetanide; niflumic and flufenamic acids, used as antiinflamatory agents) prevented NMDA receptor activation. The inhibitory effect was dose-dependent and specific for this class of glutamate receptor, as kainate- and AMPA-activated currents were only slightly altered. Antagonism of NMDA mediated currents by chloride transport blockers was voltage-independent and showed fast on-off kinetics. The action was non-competitive with NMDA and did not arise from interaction with the Zn^{2+} inhibitory site since blockade of NMDA induced responses by furosemide and Zn^{2+} was additive. The inhibition was greater in low concentration of glycine but it could not be overcome by increasing glycine concentration (up to 100 μM). In contrast the inhibition was attenuated by the polyamine spermine. Since the presence of spermine was not required for inhibiton to develop, we conclude that chloride transport blockers are non-competitive antagonists of the NMDA receptor acting as inverse agonists of the polyamine receptor and/or as agonists of the postulated inhibitory polyamine site. This action may explain the protective effect that has been shown for some of these drugs in neuronal degeneration.

613.11

N-METHYL-D-ASPARTATE RECEPTORS (NMDA) ARE ACTIVATED BY 4-METHYLPYRAZOLE, AN ALCOHOL DEHYDROGENASE INHIBITOR. W.M. Cintra, M. Marchioro*, Y. Aracava and E.X. Albuquerque. Lab. Mol. Pharmacol. II, IBCCF, UFRJ, RJ, Brazil, 21941; Dept. Pharmacol. Expt. Ther., Univ. Maryland Sch. of Med., Baltimore, MD, 21201.

It has been previously shown that pyrazole, an alcohol dehydrogenase inhibitor, is able to interact with NMDA receptors on hippocampal neurons (*Neurosci. Abst.*, 16:86, 1990). In this work, using the single channel patch-clamp technique we evaluated the effects of 4-methylpyrazole on outside-out patches excised from cultured fetal rat hippocampal neurons. After the outside-out configuration was achieved, the patch was placed inside a glass mini-pipe conected to a perfusion system that was used to deliver the drugs. Nominally Mg^{2+}-free solutions were used for the records. 4-methylpyrazole (1-10 μM) induced channel openings that resembled those activated by NMDA and by low concentrations of pyrazole. The voltage-current relationship showed that these channels had a predominant conductance value of 50 pS. Openings interrupted by numerous brief closures were seen and the burst pattern was more pronounced at hyperpolarized potentials. As the membrane was hyperpolarized, the number of events per burst increased and the duration of intraburst openings decreased. This channel activity was blocked by APV (100 μM), a competitive antagonist of NMDA receptors. In contrast to pyrazole, which activated and blocked the NMDA receptors at similar concentrations, the methylated derivative did not block the NMDA channels at the concentrations tested. These findings coupled to a very simple and rather rigid structures of pyrazoles make this series of compounds promising probes for structure-activity relationship analysis regarding activation and blockade of the NMDA receptors. Support: U.S. Army Med. Res. & Devel. Comm. Contr. DAMD-17-88-C-8119, FINEP/UMAB Mol. Pharmacol. Training Program and CNPq-Brazil.

613.12

NMDA STIMULATES DOPAMINE RELEASE IN THE STRIATUM OF AWAKED RATS: A MICRODIALYSIS STUDY. D. Martínez-Fong, M. Rosales*, J.L. Góngora*, S. Hernández* and J. Aceves. Dept. of Physiology, CINVESTAV-IPN, Ap. Postal 14-740, 07000 México, D. F. México.

In vitro studies have demonstrated that glutamate may modulate dopamine (DA) release in striatum (Krebs et al. J. Neurochem. 56:81, 1991). We have explored in vivo the participation of NMDA receptors in this modulation. A guide cannula was stereotaxically implanted into the right striatum of anaesthetized male wistar rats (300 g). Five days after surgery DA was recollected by 3 mm microdialysis probes and measured by HPLC.

NMDA (90-800 µM) perfused through the probe caused a dose-dependent DA release. This effect was reversible and blocked by 2-amino-5-phosphonovalerate. Also, the NMDA evoked DA release was blocked by increasing Mg^{2+} concentration to 2.5 mM in the perfusion ringer. No turning behavior was seen in spite of the finding that NMDA induced an increase in striatal DA up to 1,700% of basal value. The accurate position of the cannula was verified histologically.

These results suggest that glutamate from the cortico-striatal pathway may stimulate in vivo the DA release of nigro-striatal terminals via NMDA receptor.

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613.13

N-METHYL-D-ASPARTATE UNILATERALLY INJECTED INTO THE STRIATUM OF RATS PRODUCES CONTRALATERAL CIRCLING:ANTAGONISM BY 2-AMINO-7-PHOSPHOHEPTANOIC ACID AND CIS-FLUPENTHIXOL. P.K.Thanos, K.Jhamandas and R.J.Beninger. Department of Psychology, Queen's University, Kingston K7K 3N6, Canada.

To evaluate the contribution of striatal glutamate receptors to motor control, circling responses were observed in rats following unilateral intrastriatal microinjections of the agonist, N-methyl-D-aspartate (NMDA) or the antagonist, 2-amino-7-phosphoheptanoic acid (APH). The role of dopamine (DA) in NMDA-produced circling also was evaluated. In experiment 1, an NMDA dose of 5.0 µg (in 0.5 µl), but not 0.5 or 0.05 µg produced significant contraversive circling. In experiment 2, an APH dose 10.0 µg but not 1.0 or 0.1 µg produced significant ipsiversive circling. In experiment 3, microinjection of the ineffective 0.1 µg dose of APH or a dose (20 µg) of the DA antagonist, cis-flupenthixol, that did not produce circling when administered alone, significantly reduced the circling response produced by the 5.0 µg dose of NMDA. As NMDA produced circling in the same direction as that seen following similar unilateral injections of locomotion-stimulating DA agonists, the present results suggest that glutamate, acting via NMDA receptors in the striatum, may exert an excitatorry influence on motor systems. The observation that a DA receptor blocker antagonized the NMDA response further suggests that the observed motor excitatory effect of glutamate at NMDA receptors requires concurrent stimulation of DA receptors in the same region of the striatum.

613.14

AMINO ACID NEUROTRANSMITTER RELEASE FROM KAINATE-TREATED CEREBELLAR CULTURES. K.L. Rogers[1], M.L. Simmons[2], G.R. Dutton[2]. Departments of Psychiatry[1] and Pharmacology[2], University of Iowa.College of Medicine, Iowa City, IA 52242.

Post-natal rat cerebellar cultures were treated with 50µM kainate from day five to day nine *in vitro*. Release of endogenous amino acids in response to kainate stimulation was quantified by reverse-phase HPLC at day nine and at day 11 (following 48 hours of recovery). Release of glutamate and GABA was suppressed at both time points in the cultures exposed to kainate as compared to untreated cultures. We have previously reported that in kainate-treated cultures depolarized with 50 mM potassium, the measured glutamate efflux recovers following the removal of kainate (day 11), whereas GABA release remains suppressed due to loss of inhibitory interneurons. This would indicate that the granule cell kainate receptors may be persistently down-regulated following chronic kainate exposure since the non-specific potassium stimulation of glutamate efflux recovers. Kainate-induced neurotransmitter efflux is calcium-dependent at the doses applied, and is receptor-mediated based on inhibition of amino acid release with co-administration of CNQX but not CPP or MK-801. Supported by NS20632.

613.15

POSSIBLE INTERACTION BETWEEN NMDA AND DOPAMINE RECEPTORS. O.GANDOLFI and R.DALL'OLIO*, Dept. of Pharmacology University of Bologna, ITALY, 40126.

The administration of the NMDA non competitive antagonist MK-801 (0.25 mg/kg i.p.) produced a long lasting increase in rat locomotor activity which is blocked by D--cycloserine (3 mg/kg i.p.) or spermidine (250 µg/rat). Although these results strongly suggest that this effect is specific for NMDA receptor blockade, direct or indirect DA-ergic mechanisms could be involved. In well washed membranes the (3H)-MK-801 specific binding was decreased by the "in vitro" addition of either SKF 38393 or LY 171555 (100 µM). The stimulatory effect of (3H)-MK-801 binding by glutamic acid was inhibited by dopaminergic agents in an apparently non competitive manner. Acute treatments neither with indirect DA-ergic agents neither with specific D1 or DE DA-ergic drugs failed to change (3H)-MK-801 specific binding. Repeated administrations with MK-801 (0.25 mg/kg i.p. daily for 21 days) failed to change (3H)-MK-801 specific binding, in contrast (3H)-spiroperidol binding was decreased. Consistently LY 171555 induced hyperactivity was reduced while neither (3H)-SCH 23390 binding nor SKF 38393 induced grooming behavior were affectd.

613.16

PHARMACOLOGICAL EVIDENCE SUGGESTING MULTIPLICITY OF KAINATE RECEPTORS IN THE RAT CENTRAL NERVOUS SYSTEM. Aizawa H.*[1], Kwak S.[1], Ishida M.[2] and Shinozaki H.[2]. [1]National Institute of Neuroscience, Tokyo 187, [2]The Tokyo Metropolitan Institute of Medical Science, Tokyo 113, Japan.

Effects of various kainate analogues, including acromelic acid (ACRO) and newly synthesized kainate derivatives 4-(2-hydroxyphenyl)-2-carboxy-3-pyrroridineacetic acid (HFPA) and 4-(2-methoxyphenyl)-2-carboxy-3-pyrroridineacetic acid (MFPA), on [^{3}H]kainate and [^{3}H]AMPA binding to rat spinal cord synaptic membranes were investigated. Scatchard analysis of the saturation binding experiments revealed a single population of [^{3}H]kainate and [^{3}H]AMPA binding sites with Kd of 6.2 ± 0.35 and 7.6 ± 1.5 nM , each exhibiting similar value to Kd of the high affinity [^{3}H]kainate and [^{3}H]AMPA binding sites in the brain, respectively. The rank order of the potency to inhibit the binding of [^{3}H]kainate was domoate ≥ HFPA ≥ kainate ≥ MFPA > quisqualate ≥ ACRO B ≥ L-glutamate ≥ ACRO A >> AMPA and that of [^{3}H]AMPA was quisqualate > AMPA > MFPA ≥ ACRO A ≥ L-glutamate ≥ HFPA > domoate > kainate. ACRO, MFPA and HFPA depolarize newborn rat spinal motoneurons and dorsal root C-fibers more potently than kainate. Therefore, ACRO may exert the potent depolarization not through activating either kainate or AMPA receptors but through other receptors. Present results lend support to the presence of plural kinds of kainate receptor subtypes which we have presumed from the complicated systemic neurotoxic effects of ACRO and kainate in the rat.

614.1

SELECTIVE KAPPA ACTIVITY OF DYNORPHIN Ia (DYN Ia) IN MICE. V.K. Shukla*, M. Bansinath* and S. Lemaire. Department of Pharmacology, University of Ottawa, Ottawa, Ontario, Canada and Department of Anesthesiology, NYU Medical Center, New York, USA.

Effect of Dyn Ia (i.c.v.) was studied on the visceral pain (acetic acid-induced writhing), gastrointestinal transit and body temperature in mice. In control groups, Dyn-Ia dose dependently protected against acetic acid-induced writhing with an ED_{50} of 1.78 nmol/mouse (95% confidence limits (CL) 0.72-0.84 nmol/mouse). Nor-binaltorphimine pretreatment (10 nmol/mouse 15 min before) increased the ED_{50} of Dyn Ia to 4.61 nmol/mouse (95% CL, 3.67-5.81 nmol/mouse). The potency ratio between control and nor-binaltorphimine pretreated groups was 0.39 (95% CL, 0.27-0.55, ($P<0.05$). The ED_{50} of Dyn Ia was 2.45 nmol/mouse (95% CL 1.82-3.31 nmol/mouse) in metaphit pretreated (10 umol/mouse 24 hr before) group. The potency ratio between control and metaphit pretreated group was not significant. Dyn Ia (i.c.v., 1-10 nmol) did not have any effect on gastrointestinal transit and body temperature. These results indicate that the potent antinociceptive effect of supraspinal Dyn Ia in the writhing test is mediated by the stimulation of the κ opioid receptor and is not accompanied by any change in gut motility and body temperature. Supported by the MRCC.

614.2

PHARMACOLOGICAL CHARACTERIZATION OF NALBUPHINE, A MIXED KAPPA$_1$ AND KAPPA$_3$ OPIOID ANALGESIC. C.G. Pick, D. Paul, and G.W. Pasternak. The Cotzias Lab. of Neuro-Oncology, Memorial Sloan-Kettering Cancer Center and Depts. of Neuroscience and Pharmacology, Cornell U. Medical College, New York, NY 10021.

Nalbuphine is potent mixed agonist/antagonist analgesic with little respiratory depression, presumably due to low efficacy at μ receptors. We now present evidence suggesting that nalbuphine analgesia is mediated through a combination of κ_1 and κ_3 receptors. In CD-1 mice, nalbuphine produced a dose-response analgesia in the tailflick assay (ED_{50} 41.8 mg/kg). This analgesia was reversed by the κ_1-selective antagonist nor-BNI but not by the μ antagonist β-FNA or the δ antagonist naltrindole, implying a possible κ_1 mechanism. Nalbuphine demonstrated cross-tolerance with the κ_1 agonist U50,488H and with the κ_3 agonist NalBzoH, but not with morphine. These results suggest the importance of κ_3 receptors as well as κ_1. Both spinal and supraspinal mechanisms of action were important for nalbuphine analgesia. Nalbuphine was equipotent when injected i.c.v. or i.t. However, following systemic administration, the supraspinal mechanism predominates since Win44,441 antagonized nalbuphine analgesia over 100-fold more potently when the antagonist was administered i.c.v. than i.t. Nor-BNI effectively antagonized systemic nalbuphine analgesia when given i.t. but not i.c.v. Together, these results imply that nalbuphine analgesia involves both a supraspinal κ_3 and a spinal κ_1 opioid receptor mechanisms, with the supraspinal component playing the major role following systemic administration.

614.3

AMNIOTIC-FLUID INGESTION INHIBITS CONTRALATERAL CIRCLING AFTER UNILATERAL MORPHINE INJECTION INTO THE VENTRAL TEGMENTAL AREA. A.C. Thompson, J.M. Di Pirro, C.D. Dickerson* and M.B. Kristal. Dept. of Psychology, SUNY at Buffalo, Buffalo, NY 14260.

Ingestion of amniotic fluid (AF) or placenta has been shown in rats to enhance opioid-mediated analgesia, but not to affect opioid-mediated hyperthermia. The present study assessed the effect of ingestion of AF on another opioid-mediated behavior: contralateral circling induced by ventral tegmental (VTA) microinjection of morphine. We decided to test this opioid-induced behavior because its neural mechanism has been dissociated from that producing analgesia.

Rats were given unilateral morphine injections (0.0, 0.1, 0.3, or 1.0 μg) into the VTA, followed by orogastric infusion of 0.25 ml AF or control fluid (beef bouillon, BB). Circling was monitored for 1 hr following injection. The dependent measure was *net number of contralateral circles per hour* (NCC/hr). Pain threshold was assessed (using tail-flick latency) at 1 hr post-treatment to confirm that morphine-induced analgesia had not been produced. Each rat was tested at all doses of morphine in a balanced design.

As expected, NCC/hr among BB-infused rats (n = 4) was significantly elevated at all doses of morphine (NCC/hr = 35 - 60 after morphine injections; NCC/hr = 0 after vehicle injection). In contrast, NCC/hr among AF-infused rats (n = 4) was not elevated after any dose of morphine ($p < 0.01$) (NCC/hr ≈ 0 after all morphine injections and after the vehicle injection). Pain threshold was unaffected by any treatment (no analgesia was produced).

These results demonstrate that the effect of AF ingestion on opioid-mediated behavior is not limited to analgesia and is not mediated through a single neuroanatomic site.

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614.4

VENTRAL TEGMENTAL AREA (VTA) AND NUCLEUS ACCUMBENS (NAS) *k*- OPIOID AGONIST, U50488H, INHIBITS MALE SEXUAL BEHAVIOR. M. Leyton and J. Stewart. Center for Studies in Behavioral Neurobiology, Department of Psychology, Concordia University, Montréal, Québec, Canada H3G 1M8.

We previously reported that systemic injections of the *k*- agonist U50488H (U50) naloxone reversibly decrease both appetitive and consummatory aspects of male sexual behavior (Leyton & Stewart, 1991a,b). Moreover, selective aspects of these U50-induced deficits could be differentially reversed by infusions of the *k*- antagonist nor-binaltorphimine into the VTA, NAS and medial preoptic area (mPOA). In the present study, male rats with bilateral VTA, NAS or mPOA cannulae were centrally administered U50 (.0005-5.0 nmol/.5µl/side). Infusions of U50 into the VTA decreased the proportion of males to ejaculate, and increased the latencies to mount, intromit and ejaculate at the lowest dose. Intra-NAS U50 decreased the proportion of males to intromit and ejaculate, and, the mean number of ejaculations as well as increasing the latencies to mount, intromit and ejaculate except at the highest dose. Intra-mPOA U50 did not significantly alter any measures. Comparing these findings to our previous reports suggests that mPOA *k*- receptor activation only weakly inhibits sexual behavior in otherwise unhampered males, but, synergistically potentiates the inhibition of appetitive aspects of sexual behavior induced by VTA or NAS *k*- receptor stimulation. Conversely, *k*- receptor stimulation in either the VTA or NAS is sufficient to delay the initiation of male sexual behavior.

614.5

NEURAL SUBSTRATES UNDERLYING AMYGDALOID CONTROL OF DEFENSIVE RAGE ELICITED FROM THE PERIAQUEDUCTAL GRAY IN THE CAT. M.B. Shaikh and A. Siegel. Department of Neurosciences, N.J. Medical School, Newark, NJ 07103.

Recently, we have shown that opioid peptides powerfully suppress feline defensive rage (DR) from the midbrain periaqueductal gray (PAG) and that a primary source of such input is the central amygdaloid nucleus (CE). This study provides anatomical/immunocytochemical evidence in support of this hypothesis. Cannula electrodes were implanted into the PAG for elicitation of DR as well as for infusion of non-selective and selective opioid antagonists and the retrograde tracer, Fluoro-Gold (FG)[4-8%]. Monopolar electrodes were implanted into amygdaloid sites from which suppression of DR was obtained. Brain tissue was processed for identification of met-enkephalinergic (met-enk) cell bodies within the amygdala. CE suppression of DR was blocked by infusion of naloxone or B-FNA into the PAG. Met-enk cells were located within the CE and retrogradely labelled cells within the ventral CE and adjoining regions of the lateral and basal nuclei. Neurons double labelled with FG and met-enk were identified within the rostral half of CE and adjoining portions of the lateral nucleus. Sites from which suppression of DR was obtained corresponded to the same general regions where single or double labelled neurons were identified. The results provide evidence that met- enk neurons from the CE can directly inhibit DR at the level of the PAG.
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614.6

ENHANCEMENT OF MET-ENKEPHALIN STAINING IN THE RAT BRAINSTEM AS A RESULT OF SOCIAL STRESS. C.A.Cohen, R.M. Kream*, J.E. Marchand, and K.A. Miczek. Department of Psychology, Tufts University, Research Building, 490 Boston Ave., Medford, MA 02155 and the Department of Anesthesiology, Tufts University School of Medicine, 136 Harrison Ave., Boston MA 02111.

Immunohistochemistry was used to assess the effects of social stress on the endogenous opioid system. Similar studies have already indicated that socially stressed animals show marked potentiation of the analgesic effect of morphine, as demonstrated by a shift in the dose response curve to the left, during the social stress experience. Within 24 hours after social confrontation, there is also evidence for mild tolerance to opiate analgesia, and tolerance continues to develop as a function of time. In the current experiment an intruder male rat was placed into the home cage of a resident and subsequently exposed to attack and threat. Once the animal showed unambiguous signs of submission (i.e. through ultrasounds and supine postures), the intruder was placed into a protective cage for one hour while being exposed to the threat of an attack. No physical harm could come to the intruder at this time. The intruders were perfused with saline followed by 4% paraformaldehyde, at various time intervals after the social stress experience. The brains were removed and placed in fixative for two hours followed by a solution of 20% sucrose and 0.1M phosphate buffer where they remained until sectioning. The control animals were housed in the same room and were located in proximity to the defeated animals. They were perfused and their brains handled in the same manner as mentioned above. All brains were sectioned (32 µm) and prepared for immunohistochemistry using antibodies for met-enkephalin. Preliminary observations suggest that at the mesencephalic and pontine levels there was an increase in met-enkephalin immunoreactive elements in the central gray surrounding the aqueduct in the socially stressed animals three hours after the encounter, in comparison to controls. These changes are being monitored via radioimmunoassay and molecular biological techniques.

614.7

DIFFERENTIAL EFFECTS ON MOUSE LOCOMOTOR ACTIVITY OF MU-SELECTIVE VS DELTA-SELECTIVE OPIOIDS. M. E. Nevins and S. A. Nash* Dept. of CNS Diseases Research, Searle, Skokie, IL 60077.

It has been suggested that mu-selective and delta-selective opioids produce different profiles of locomotor activation after i.c.v. administration to mice. Michael-Titus, et al. [Neuropeptides (1989) 13,235-242] and Mickley, et al. [Psychopharmacology (1990) 101,332-337] showed that while mu-selective opioids such as morphine and [D-Ala^2 Me Phe^4 Glyol] enkephalin (DAMGO) produce large increases in horizontal movements and decreases in vertical movements, the delta-selective opiate [D-Pen^{2,5}] enkephalin (DPDPE) increases horizontal movements to a lesser extent and either increases (Michael-Titus, et al.) or does not affect (Mickley, et al.) vertical movement. The current study was conducted to determine whether the profile of locomotor activation reported for DPDPE would extend to other delta-selective peptides. The locomotor activity of male CD-1 mice was monitored by use of infrared photocell-based activity monitors (Omnitech Electronics). Mice were habituated to the monitors for 20 min. prior to i.c.v. drug administration. The results show that [D-Ala^2] Deltorphin I produced a locomotor profile similar to that of DPDPE, while [D-Ser^2-Leu] enkephalin (DSLET), [D-Thr^2-Leu] enkephalin (DTLET), and [D-Ala^2-D-Leu^5] enkephalin (DADLE) produced profiles similar to the mu-selective agents. The results are discussed in terms of delta-receptor selectivity.

614.8

MECAMYLAMINE ALTERS DIFFERENTLY THE MORPHINE- AND METHADONE-INDUCED CATALEPSY AS WELL AS CHANGES IN STRIATAL DOPAMINE METABOLISM. L. Ahtee, M. Attila and P. Lehtonen*. Div. of Pharmacology and Toxicology, Dept. of Pharmacy, Univ. of Helsinki, SF-00170 Helsinki, Finland.

Morphine and methadone induce catalepsy in rats and elevate the acidic dopamine metabolites. The nicotinic antagonist, mecamylamine, reduces methadone catalepsy (Ahtee, Eur.J.Pharmac. 1976:**39**:203) but prolongs morphine catalepsy (Ahtee & Kaakkola, Br.J.Pharmac. 1978:**62**: 213). In the present experiments mecamylamine (4 mg/kg i.p.) given 20 min before the opioids potentiated and prolonged the cataleptic effect of morphine (10 mg/kg s.c.) but reduced that of methadone (6 mg/kg s.c.). Both morphine (20 mg/kg) and methadone (10 mg/kg) elevated the striatal DOPAC and HVA concentrations. Mecamylamine pretreatment antagonized these effects of morphine (by 70-80%) more than those of methadone (by 30-35%). However, mecamylamine antagonized the methadone-induced decrease of striatal 3-MT concentration but tended to potentiate the morphine-induced decrease of 3-MT. Thus the mecamylamine-induced alterations in morphine and methadone catalepsy could be related to changes in striatal dopamine metabolism.

614.9

MU OPIOID ACTIVITY IN MEDIAL THALAMUS: EFFECTS ON BEHAVIOR AND DOPAMINE UTILIZATION. K.D. Carr, S. Uysal, J.W. Schweitzer* and A.J. Friedhoff. Millhauser Labs, NYU Medical Center, NY, NY 10016.

Subcataleptic doses of the mu-selective opioid agonist DAMGO, infused into the lateral segment of the thalamic dorsomedial nucleus, elevate stimulus thresholds for pain vocalization and brain stimulation reward (Carr and Bak, 1988). Higher doses produce naloxone-reversible catalepsy. In the present study, bilateral medial thalamic (MT) infusions of DAMGO (1.0 ug) increased latencies to shuttle-escape from gridshock, while the kappa-selective agonist, U50,488 (25.0 ug) and the delta-selective agonist, DPDPE (10.0 ug) had no effect. Pre-infusion of the mu-selective antagonist, CTP (5.0 ug), blocked the DAMGO-induced increase in escape latency while pre-infusion of the delta-selective antagonist, ICI 174,864 (5.0 ug), did not. Lower doses of DAMGO (0.1 to 0.25 ug) sometimes reduced conditioned avoidance without affecting escape from gridshock. Many of the above mentioned behavioral effects of MT DAMGO are also produced by systemically administered neuroleptics. Moreover, MT has strong anatomical and functional connections with several forebrain dopamine (DA) terminal regions. To investigate whether modulation of dopaminergic activity may account for the behavioral effects of MT DAMGO infusion, we used HPLC to measure DA and DA metabolite levels in striatum, nucleus accumbens, and medial prefrontal cortex (mpfc) of rats sacrificed 20 minutes after MT DAMGO (1.0 ug) infusion. MT DAMGO elevated DOPAC levels in mpfc (saline = 0.39 $\pm$ 0.015 ng/mg protein vs DAMGO = 0.54 $\pm$ 0.03 ng/mg protein; $t(6) = 4.37$, $p<.005$) and tended to elevate DOPAC levels in striatum and accumbens as well. Systemically administered neuroleptics also increase DA utilization and elevate DA metabolite levels but do so as a compensatory response to postsynaptic receptor blockade. Whether MT DAMGO alters postsynaptic sensitivity to DA release remains to be determined.

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614.10

EVIDENCE FOR THE CONTRIBUTION OF NON-OPIOID MECHANISMS IN THE ANTINOCICEPTION PRODUCED BY THE CENTRAL ANALGESIC TRAMADOL. W.S. Reimann, N. Selve, and J.L. Vaught, Grunenthal Gmbh, Dept. Pharmacology, W-5100 Aachen, FRG, and The R.W. Johnson Pharmaceutical Research Institute, Welsh & McKean Roads, Spring House, PA 19477-0776.

Tramadol is a centrally acting analgesic with low affinity for opioid receptors and the ability to inhibit norepinephrine (NE) and serotonin (5-HT) uptake as well as facilitate 5-HT release. The antinociceptive effects of tramadol are only partially reversed by naloxone (Nlx) in some tests. Thus, the contribution of potential non-opioid mechanisms was the focus of the present research. In the Randall-Selitto test, tramadol produced a dose-related antinociception with an ED50 value of 4.6 mg/kg i.v. Nlx (1 mg/kg i.v.) only partially reversed tramadol-induced antinociception suggesting a non-opioid component. In the rat tail flick test, i.t. tramadol and morphine prolonged tail flick latencies. Nlx blocked morphine but not tramadol-induced antinociception. In contrast, the serotonin antagonist ritanserin (1 mg/kg i.p.) antagonized the effects of tramadol but not those of morphine. This antagonism was very similar to that previously reported for the alpha$_2$-adrenergic antagonist yohimbine. These results demonstrate the probable contribution of a serotonergic component to tramadol antinociception at spinal sites. In concomitant experiments we tested a small and ineffective dose of i.t. serotonin. When added to a subthreshold dose of the combination of morphine and desipramine, pronounced antinociception was observed demonstrating the potentiation of noradrenergic and opioid effects by a weak serotonergic stimulus.

CATECHOLAMINES: LOCUS COERULEUS

615.1

AFFERENTS TO THE RAT LOCUS COERULEUS (LC) USING CHOLERATOXIN B SUBUNIT (CTb) AS A RETROGRADE TRACER P.H Luppi,[1] G. Aston-Jones, H. Akaoka, P. Charléty[1], C. Kovelowski, M. T. Shipley[2], Y. Zhu, M. Ennis[2], P. Fort[1], G. Chouvet[1] and M. Jouvet[1]. Dept Mental Health Sci, Hahnemann Univ, Philadelphia, 19102; [1]CNRS UA 1195, INSERM U52 & 171, Lyon, France;[2]Univ. Cincinnati, Dept Anat Cell Biol, OH.

CTb when iontophoretically applied and visualized with streptavidin-HRP immunohistochemistry has been reported to be a highly sensitive retrograde and anterograde tracer (Luppi et al., 1990). Using CTb, we have reexamined the afferents to the LC. Physiologic recordings were made through glass micropipettes containing CTb (4µm tip, 1% CTb in PB 0.1M, pH 6) and the LC identified by its characteristic discharge properties. Iontophoretic application (0.5µA, 5min, n=20) of CTb produced injection sites that appeared to be restricted to the LC. The present results confirm major inputs from the nuclei paragigantocellularis and prepositus hypoglossi, and minor projections from the hypothalamic paraventricular nucleus (Aston-Jones et al., 1986). After some injections, that previous study noted occasional retrogradely labeled neurons in the rostral and posterior hypothalamic areas, median (MnR) and dorsal raphe (DR) nuclei, and periaqueductal gray (PAG). In contrast, after all CTb injections in the LC, we found numerous retrogradely labeled neurons in the lateral preoptic area (LPO), the posterior, dorsal and lateral areas of the caudal hypothalamus, the area ventrolateral to the MnR (B9 area), the area of the Kölliker-Fuse nucleus (KF), and the ventrolateral part of the PAG. Small numbers of cells were also observed in other nuclei including rostral DR, the area of the A5 noradrenergic cell group and nucleus parabrachialis lateralis. Control CTb injections in peri-LC areas (Barrington nuc., n=1, and mesencephalic trigeminal nuc., n=2) yielded a pattern of labeled cells different in some areas from that after LC injections indicating specificity of LC innervation. Anterograde tracing using CTb (n=3) and PHAL (n=3) from the LPO and the KF yielded numerous labeled fibers in the LC while injections in the ventrolateral PAG, the posterior hypothalamic areas and the B9 area gave rise to a moderate amount of fibers in LC. For PAG and hypothalamic labeling in particular, this labeling was more prominent in peri-LC areas and dendritic fields than within LC itself, possibly accounting for some retrograde labeling following LC injections. The demonstration of these additional afferents may be explained by higher sensitivity of CTb compared to the previously used WGA-HRP. Supported by grant NS 24698.

615.2

AUDITORY-EVOKED RESPONSES OF LOCUS COERULEUS (LC) NEURONS ARE ATTENUATED BY EXCITATORY AMINO ACID (EAA) RECEPTOR ANTAGONISTS IN THE AWAKE RAT. C. Chiang, A.L. Curtis, G. Drolet, R.J. Valentino, and G. Aston-Jones. Division of Behavioral Neurobiology, Dept. Mental Health Sciences, Hahnemann University, Philadelphia, PA 19102

LC neurons of behaving rats respond to multimodal non-noxious stimuli (Aston-Jones and Bloom, 1981). This study was designed to elucidate the transmitter mechanisms through which auditory stimuli exert their effects on LC. Experiments were performed in unanesthetized rats 5-7 days after implantation surgery. Extracellular single- and multi-unit activity were differentially recorded through stainless steel wires in the LC, and drug solutions (5 µl) were injected into the lateral ventricle. Activation of LC neurons by auditory stimuli (2-3 KHz, 50 or 100 ms duration, 90 dB) was significantly attenuated by the broad spectrum EAA antagonist kynurenate (KYN: 175 nmoles, 20.1 $\pm$ 4.9% of control, $n = 5$, $p < 0.05$). Similarly, the selective NMDA antagonist, 2-amino-5-phosphonopentanoic acid (AP5: 70 nmoles), also attenuated this activation (28.2 $\pm$ 7.2% of control, $n = 3$). The preferential non-NMDA antagonist, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX: 18 nmoles) caused a smaller reduction in the auditory-evoked response (65.6 $\pm$ 16.3% of control, $n = 4$), which was significantly different from the attenuations produced by KYN and AP5 ($F_{2,9} = 5.6$, $p < 0.05$). Recovery of the response was seen 30 to 60 min post-KYN and at 15 min post-CNQX; the response post-AP5 did not recover within 30 min. Vehicle injections of artificial CSF were largely ineffective. These preliminary results suggest that LC discharge evoked by phasically presented auditory stimuli requires EAA neurotransmission that may act primarily at NMDA receptors. Additional work is underway to localize the site(s) of EAA neurotransmission mediating these responses. Supported by PHS grants NS24698, DA06214, MH 40008, MH00840, MH42796, NARSAD (A.L.C.) and FRSQ (G.D.).

615.3

PUTATIVE GLUTAMATERGIC AFFERENTS TO THE NUCLEUS LOCUS COERULEUS FROM THE NUCLEUS PARAGIGANTOCELLULARIS: IMMUNOHISTOCHEMISTRY AND TRACT-TRACING.

G. Drolet and G. Aston-Jones, Division of Behavioral Neurobiology, Dept. Mental Health Sciences, Hahnemann University, Philadelphia, PA 19102

Recent investigations have found that the nucleus paragigantocellularis (PGi) in the ventrolateral medulla provides a major excitatory input to the nucleus locus coeruleus (LC). Morever, pharmacological studies revealed that this excitatory pathway, which mediates somatic- and visceral-evoked activation of the LC, uses an excitatory amino acid (EAA) neurotransmitter. The endogenous EAA used in this pathway is not known, but glutamate is a good candidate. In the present study, we combined retrograde transport and immunohistochemical techniques to identify the source of possible glutamatergic inputs to the LC. The retrograde tracer, a wheat germ agglutinin conjugate coupled to colloidal gold particles (WGA-apoHRP-Au), was injected into the electrophysiologically identified LC nucleus of two rats. After survival for 5-7 days, the rats received colchicine (100 µg, icv) and were perfused 24 hours later. A mouse monoclonal IgM antibody against phosphate-activated glutaminase (PAG, kindly provided by Dr. T. Kanebo, Kyoto University, Japan) which is a major synthetic enzyme of glutamate, was used to tentatively identify glutamatergic neurons. Numerous PAG-immunoreactive (ir) cell bodies were observed in the PGi. Preliminary double labeling studies indicated that PAG-ir neurons represent a substantial proportion of the LC-projecting neurons in the PGi. These results provide anatomical support for the existence of an excitatory amino acid pathway, putatively, glutamatergic, from PGi to LC. Supported by PHS grants NS 24698, DA 06214 and Le Fonds de la Recherche en Santé du Québec.

615.4

ELECTROPHYSIOLOGICAL CHARACTERISTICS OF LOCUS CERULEUS NEURONS IN MAUDSLEY REACTIVE AND NON-REACTIVE RAT STRAINS. J. S. Verbanac[2], R.L. Commissaris[2,3] H.J. Altman[3], G.M. Harrington[1] and D.K. Pitts[2]. [1]Univ. N. Iowa, Cedar Falls, IA, [2]Dept. of Pharm. Sci., Coll. Pharmacy & A.H.P., [3]Dept. of Psychiatry, Sch. Med., Wayne State Univ., Detroit, MI 48202.

The Maudsley Reactive (MR) and Non-Reactive (MNRA) inbred rat strains, have been shown to perform differently in various animal models for anxiety. Changes in noradrenergic function have been implicated in anxiety states. In the present study extracellular single-unit recording techniques were used to examine the physiology and pharmacology of noradrenergic locus ceruleus (LC) neurons in urethane anesthetized rats. The basal discharge rate of LC neurons in MR ("anxious"), MNRA ("non-anxious") and Sprague-Dawley (SD; outbred control) rats was 2.6 + 0.8 (n=7), 4.6 + 0.8 (n=6) and 3.0 + 0.8 (n=5) respectively. The sensitivity of somatodendritic alpha-2-adrenergic receptors to i.v. clonidine was also examined. An analysis of covariance (basal discharge rate=covariate) indicated that the dose-response (DR) curves for both Maudsley strains were significantly ($P< 0.01$) shifted to the right relative to SD rats and that MR and MNRA DR curves were not significantly different from each other ($P=0.23$). Although these studies are preliminary, a clear difference in clonidine sensitivity was observed between Maudsley and SD rats. A more complete analysis of the physiological and pharmacological characteristics is currently underway. (Supported by MH47181).

615.5

DECREASED TONIC DISCHARGE AND INDUCTION OF PERIODIC BURSTING OF LOCUS COERULEUS (LC) NEURONS AFTER ACUTE MORPHINE IN WAKING MONKEYS J.Rajkowski, H. Akaoka, C.J. Kovelowski, II and G. Aston-Jones Div. Behav. Neurobiol., Dept. Mental Health Sci., Hahnemann Univ. Philadelphia, PA 19102.

Substantial evidence indicates that the LC may be an important site of action for exogenous opiates. However, the effects of opiates on the electrical activity of LC neurons in conscious animals remain controversial, and never have been reported in primates. Here, the discharge activity of 11 neurons located in the LC region and tentatively identified as noradrenergic cells (>2 ms spike, low frequency, burst-pause response to salient stimuli) was recorded before, and up to 4 hours after i.m. injections of morphine sulphate in a chair restrained Cynomolgus monkey (3 cells each with 0.3 mg/kg, 1 mg/kg or 3 mg/kg, and 2 cells with 10 mg/kg). After injection, the animal sat quietly appearing sedated with his eyes open. One-half to 1 hour following morphine administration, the animal's eyes exhibited episodic slow drifts, the pupil diameter oscillated widely, and there were occasional short periods of drowsiness.

LC activity prior to drug administration was characteristically tonic and regular; closer analysis revealed that there were small oscillations in discharge rate occuring at a frequency of about 0.04 Hz. At 3 to 7 min after morphine injection, LC neurons showed pronounced periodic bursting activity which continued for the duration of the recording session. Such bursting also occurred with a frequency of 0.04 -0.05 Hz, as detected by autocorrelation of unit discharge, and was most pronounced about 20 min after injection. Interspike intervals increased with time after injection, resulting in an overall decrease of impulse activity. By 2 hrs following a high dose of morphine, LC neurons were nearly silent, even though the animal's eyes were fully open. Although most pronounced for higher doses of morphine, the above effects were observed for all cells tested. Supported by AFOSR grant 90-0147 and PHS grant DA 06214.

615.6

PHASIC LOCUS COERULEUS STIMULATION: EFFECTS IN THE RAT LATERAL GENICULATE NUCLEUS. R.N. Holdefer and B.L. Jacobs. Psychology Dept., Princeton Univ., Princeton, NJ 08544

Neuronal discharge in the locus coeruleus (LC) conveys to the CNS both a tonic information code related to behavioral state, and a phasic code elicited by sensory stimuli. Phasic activation of LC (0.26-0.62 mA at 30 Hz over 200 ms) in urethane-anesthetized rats increased spontaneous activity in the LGN by 9.4±6.0 spikes/s (mean±s.d.) in 13 out of 15 cells (onset latency: 299±70 ms, duration < 3.3 s). In some LGN neurons this increased discharge was preceded by a change from burst firing mode to a predominantly single spike mode. LGN activation was much weaker in animals with stimulating electrodes medial to the LC (2.5±2.2 spikes/s, $t=2.41$, $p<.05$). Microiontophoretic phentolamine (α-receptor antagonist, 5-10 nA) in the LGN partially reversed the increased firing after LC stimulation, but also decreased spike amplitude, making interpretation difficult. Increased LGN neuronal activity after LC stimulation was significantly reduced (2.9±4.4 spikes/s, $F=4.47$, $p<.05$) in animals pretreated (3-11 h) with a dose of AMPT (175-200 mg/kg, i.p.), known to deplete NE in the brain. We conclude that phasic LC activation can phasically activate LGN neurons in urethane-anesthetized animals. Supported by NSF BNS8921348.

615.7

MODULATION OF FOREBRAIN EEG BY THE LOCUS COERULEUS-NORADRENERGIC (LC/NE) SYSTEM. C.W. Berridge, *M. Page, *R.J. Valentino, S.L. Foote. UC San Diego; *Hahnemann Univ.

We previously reported that peri-LC infusions of the cholinergic agonist, carbachol (CARB) enhance LC neuronal activity and elicit forebrain EEG activation in halothane-anesthetized rats. These effects were observed only when the infusion needle is placed within 500 µm of the LC. To determine whether β-receptors are involved in these effects, we examined the effects of the β-receptor antagonist, DL-propranolol (PRO, 200 µg; ICV). Animals received a peri-LC CARB infusion (100 nl; 1ng/nl) 15 min after an ICV injection of PRO (3 µl). Whereas PRO did not affect the CARB-induced activation of LC, PRO blocked or severely attenuated the LC-stimulated activation of neocortical and hippocampal EEG. Preliminary data suggest an involvement of β_2-receptors in mediating the LC-induced activation of forebrain EEG. The effects of inhibition of LC neuronal activity by peri-LC infusions of the α_2-agonist, clonidine (125 nl; 1 ng/nl) were also examined. These infusions completely inhibited LC neuronal discharge activity for a 40-60 min period. Unilateral infusions typically had no obvious effects on cortical EEG activity. Bilateral inhibition of LC activity significantly increased the incidence and amplitude of slow-wave activity. Recovery of EEG activity was closely correlated with recovery of LC activity. These results suggest the LC/NE system is a potent modulator of forebrain EEG activity.

615.8

PACEMAKER ACTIVITY OF LOCUS COERULEUS (LC) NEURONS: DEPENDENCE ON cAMP AND PROTEIN KINASE AS SHOWN BY WHOLE-CELL RECORDINGS IN BRAIN SLICES.

M.Alreja and G.K. Aghajanian. Depts. of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06508.

Noradrenergic neurons of the rat LC are endogenous pacemakers that exhibit slow, tonic firing even in the complete absence of synaptic inputs. Conventional extracellular and intracellular recordings in LC neurons show persistent basal firing rates of ~1-2 Hz both in *in vivo* and *in vitro* preparations. In contrast, we now report a time-dependent decline in LC spontaneous firing rates during whole-cell recording with low-resistance patch electrodes. A majority of the neurons tested gradually stopped firing after establishing whole-cell configuration, presumably due to wash-out of an intracellular molecule. Inclusion of PKI_{5-24} (a specific inhibitor of cAMP-dependent protein kinase) in the patch pipette accentuated the washout phenomenon: in all the cells tested there was a rapid cessation of firing accompanied by a 2-7 mV hyperpolarization, suggesting the involvement of endogenous cAMP and protein kinase in pacemaker activity. The inclusion of cAMP, 8-Br-cAMP or the catalytic subunit of cAMP-dependent protein kinase (PKA_{cat}) in the patch-pipette prevented the loss of firing and dose-dependently increased LC firing rates; PKI_{5-24} blocked both 8-Br-cAMP and PKA_{cat}-induced firing in LC neurons.

We conclude that endogenous cAMP drives tonic pacemaker activity in LC neurons via cAMP-dependent protein kinase and its phosphorylation pathway.

615.9

Both Transient and Tonic Activation Elevates C-Fos Levels in the Locus Coeruleus. K. Bittman*, S. Grant, D.A. Highfield, M. Iadarola, B. Benno Dept. Psychology and Prog. in Neuroscience, Univ. Delaware, Newark, DE 19716; NIDR; and Wilm. Patterson College.

Increased neuronal activity induces the early intermediate gene product c-fos. The nuc. locus coeruleus (LC) is ideal for studying mechanisms coupling neuronal activity to c-fos induction. The LC has a homogenous (noradrenergic) composition, clear nuclear boundaries and well established pharmacological and physiological response patterns. Since past studies used treatments that elicit tonic elevations in firing rates, we immuhistochemically compared c-fos levels in LC neurons after transient sensory evoked and tonic pharmacological increases in neuronal activity.

Halothane anesthetized rats received either Yohimbine (5mg/kg) to tonically elevate LC firing, 30 min of brief intermittent contralateral footshocks (0.5 Hz, 1 msec, 1-9 mAmp) to evoke transient LC activation or no stimuli (controls). Rats were sacrificed 3 hours later. C-fos like immunoreactivity was stained using the ABC-peroxidase method and a polyclonal antibody that detects both c-fos and fos -related proteins.

C-fos like staining was present in the nuclei of cells in the LC (and surrounding areas) in response to both classes of stimuli, but not controls. Not all neurons in the LC exhibited c-fos like staining, even though presumably all LC neurons respond neurophysiologically to these treatments.

It is concluded that transient neuronal activation elevates c-fos levels, and that c-fos induction does not require high tonic firing rates. Furthermore, since LC neurons are spontaenously active and virtually no c-fos like staining was seen in controls, neuronal activity alone is insufficient for c-fos induction. Many cellular substrates of LC neuronal activation are known; this should facillitate determination of the critical events that link neuronal activation to c-fos expression in LC neurons.

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SEROTONIN IV

616.1

8-OH-DPAT PERFUSION INTO THE DORSAL RAPHE NUCLEUS OF THE FREELY MOVING RAT DECREASES TERMINAL BUT NOT SOMATODENDRITIC 5-HT RELEASE. *A. Adell and S. Hjorth. Dept. of Pharmacology, University of Göteborg, Box 33031, S-400 33 Göteborg, Sweden.*

Previous research has described the occurrence of extracellular serotonin (5-HT) in the vicinity of midbrain raphe nuclei of freely moving rats (Adell & Artigas, NSAP **343**: 237, 1991). The present work was undertaken to investigate whether the 5-HT release in the dorsal raphe nucleus (DRN) is regulated by cell body 5-HT_{1A} autoreceptors. For this purpose a microdialysis probe (1 mm membrane length) was stereotaxically implanted in the DRN. Approximately 24 h later probes were perfused with artificial CSF. Cumulative doses of 8-OH-DPAT (10 nM-1 μM) were then perfused. For every dose, the effect of a single 20-min pulse of K^+ (100mM) was tested. Dialysate 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) were analyzed by HPLC(EC). The basal extracellular levels of 5-HT and 5-HIAA were 1.62 ± 0.3 nM and 6.8 ± 0.5 μM, respectively, corrected for *in vitro* probe recovery (5%). K^+-stimulation increased dialysate 5-HT to 438% and decreased 5-HIAA to 56% of respective basal values. 8-OH-DPAT did not affect the spontaneous release of 5-HT in the DRN, but at concentrations ≥ 1 μM clearly enhanced the K^+-evoked 5-HT release (to 1670%). As this drug is known to inhibit the firing of 5-HT neurons in DRN, our results suggest that the spontaneous 5-HT output in this nucleus is not dependent on the firing of 5-HT neurons. In a second experiment rats were implanted with two dialysis probes, one in the DRN and the other in ventral hippocampus. Perfusion of 8-OH-DPAT (1 mM) through the probe located in the DRN decreased 5-HT and 5-HIAA output in the ventral hippocampus. The findings confirm that terminal, but not cell body, 5-HT release is regulated by somatodendritic 5-HT_{1A} autoreceptors.

616.2

ANTIDEPRESSANT-LIKE ACTIVITY OF COMPOUNDS WITH EFFICACY FOR THE 5-HT_{1A} RECEPTOR. A. Singh, J.N. Wolfe* and I. Lucki. Departments of Psychiatry and Pharmacology, University of Pennsylvania, Philadelphia, PA 19104.

The present study examined the ability of three novel compounds with high affinity but with varying efficacies for the 5-HT_{1A} receptor to produce antidepressant-like behavioral effects in the forced swim test in rats. The compounds used were: WY 48,723, WY 50,324 and WY 47,846, respectively an agonist, a partial agonist and a putative antagonist at the 5-HT_{1A} receptor. WY 50,324 is also a 5-HT_2 receptor antagonist. The rats received three subcutaneous injections of WY 48,723 (0.06-0.5 mg/kg), WY 50,324 (0.125-20 mg/kg) or WY 47,846 (10-20 mg/kg) after the 15-min pretest swim session. Behavioral immobility was measured during a 5-min test session, which occured one hour after the last injection. The 5-HT_{1A} agonist 8-OH-DPAT (0.5 mg/kg) and the tricyclic antidepressant desipramine (10 mg/kg) served as positive controls.

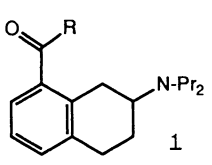
WY 48,723 and WY 50,324 significantly reduced behavioral immobility as compared to saline-treated controls. These results were similar to those obtained with desipramine and 8-OH-DPAT. In contrast, WY 47,846 failed to alter behavioral immobility when tested alone, and antagonized the reduction in behavioral immobility produced by the 5-HT_{1A} agonist. These results support our previous findings that compounds with agonist or partial agonist activity at the 5-HT_{1A} receptor may possess antidepressant-like activity. Further studies are currently underway to ensure that the activity of these compounds in the forced swim test was not due to their effects on locomotor activity.

616.3

8-ACYL-2-DIPROPYLAMINOTETRALINS: A NEW SERIES OF POTENT 5-HT_{1A} AGONISTS J. M. Schaus, D. L. Huser*, R. D. Titus*, C. S. Hoechstetter*, D. T. Wong, R. D. Marsh*, R. W. Fuller, and H. D. Snoddy*
Lilly Research Laboratories, Eli Lilly and Co. Indianapolis, IN 46285

8-Hydroxy-2-dipropylaminotetralin (8-OH-DPAT) is a potent and selective 5-HT_{1a} agonist which has limited oral activity. In an effort to obtain orally active 5-HT_{1a} agonists, we prepared a series of 8-substituted-2-dipropylaminotetralins. We report here the synthesis and phamacologic activity of a series of 8-acyl-2-dipropylaminotetralins (1, R = alkyl).

The 8-acyl-2-dipropylaminotetralins (1, R = Me, Et, i-Pr, t-Bu, and Ph) were found to have high affinity *in vitro* for the 5-HT_{1a} receptor (IC_{50}'s varied from 0.4 - 9 · M). The compounds were tested for 5-HT_{1a} agonist activity *in vivo* by their ability to lower brain 5-HIAA levels and increase serum corticosterone levels in rats. All of these compounds (except 1, R = Ph) were found to exert one or both of these effects at doses between 0.1 and 1.0 mg/kg, sc. The most potent compounds were found to lower brain 5-HIAA levels following oral dosing with the i-Pr and t-Bu ketones having the longest duration of action.



1

Molecular modelling studies of this series of ketones show that conformations in which the carbonyl system is coplanar with the aromatic ring are not accessible for 1, R = i-Pr and 1, R = t-Bu. Hence it is likely that the hydrogen bonding interaction between the carbonyl group of compounds 1 and the 5-HT1a receptor are out of the plane of the tetralin ring system.

616.4

COMPARATIVE EFFECTS OF 5-HT_{1a} AGONISTS ON SEXUAL BEHAVIOR OF MALE RATS. M. M. FOREMAN, K. RASMUSSEN, J. M. SCHAUS* AND M. E. FLAUGH*, The Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN, 46285.

Sexual behavior in the male rat is suppressed by most pharmacologic agents that amplify 5-HT receptor activity. The only known serotonergic agonists that stimulate this behavior are the 5-HT_{1a} agonists. These agonists have been proposed to be autoreceptor agonists which act on the 5-HT soma to suppress the activity of these neurons. The present studies have compared effects of different types of 5-HT_{1a} agonists including pyrimidinylpiperazines (PP), aminotetralins (AT) and "ABC" partial ergolines (PE). These agonists amplified sexual behavior, but at higher, postsynaptic doses sexual behavior was suppressed coincidental with the amplification of the 5-HT behavioral syndrome responses. The PP agonists were less potent, efficacious and shorter acting than either AT or PE agonists. The efficacy and duration of action of the AT and PE agonists varied with the aromatic substitution patterns.

616.5

5-HYDROXYTRYPTAMINE HYPERPOLARIZATION IN CA3 HIPPOCAMPAL PYRAMIDAL CELLS MAY NOT BE 5-HT_{1A} RECEPTOR MEDIATED K. Choi*, H. Read and S.G. Beck, Department of Pharmacology, Loyola University Chicago Stritch School of Medicine, Maywood, Il 60153.

Intracellular recording techniques were used to characterize 5HT actions in area CA3 of the hippocampal slice. 5HT and the 5HT analog 5-carboxyamidotryptamine elicited a hyperpolarization: Emax 15±5 mV, EC_{50} 10±5 µM and slope 1.9±0.7 and Emax 13.7±3.4 mV, EC_{50} 0.050±2 µM, slope 1.8±0.7 respectively. The K_d of the $5HT_{1A}$ antagonist spiperone from a Schild plot analysis was 72 nM. The K_d of the 5-HT_{1A} partial agonist BMY 7378 was 87 nM, but BMY also decreased the Emax, an effect that was reversible. The hyperpolarization was obtained with 2M KCl or 2M K Methyl Sulphate electrodes and in the presence of 1 µM TTX. The reversal potential was -105 mV in 3 mM extracellular KCl buffer and -85 mV in 5 mM KCl. We conclude that the 5HT hyperpolarization is mediated by an increase in potassium conductance. The identity of the 5HT receptor mediating the hyperpolarization has not been conclusively identified; however, evidence against the 5-HT_{1A} receptor includes: 1) The difference in the affinity of 5HT and 5CT is 200 fold, 2) the K_d of spiperone is low for a 5-HT_{1A} receptor, 3) the rank order of BMY and Spiperone is reversed and 3) BMY acts as a non-competitive antagonist. Other agonists and antagonists are being tested to obtain rank order potencies of agonists and K_d's of antagonists. USPHS grants MH41917, NS28512, KO2-MH00880 to SGB.

616.6

THE 5-HT_{1A} AGONISTS IPSAPIRONE AND 8-OH-DPAT DIFFERENTIALLY INFLUENCE THE 5-HT_2 AGONIST DOI-INDUCED INCREASE IN PLASMA ACTH, CORTICOSTERONE AND RENIN. Q. Li, P.A. Rittenhouse, A.D. Levy & L.D. Van de Kar. Dept. Pharmacology, Loyola Univ. Stritch Sch. Med. Maywood IL 60153

Evidence suggests that activation of 5-HT_{1A} receptors leads to inhibition of 5-HT_2 mediated behaviors. The purpose of this study was to investigate the interaction between 5-HT_{1A} and 5-HT_2 receptors. 5-HT_2, but not 5-HT_{1A} receptors are involved in the regulation of renin secretion. On the other hand, stimulation of 5-HT_{1A} and 5-$HT_{1C/2}$ receptors increases ACTH and corticosterone secretion. In the present experiment, rats were pretreated with the 5-HT_{1A} agonists ipsapirone (0, 1.0, 2.5 mg/kg ip) or 8-OH-DPAT (0, 50, 200 µg/kg sc) 45 min. before decapitation. The 5-$HT_{1C/2}$ agonist DOI was administered 15 min. following injection of the 5-HT_{1A} agonists, in a dose response manner (1-10 mg/kg ip). Plasma renin activity (PRA), plasma renin concentration (PRC), plasma corticosterone and plasma ACTH were measured by radioimminoassay.

Neither ipsapirone nor 8-OH-DPAT influenced the basal level of PRA/PRC or ACTH, but 8-OH-DPAT increased basal level of plasma corticosterone. Ipsapirone, but not 8-OH-DPAT, potentiated the effect of DOI on renin secretion. In contrast, 8-OH-DPAT, but not ipsapirone, potentiated the DOI-induced increase of plasma ACTH. In conclusion, the results suggest that activation of 5-HT_{1A} receptors does not inhibit the effect of a 5-HT_2 agonist on hormone secretion. Furthermore, the data suggest that ipsapirone and 8-OH-DPAT have distinct pharmacological profiles. (Supported by NIDA DA04865, NIMH MH 45812 and American Heart Association of metropoloton Chicago).

616.7

EVIDENCE THAT SOMATODENDRITIC 5-HT_{1A} AUTORECEPTORS IN THE DORSAL/MEDIAL RAPHE ARE FUNCTIONAL IN PREWEANLING RAT PUPS. G. A. Goodwin, C. J. Heyser, and L. P. Spear. Center for Developmental Psychobiology, SUNY Binghamton, Binghamton, NY 13902-6000.

Brainstem raphe nuclei such as the dorsal/medial raphe, appear to be regulated in adulthood by somatodendritic autoreceptors of the 5-HT_{1A} subtype. (Hjorth and Magnusson, 1988). Previous work from our laboratory examining the ontogeny of low dose effects of the 5-HT_{1A} agonist 8-OH-DPAT has led to the suggestion that 5-HT_{1A} autoreceptors are functional in preweanling but not infant rat pups (Frambes, Spear, & Goodwin, submitted). To further assess this possibility, the present study examined the neurochemical and behavioral effects of microinjections of 8-OH-DPAT into the dorsal/medial raphe of postnatal day 17 (P17) rat pups. Local microinjection of 1.0 and 5.0 µg of 8-OH-DPAT was observed to decrease 5-HIAA/5-HT ratios, with the higher dose inducing clear behavioral alterations, including the induction of flat body posture and an increase in sniffing. These data provide evidence that 5-HT_{1A} somatodendritic autoreceptors in the dorsal raphe are functional during the late preweanling period (i.e. by 17 days of age). Future studies will explore the consequences of local microinjection of 8-OH-DPAT in younger rat pups in order to determine when these autoreceptors first become functional.

616.8

THE EFFECT OF 8-HYDROXY-2-(DI-N-PROPYLAMINO) TETRALIN ON CHEMICALLY INDUCED DEFENSIVE BEHAVIOUR. S. R. C. Beckett*, A. J. Lawrence*, C. A. Marsden* and P. W. Marshall*, Dept. of Physiology and Pharmacology, Queen's Medical Centre, Nottingham, NG7 2UH. U. K. and ICI Pharmaceuticals, Bioscience II, Mereside, Alderley Park, Macclesfield, Cheshire, U. K. (SPON: Brain Research Association).

The ability of the 5HT1A receptor agonist 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) to modulate a chemically induced defence response has been studied in Lister hooded rats. Microinjections of the excitatory amino acid D, L-Homocysteic acid (DLH: 5nmol in 250nl) in both the rostral and caudal dorsal periaqueductal gray matter (DPAG) caused explosive motor behaviour characteristic of defence. Pretreatment with 8-OH-DPAT (10nmol in 250nl) resulted in a significant attenuation, and in some cases a complete abolition, of the DLH evoked response. Thus the results suggest that in vivo activation of 5HT1A receptors mediates an antiaversive response with respect to defensive behaviour elicited by specific chemical stimulation of the DPAG.

616.9

REDUCTIONS OF STRIATAL SEROTONIN RELEASE BY ACTIVATION OF 5-HT_{1A} RECEPTORS. D.S. Kreiss & I.Lucki. Institute of Neurological Sciences, Depts of Pharmacology & Psychiatry, University of Pennsylvania, Philadelphia, PA 19104.

The regulation of serotonin (5-HT) release in the striatum by somatodendritic 5-HT_{1A} autoreceptors was investigated using in vivo microdialysis. 5-HT release was measured in the ventral caudate nucleus of rats maintained under chloral hydrate anesthesia. Dialysate samples, collected at 20 min. intervals, were analyzed by an HPLC equipped with a microbore column. The sensitivity of detection was approximately 0.5 fmoles 5-HT/5 ul (2 X background noise), enabling reductions of 5-HT release to be measured without a 5-HT uptake inhibitor in the perfusion medium.

The major portion of dialysate 5-HT content was neuronal in origin, as determined by sensitivity to blockade of sodium ion channels with 1.0 uM TTX (mean ± s.e.m.: -69.2% ± 7.5) and to removal of calcium from the perfusion medium (-52.0% ± 11.2).

Systemic administration of the selective 5-HT_{1A} receptor agonist 8-OH-DPAT reduced striatal dialysate 5-HT content dose-dependently. 8-OH-DPAT was infused directly into the dorsal raphe nucleus, the origin of most of the serotonergic innervation of the striatum, reduced striatal dialysate 5-HT content to a similar magnitude. In contrast, infusion of 1.0 uM 8-OH-DPAT into the striatum via the probe perfusion medium, did not alter striatal dialysate 5-HT content. These observations suggest reductions in striatal 5-HT release are mediated by activation of somatodendritic 5-HT_{1A} autoreceptors in the dorsal raphe nucleus. Further experiments are being conducted to evaluate the pharmacological and anatomical specificity of this effect. This work was supported by MH36262 & MH48125.

616.10

ELECTROPHYSIOLOGICAL RESPONSES OF HIPPOCAMPAL, SEPTAL AND RAPHE NEURONS TO MDL 73,005EF, A POTENT AND SELECTIVE 5-HT_{1A} RECEPTOR LIGAND. J. Sprouse, D. McCarty, P. van den Hooff and M. Galvan, Marion Merrell Dow Research Institute, Cincinnati, OH and Strasbourg, France.

MDL 73,005EF exhibits anxiolytic-like effects in behavioral paradigms, but like other 5-HT_{1A} ligands, its mode of action is uncertain. A preferential agonist effect on 5-HT_{1A} receptors located on raphe cell bodies over those located postsynaptically in raphe projection areas has been proposed. The purpose of the present study was to examine this possibility by comparing the actions of MDL 73,005EF on single neurons in the dorsal raphe (DR), hippocampus (HC) and lateral septum (LS).

In DR slices, MDL 73,005EF produced a concentration-dependent inhibition of cell firing. Based upon IC_{50} values, MDL 73,005EF was equipotent with buspirone (129 ± 34 vs 97 ± 8 nM; means ± S.E.M.). Pretreatment with (-)-propranolol (1 µM), a mixed 5-$HT_{1A/B}$ antagonist, blocked MDL 73,005EF-induced inhibition. In contrast, in HC and LS slices, MDL 73,005EF (1 - 30 µM) did not change membrane potential but did antagonize hyperpolarizations induced by 5-HT. Buspirone induced a slowly developing hyperpolarization which was small in HC cells (30 µM: 1.4 ± 0.3 mv) but significantly larger in LS neurons (10 µM: 7.0 ± 0.7 mv).

These data indicate that MDL 73,005EF is an agonist at 5-HT_{1A} autoreceptors on DR cell bodies. Little or no agonist activity was detected on HC or LS neurons and instead MDL 73,005EF acted as an effective antagonist of 5-HT-induced hyperpolarization. Buspirone also inhibited DR cell firing; however, the small but significant activations of HC and LS postsynaptic 5-HT_{1A} receptors by buspirone signify potential differences between its mode of action and that of MDL 73,005EF.

616.11

ANTIDEPRESSANT ACTIVITY OF FLESINOXAN IN ANIMAL MODELS. J. Schipper, M.Th.M. Tulp*, H.S. Berkelmans*, F.N.C. Krijzer*, J.A.M. van der Heyden and B. Olivier. Dept. Pharmacology, Duphar B.V., P.O. Box 900, 1380 DA Weesp, The Netherlands.

The antidepressant properties of flesinoxan, a selective 5-HT_{1A} agonist, have been investigated in a number behavioural and neurochemical models. Flesinoxan, like clinical active antidepressants, prolongs the period of active resistance of rats in the forced swimming test (0.2 to 1.8 mg/kg). The effects of flesinoxan occur at much lower doses and the active period of resistance lasts longer compared with the classical antidepressants. Similar effects have been found with other 5-HT_{1A} agonists such as 8-OH-DPAT and ipsapirone. Longterm treatment with flesinoxan (1 mg/kg/day p.o. during 7 days) does not show tolerance on immobility in the forced swimming test.

Two weeks treatment with flesinoxan (6 mg/kg/day i.p.) shows no significant changes in the β-receptor number (B_{max}) nor affinity (K_d). Also autoradiographic studies reveal no significant change in the β-receptor binding in any of the brain regions studied. However, noradrenaline stimulated c-AMP production in brain slices is reduced, whereas isoprenaline stimulated c-AMP production is not changed after chronic flesinoxan. After chronic desmethylimipramine, both noradrenaline and isoprenaline stimulated c-AMP production is reduced. These data indicate that flesinoxan induces adaptive changes in β-adrenoceptor mediated c-AMP response, but in a different way compared with the tricyclic antidepressants.

In the rat, the EEG profile of flesinoxan shows characteristics of that of both antidepressants and anxiolytics, with a slightly greater similarity to that of anxiolytics. Similar EEG profiles are seen with 8-OH-DPAT and ipsapirone.

In conclusion, the animal pharmacology data on flesinoxan strongly suggest that this potent and selective 5-HT_{1A} agonist will have potential antidepressant activity in man.

616.12

Effects of 5-HT_{1A} agonists in the conflict test. A. Meneses and E. Hong. (Spon: J.E. Villarreal). Terapéutica Experimental, Depto. de Farmacología y Toxicología, CINVESTAV-IPN, Mexico City, México 14370.

Contradictory evidence exists concerning the anxiolytic effects of 5-HT_{1A} agonists in the conflict test. In the present work a modification of the Vogel conflict model was used to assess different doses of diazepam (0.1-10.0 mg), ipsapirone (0.56-17.8 mg), buspirone (1.0-17.8 mg) and indorenate (0.56-17.8 mg) in rats receiving two different electric shock intensities (0.16 and 0.32 mA). The results show that the three 5-HT_{1A} agonists had a smaller anticonflict effect than diazepam. The anticonflict effect with each compound was of a greater magnitude at 0.16 mA intensity than at 0.32 mA. This study shows that using different electric shock intensities, compounds produce a differential effect: the anticonflict effects were more pronounced with the lower electric shock intensity than with the higher intensity. The present results suggest that the use of different shock intensities, can play distint roles over the drug's effect in the conflict test.

616.13

VARIATION IN CEREBRAL METABOLISM OF EXOGENOUS L-TRYPTOPHAN IN BALB/C AND C57/BL MICE. D. Ghosh and B.O. Anyanwu*, Department of Biology, Texas Southern University, Houston, Texas 77004

L-Tryptophan, an essential amino acid, is metabolized in two major (i) kynurenine and (ii) serotonin (5-HT) pathways in mammalian cells. In the brain, the tryptophan →5-HT→5-hydroxyindole-3-acetic acid (5-HIAA) pathway is predominant, and 5-HT so synthesized serves as a crucial neurotransmitter in some discrete areas of the brain. Experiments were conducted to explore the existence of any difference in the cerebral metabolism of exogenous L-tryptophan (0.375 mg/g body weight/intraperitoneal/2 hrs) in two inbred [BALB/C (albino) and C57/BL6(dark-coat color)] lines of male mice which were 12 weeks of age. Each single whole mouse brain was extracted in 0.05 M perchloric acid (16.7% homogenate). HPLC/electrochemical detection was performed on supernatants obtained from crude homogenates centrifuged at 15,000 g for 15 minutes. Our results indicate that C57/BL6 mice produced significantly higher levels of 5-HT and 5-HIAA than BALB/C mice. [5-HT : C57/BL6 = 394 ± 12.5 ng/g (=100%) & BALB/C = 336 ± 6.9 ng/g (=85%); N = 7; P<.05/5-HIAA: C57/BL6 = 742 ± 1.1 ng/g (=100%) & BALB/C = 476 ± 8.8 ng/g (=64%); N = 7; P <.01.] Central 5-HT and its turnover regulate a number of physiological states in mammals, and this biogenic amine assumes added importance if the underlying cause of its tissue level is genetic. (Supported by NIH grant RR03045).

616.14

CENTRAL EFFECT OF METHYSERGIDE ON SUCKLING. J. Zhang and C. P. Cramer. Department of Psychology, Dartmouth College, Hanover, NH 03755

5-HT antagonists, such as methysergide, administrated systematically to 20-day-old rats facilitate nipple attachment behavior. Conversely, 5-HT agonists inhibit attachment at this age. We have recently demonstrated that the agonist-induced inhibition is peripheral. Here we examine the effect of methysergide, injected ICV, on nipple attachment of 20-day-old rats.

20-day-old rats were anesthetized by metophane, and injections of drug or vehicle were made directly into the left lateral ventricle. In the first experiment, 4 pups were taken from each of 8 litters. 1 male and 1 female served as controls and received only isotonic saline (0.03 ml). The other two (M, F) received methysergide (0.010 mg/ml) in isotonic saline (0.03 ml). 10 min after injection, the pups were placed for 31 min with their anesthetized mother (Nembutal sodium, 1 ml/kg, IP). In the second experiment, dye (Evans Blue, 0.010 mg/ml) was added to each injection to confirm injection location. The same testing procedure as in the first experiment was used and immediately followed by a histological examination.

Result of the first experiment showed a significant facilitating effect of methysergide in terms of percentage of attachment, latency and duration (all p<.01). In the second experiment, only those subjects whose injections were accurately placed were included. (67.5% of these injected, confirming the first experiment). In this case, methysergide produced a weak facilitating effect.

The results suggest that the facilitating effects of methysergide are mediated centrally and that the dye may reduce the effect perhaps by binding the drug. Further experiments with other 5-HT drugs are underway.

616.15

ZATOSETRON, A SELECTIVE 5-HT_3 ANTAGONIST, DECREASES THE NUMBER OF SPONTANEOUSLY ACTIVE A10 DOPAMINE NEURONS. *M. E. Stockton* and K. Rasmussen*. *Lilly Research Labs, Eli Lilly and Company, Indianapolis, IN 46285.*

5-HT_3 receptor antagonists have been shown to have a modulatory effect on the mesolimbic dopaminergic system, leading to the hypothesis that 5-HT_3 antagonists could have antipsychotic actions in schizophrenic patients. Previous studies have shown that chronic treatment with antipsychotics (e.g., haloperidol) decreases the number of spontaneously active A9 and A10 dopamine (DA) cells. However, chronic treatment with atypical antipsychotics (e.g., clozapine), which lack extrapyramidal motor side-effects, only decreases the number of spontaneously active A10 DA neurons. Apomorphine, a DA agonist, reverses these inhibitory effects of antipsychotics. Previous studies of the effects of 5-HT_3 antagonists on the number of spontaneously active DA cells have produced divergent results. This study evaluated the effects of zatosetron, a 5-HT_3 antagonist belonging to a different structural class than previously tested 5-HT_3 antagonists, on A9 and A10 single unit activity. Extracellular, single-unit recordings were made in chloral hydrate anesthetized rats and the number of spontaneously active A9 and A10 DA cells was counted. Both chronic (21 days) and acute administration of zatosetron resulted in a dose-related suppression of the number of spontaneously active A10 neurons , with no change in A9. Apomorphine did not reverse the effects of zatosetron, indicating that the response may not be mediated via depolarization inactivation. These data indicate that zatosetron's effects on spontaneously active DA neurons: (1) differs from other 5-HT_3 antagonists; (2) may not be mediated by depolarization inactivation; and, (3) may be predictive of an atypical antipsychotic action without delayed onset.

616.16

LACTATION-INDUCED ENHANCEMENT OF GASTRIC CONTRACTILITY TO SEROTONIN IN VITRO

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Studies have shown that lactating rats exhibit enhanced absorptive and motor activity of the gastrointestinal tract. In order to explore the possible changes associated with lactation, the contractility of gastric fundus to serotonin (5-HT) was studied. The serotonin-evoked isometric contractions of rat fundal strip preparations obtained from four groups viz., lactating rats (LR), non-lactating non-pregnant rats (NLR), pregnant non-lactating rats (PR) and adult male rats (MR), were measured and compared. An analysis of variance revealed that the fundal tissues from LR are more sensitive to 5-HT than those of NLR ($F = 18.3$; $P < 0.001$), while those from PR are less sensitive ($F = 8.04$; $P < 0.001$). Furthermore, the preparations from MR were more sensitive to 5-HT than those of NLR ($F = 6.5$; $P < 0.001$). While preliminary, these data suggest enhanced sensitivity of fundal strips to 5-HT in lactating rats. (Supported by ICMR Grant No. 5/3-5(17)/89-BMS-II).

616.17

GASTRIC EMPTYING IN THE RAT: EFFECTS OF CENTRAL AND PERIPHERAL TREATMENT WITH AGENTS ACTIVE AT SEROTONIN-3 AND/OR 4 RECEPTORS. B. J. Chase, D. J. Rossi, Jr. and G. E. Martin, Rhône-Poulenc Rorer Central Research, King of Prussia, PA 19406

The effects on gastric emptying (GE) of intracerebroventricularly (ICV) and IP administered agents acting at 5-HT-3 or 4 receptors was compared. In male rats, GE of a semi-solid meal was compared between treated and control animals 60 min post meal administration (p.o.). ICV -15 min and IP injections -30 min were given prior to the meal.

	5-HT	Agonist/	Maximal Effect on Emptying	
Agent	Receptor(s)	Antagonist	ICV	IP
RG 12915	3	Ant	+	++
2-methyl-serotonin	3	AG	++	NT
Phenylbiguanide	3	AG	-	--
Zacopride	3,4	Ant/AG	+	+++
Renzapride	3,4	Ant/AG	NE	+++
ICS 205,930	3,4	Ant/Ant	+	++
Reglan	3,4	Ant/AG	-	++
Serotonin	ALL	AG	--	NT

-,+>25%,--,++>50%,---,+++>75%,NE=No effect,NT=Not Tested

5-HT-3 antagonists increased gastric emptying in this model whether given ICV or IP, but compounds with 5-HT-4 properties were more potent. Both central and peripheral receptors play a role in modulating GE in the rat.

ADENOSINE

617.1

IDENTIFICATION AND CHARACTERIZATION OF ADENOSINE A2 AND A1 RECEPTORS cDNAs. LOCALISATION OF THE A2 RECEPTOR IN THE HUMAN STRIATUM AND SELECTIVE EXPRESSION BY THE RAT ENKEPHALINERGIC STRIATAL SUBPOPULATION. SN Schiffmann1*, F Libert2*, C Maenhaut2*, JE Dumont2*, G Vassart2* and J-J Vanderhaeghen1 [1]Lab. Neuropeptide Res. and [2]IRIBHN, Brussels Univ., 1070 Brussels, Belgium.

We have recently cloned RDC8 and RDC7, two orphans G protein-coupled receptors (Libert et al., *Science* (1989) 244:568-572). RDC8 has been identified as an adenosine A_2 receptor (A_2r) (Maenhaut et al., *Biochem. Biophys. Res. Comm.* (1990) 173:1169-1178) and we have now identified RDC7 as an A_1 receptor (A_1r). RDC8 and RDC7 were expressed in several mammalian cells. This confer to these cells the capacity to bind specifically either an A_2 ([^{3}H]CGS 21680) or an A_1 agonist ([^{3}H]CHA); and to stimulate or inhibit adenylyl cyclase in response to A_2 or A_1 agonists, respectively.

Using *in situ* hybridization (ISH), the mRNA encoding for this A_2r is exclusively detected in the medium-sized neurons of the rat and dog striatum (Schiffmann et al., *Brain Res.* (1990) 519:333-337). The human A_2r mRNA is also exclusively expressed by the medium-sized striatal neurons. A_2r is homogeneously distributed in the adult striatum, whilst it exhibits a striosomial pattern during the early postnatal development. Morover, a ISH colocalization study revealed that in the rat caudate-putamen A_2r is mainly expressed by the GABAergic-enkephalinergic subpopulation as compared to the substance P or the cholinergic neurons. The dopamine D_2 receptor is also selectively expressed by these neurons and, conversely to the A_2r, inhibits cAMP formation.

617.2

MOLECULAR CLONING AND CHARACTERIZATION OF A RAT A1 ADENOSINE RECEPTOR THAT IS WIDELY EXPRESSED IN BRAIN AND SPINAL CORD. D.R. Weaver, S.A. Rivkees, J.H. Stehle*, and S.M. Reppert. Laboratory of Developmental Chronobiology, Children's Service, Massachusetts General Hospital, Boston, MA, 02114.

Libert et al. (Science 244: 569, 1989) reported the cloning of four unidentified receptors belonging to the superfamily of G protein-coupled receptors. Two of these receptors, RDC7 and RDC8, appear to comprise a receptor subfamily. With the identification of RDC8 as an A_2-adenosine receptor (BBRC 173:1169, 1990; see also abstract by J.S. Fink et al.), it seemed likely that RDC7 encodes an adenosine receptor subtype. We used the polymerase chain reaction to clone a fragment of the rat homolog of RDC7, leading to isolation of a full-length clone from rat brain. The rat cDNA encodes a protein of 327 amino acids which shares several structural features with the A_2-adenosine receptor. Expression studies in COS-6M cells demonstrated that the cloned cDNA encodes a protein with high affinity for the A_1-adenosine specific ligand ^{3}H-CCPA (K_d = 882 ± 139 pM; n = 3) and appropriate pharmacological specificity (rank order for inhibiting ^{3}H-CCPA binding: CPA > R-PIA > NECA > S-PIA). In situ hybridization studies revealed a widespread distribution of the receptor mRNA, with high levels in cerebral cortex, hippocampus, cerebellum, thalamus, brainstem and spinal cord. The distribution of the receptor mRNA is generally in good agreement with the distribution of A_1-adenosine receptors as revealed by autoradiography, but several receptor/mRNA mismatches exist. The cloning and identification of A_1- and A_2-adenosine receptor cDNA's represents an important advance toward defining the role of adenosine receptor subtypes in CNS function.

617.3

MOLECULAR CLONING OF AN A_1 ADENOSINE RECEPTOR. E.M. Smyk-Randall*, L.D. McVittie*, H. Nakata*, F.J. Monsma, Jr, C.R. Gerfen, D.R. Sibley & L.C. Mahan*. ETB, NINDS and LCB & LCS, NIMH, NIH, Bethesda, MD 20892.

We have utilized the polymerase chain reaction technique to selectively amplify G protein-coupled receptor cDNA sequences from rat striatal mRNA. A novel cDNA fragment was identified which exhibits considerable homology to various members of the G protein-coupled receptor family. This fragment was used to isolate a full-length cDNA from a rat striatal library. A 2.2 kb clone was obtained encoding a protein of 326 amino acids with seven transmembrane domains as predicted by hydropathy analysis. Stably transfected mouse A9-L cells and chinese hamster ovary cells that express mRNA for this clone were screened with putative receptor ligands. Saturable and specific binding sites for the A_1 adenosine antagonist, [^{3}H]DPCPX, were identified on membranes from transfected cells. The rank order of potency and affinities of various adenosine agonist and antagonist ligands confirmed the identity of this cDNA clone as an A_1 adenosine receptor. The high affinity binding of A_1 adenosine agonists was shown to be sensitive to the non-hydrolyzable GTP analogue, Gpp(NH)p. In adenylyl cyclase assays, adenosine agonists inhibited forskolin-stimulated cAMP production by greater than 50% in a pharmacologically specific fashion. Northern blot and *in situ* hybridization analyses of receptor mRNA in brain tissues revealed two transcripts of 5.6 kb and 3.1 kb, both of which were abundant in cortex, cerebellum, hippocampus, and thalamus with lower levels in olfactory bulb, striatum, mesencephalon and retina. These regional distribution data are in good agreement with previous receptor autoradiographic studies involving the A_1 adenosine receptor. We conclude that we have cloned a cDNA encoding an A_1 adenosine receptor linked to the inhibition of adenylyl cyclase activity.

617.4

THE RAT A2 ADENOSINE RECEPTOR: MOLECULAR CLONING, EXPRESSION AND LOCALIZATION IN BASAL GANGLIA. J.S. Fink, D.R. Weaver, S. A. Rivkees, R. Peterfreund*, E. M. Adler* and S. M. Reppert, *Molecular Neurobiology and Developmental Chronobiology Labs, Massachusetts General Hospital, Boston, MA 02114.*

Selective ligand binding and receptor autoradiography have demonstrated that the A2 subtype of adenosine receptor is expressed exclusively in striatum, nuc. accumbens and olfactory tubercle in rat (Eur J Pharm 1989; 168:243). Using the technique of PCR cloning with homologous sequences we have isolated the cDNA encoding the rat A2 adenosine receptor. With degenerate oligonucleotide primers corresponding to conserved transmembrane domains of G protein-linked receptors and first strand cDNA from rat brain as template, 230 bp fragments of G protein-linked receptor cDNAs were amplified using PCR. One of these receptor fragments hybridized to mRNA in rat striatum. Using this receptor fragment as probe, a full-length cDNA was isolated from a gt10 rat striatal library. This rat receptor was highly similar to the canine G protein-linked receptor RDC8 (Science 1989; 244:569) which has recently been shown to be a canine A2 adenosine receptor (BBRC 1990; 173:1169). Identification of the rat cDNA as an A2 receptor was achieved by expression in COS cells. In situ hybridization demonstrated abundant and exclusive expression of the rat A2 receptor mRNA in striatum, nuc. accumbens and olfactory tubercle. The abundant expression of the rat A2 receptor mRNA in basal ganglia may underlie the known interaction between dopaminergic and adenosinergic systems on behavior.

617.5

CONTRIBUTION OF THE SECOND ADENOSINE GROUP TO THE TOTAL BINDING OF BIS(N^6-ADENOSYL)ALKANES TO THE ADENOSINE A$_1$ RECEPTOR. J. B. Wollack and R. M. Collins, Jr.* Dept. of Pediatrics, School of Medicine, Univ. of Maryland at Baltimore, Baltimore, MD 21201

Bis(N^6-adenosyl)alkanes are "bivalent" analogs of adenosine, in which the two adenosyl groups are separated by a spacer of methylene groups. We have previously reported a series of these compounds whose affinities for the A1 adenosine receptor range from approximately 3 to 300 nanomolar. In order to assess the contribution that the presence of the second adenosyl group makes to this binding, we have compared one of the best bivalent compounds, bis(N^6-adenosyl)decane, with its monovalent counterpart, N^6-decyladenosine. Since N^6-decyladenosine contains not only one of the adenosyl groups, but also the bridging structure from bis(N^6-adenosyl)-decane, any differences in binding affinity should be attributable to the second adenosine group.

N^6-decyladenosine and bis(N^6-adenosyl)decane were synthesized by the reaction of 6-chloropurine riboside with either decylamine or diaminodecane. Binding to the A$_1$ adenosine receptor in rat brains was assessed using ^{3}H-cyclohexyladenosine as the radioligand. The binding of the monovalent N^6-decyladenosine was 30 nanomolar, 12 - fold less than the bivalent bis(N^6-adenosyl)decane. These results suggest that the presence of the second adenosyl group contributes significantly to the overall binding of the bivalent analog. Supported by grants from the NIH (1-KO8-NS01514) and the American Paralysis Association.

617.6

[^{3}H]CV 1808 LABELS A NOVEL NON-ADENOSINE A$_2$ BINDING SITE IN RAT BRAIN. L. J. Cornfield, P.S. Loo, S. D. Hurt and M. A. Sills. CIBA-GEIGY Corp., Research Dept., 556 Morris Ave., Summit, NJ 07901, and NEN-DuPont, 549 Albany St., Boston, MA 02118.

The vasoactive agent CV 1808 (2-phenylaminoadenosine) has previously been shown to possess 10-fold selectivity for the adenosine A$_2$ receptor (Jarvis et al., J. Pharmacol. Exp. Ther., 251:888, 1989). CV 1808 was subsequently radiolabeled and binding was evaluated in membranes prepared from Sprague-Dawley rat striatum, cortex and hippocampus. Specific binding accounted for 65-70% of total binding. Using 5 nM [^{3}H]CV 1808 in a series of competition studies, CV 1808 produced shallow inhibition curves in all three tissues. Computer analysis of the data indicated that CV 1808 inhibited 61-75% of the binding in the three brain areas with IC$_{50}$ values of 16-24 nM, and 25-37 % of the binding with IC$_{50}$ values of 590-1130 nM. The adenosine A$_1$ selective compounds, CPA and CHA, inhibited only ~35% of total specific binding with IC$_{50}$ values of 270-1750 nM and 960 - 2560 nM, respectively. Interestingly, the adenosine A$_2$ selective agonist CGS 21680 and the non-selective agonists NECA and MECA inhibited < 20 % of total specific binding at a concentration of 10 µM. In contrast, CGS 15943, an adenosine antagonist, inhibited 56-68 % of total binding, with an IC$_{50}$ value of 128-310 nM. In addition, several novel adenosine analogs, such as CGS 22988, inhibited 100% of specific binding with IC$_{50}$ values of 370 - 430 nM in the three brain areas. The adenosine uptake inhibitor, dipyridamole, and the guanine nucleotide analog, GTPγS, were unable to inhibit the binding of [^{3}H]CV 1808. These results suggest that [^{3}H]CV 1808 labels two binding sites in rat brain, neither of which appear to be the adenosine A$_2$ receptor. One site appears to be an adenosine A$_1$-like component, whereas the second site appears to be a novel adenosine binding site. Further studies to identify a functional correlate for this novel binding site are presently in progress.

617.7

TRANSPORT KINETICS AND METABOLISM OF ACCUMULATED [^{3}H]D-ADENOSINE AND [^{3}H]L-ADENOSINE IN RAT CEREBRAL CORTICAL SYNAPTONEUROSOMES. **J.G. Gu., A.N. Sawka* and J.D. Geiger. Dept of Pharmacology and Therapeutics, Faculty of Med., Univ. of Manitoba, Winnipeg, Manitoba, Canada, R3E 0W3.**

[^{3}H]L-Adenosine was previously found to be more metabolically stable than [^{3}H]D-adenosine and to be a substrate for CNS adenosine transporters [Gu et al., J. Neurochem. 56 (1991) 548-552]. Here we examined the degree to which accumulated [^{3}H]D- and [^{3}H]L-adenosine were metabolized and the effects that this metabolism had on transport characteristics. For [^{3}H]D-adenosine, there was an inverse relationship between transport reaction times and Km values; for 5, 15 and 600 sec there were 24, 32, and 76% conversions to nucleotides; the Km values (uM) were 9.4±4.2, 8.4±2.3, and 4.5±0.6, respectively. Ten minute preincubations of tissue with 10 uM EHNA, an adenosine deaminase inhibitor, and 5'-iodotubercidin, an adenosine kinase inhibitor, resulted in significantly higher Km values of 36±1 for 5 sec and 44±13 for 15 sec incubations; metabolism of accumulated [^{3}H]D-adenosine to nucleotides was decreased to 6.4% at 5 sec and 9.0% at 15 sec. In the presence of these inhibitors, 32% of accumulated [^{3}H]D-adenosine was metabolized to nucleotides for 600 sec incubations and Km value of 15.8±4.7 were not significantly different from control value of 4.5±0.6. For [^{3}H]L-adenosine, Km value for 20 sec incubations were 38.7±6.8. Metabolism of accumulated [^{3}H]L-adenosine to nucleotides was 2.5, 4.8, and 30.9% for 5, 15 and 600 sec incubations, respectively. Metabolism of accumulated adenosine affects measured kinetic parameters for adenosine transport and the Km value for adenosine transport in rat cerebral cortical synaptoneurosomes is approximately 40 uM.

617.8

THE ADENOSINE REUPTAKE ANTAGONIST SOLUFLAZINE REDUCES AND DELAYS POPULATION ANOXIC DEPOLARIZATION IN RAT HIPPOCAMPAL NEURONS IN VITRO. C.G. Boissard and V.K. Gribkoff. Neuropharmacology, Bristol-Myers Squibb Pharmaceutical Research Institute, 5 Research Parkway, Wallingford, CT 06492.

Endogenous adenosine, released by hippocampal neurons during hypoxia, mediates hypoxic depression of synaptic transmission, a response which may be neuroprotective (Gribkoff et al., Brain Res. 512: 353, 1990). Application of metabolically stable adenosine analogs prior to an ischemic event results in a reduction in ischemic neuronal death (Evans et al., Neurosci. Lett. 83:287, 1987), while application of an adenosine antagonist exacerbates ischemia-related hippocampal injury (Rudolphi et al., J. Cereb. Blood Flow Metab. 7 :74, 1987). In the present experiments we have begun to examine the ability of an adenosine reuptake antagonist, soluflazine, to potentiate the potentially protective effects of endogenous adenosine release; this compound has been previously shown to increase the inhibitory effects of exogenously applied adenosine in hippocampal slices (Ashton et al., Eur. J. Pharm. 142:403, 1987).

Population anoxic depolarizations, recorded from stratum radiatum in area CA$_1$ of rat hippocampal slices in response to exposure to N$_2$ gas, were reduced in amplitude in slices incubated in 10 µM soluflazine (to 39.8% of control); this effect was less pronounced at 20 and 50 µM soluflazine, although the higher concentrations of this reuptake antagonist produced a clearly concentration-dependent increase in synaptic inhibition. In similar fashion, the latency to the onset of anoxic depolarization was significantly increased by the lowest concentration of soluflazine (to 227.4% of control). These data suggest that increasing extracellular adenosine levels by blocking reuptake of the nucleoside can ameliorate a potentially cytotoxic neuronal response to hypoxia.

617.9

AUTORADIOGRAPHIC VISUALIZATION OF ADENOSINE A1 RECEPTORS IN HUMAN HIPPOCAMPUS. J.Deckert, W.Berger*, K.Kleopa*, S.Heckers*, G.Ransmayr*, H.Heinsen*, A.Wree*, H.Beckmann* and P.Riederer*. Dep. of Psychiatry, University of Würzburg, FRG.

20 micron-sections from autoptic samples of the hippocampus and parahippocampal gyrus were cut and thaw-mounted on gelatine-coated slides.

After preincubation (30 minutes, 20 °C) in 150 mM Tris-HCl buffer pH 7.4 with 1.5 IU/l ADA the sections were incubated (2 hours, 20° C) with (^{3}H)DPCPX (0.2 to 20 nM) followed by 2 rinses with buffer (4° C, 120 sec). Non-specific binding in the presence of 20 uM R-PIA was 15%.

Kinetic parameters as determined by wiping the incubated sections off the slide and liquid scintillation spectophotometry were Kd=1.6 nM and Bmax=169.9 fmol/section.

Saturation analysis using quantitative autoradiography and determination of cell density in adjacent Gallocyanin-stained sections provided receptor density corrected for cell density in subregions and individual layers of hippocampus.

617.10

RADIOLIGAND BINDING STUDIES OF CGS 21680 TO ADENOSINE RECEPTORS IN SPINAL CORD AND STRIATAL MEMBRANES. V. Pirec, H. K. Proudfit, J. I. Choca. Dept. of Pharmacology, Univ. of Illinois College of Medicine at Chicago, Chicago, IL 60612

To better characterize adenosine A$_2$ receptors in the spinal cord we performed parallel studies in membranes of spinal cord and striatum. In both preparations CGS 21680 displaced [^{3}H]NECA from two sites with similar K$_i$ values (K$_{i1}$ = 37.4 and 22.0nM; K$_{i2}$ = 6.4 and 4.0 µM for cord and striatum, respectively). In the spinal cord 20% of these [^{3}H]NECA sites have characteristics of A$_2$ receptors. Additional displacement studies with R-PIA, CPA, CHA, CADO, DMPA, DMPX, confirmed that 12 to 30% of the [^{3}H]NECA binding sites show characteristics of an adenosine A$_2$ receptor.

However we have been unable to detect direct binding of [^{3}H]CGS 21680 to spinal cord membranes using concentrations in the range of its K$_d$ (4 to 80 nM). At higher concentrations (30 to 400 nM) [^{3}H]CGS 21680 binds to a site which has a K$_d$ of 125 nM. Various displacement studies (CGS 21680, DMPA, CPA, CPX), suggest that 90 to 99.9% of this binding is to a site which has characteristics of adenosine A$_1$ receptors. The remaining 0.1 to 9% of the displacable binding appears to be an A$_2$-like receptor.

These conflicting results suggest that A$_2$ receptors in the spinal cord differ from those in striatum. Perhaps the binding kinetics of [^{3}H]CGS 21680 are much faster in the spinal cord than in the striatum. Faster kinetics could explain our inability to observe direct binding. (Supported by USPHS Grant DA03980.)

617.11

EFFECT OF NEONATAL CAFFEINE ON ADENOSINE A1 RECEPTOR ONTOGENY USING AUTORADIOGRAPHY. Barbara A. Etzel and Ronnie Guillet. Department of Psychology, University of Rochester, Rochester, NY 14627.

Caffeine (C), an adenosine antagonist, is commonly administered as treatment for apnea of prematurity in human infants. To investigate the long term effects of this treatment, an animal model was developed. Homogenate assay of 14-90 day old rat brain has shown that neonatal C exposure significantly increased specific adenosine A1 receptor (A1R) binding in cerebellum (CBL) and hippocampus (H), two areas in which A1R binding increases with age. To further specify the subregional effects of neonatal C treatment on ontogeny, autoradiography was performed. The brains of unhandled (NH) or neonatally C-exposed (C-ex) 14 and 30-31 day old rats were removed, quick frozen, sectioned and treated with adenosine deaminase. Sections were then incubated with 1 nM 3H-cyclohexyladenosine (3H-CHA). Nonspecific binding was determined in adjacent sections with L-phenylisopropyladenosine. The sections were apposed to LKB Ultrofilm for 4 weeks. Quantification was performed using Analytical Imaging Concepts software.

3H-CHA binding was greatest in the H. Within H, binding was more pronounced in CA1 and CA3 than in dentate gyrus. In all H subregions there was an age by treatment interaction ($p<0.01$). Binding was less in 14d C-ex rats than in controls. However, by 30d, binding in C-ex rats equalled or exceeded that in controls. In CBL more labelling was apparent in the molecular layer (M) than in the granular layer (G), especially in the older rats. The layers could not be distinguished in the 14d C-ex CBL in which there was minimal binding. In M and G, 3H-CHA binding increased with age in the NH groups ($p<.01$). Binding was greater in C-ex than in NH rats at 30d (M, $p=0.05$; G, $p<0.01$). These studies corroborate and enlarge upon the homogenate binding studies by demonstrating differences in subregional A1 adenosine binding as a function of age and early developmental caffeine exposure. Grant No. HD22782

617.12

PENETRATION OF ADENOSINE ANTAGONISTS INTO MOUSE BRAIN AS DETERMINED BY EX VIVO BINDING. K. A. Jacobson,+ O. Nikodijevic,+ and J. Baumgold,* +NIDDK, NIH, Bethesda, MD, *Dept. of Radiology, George Washington Univ., Washington, DC.

The penetration of peripherally-administered adenosine antagonists into mouse brain was determined using ex vivo binding and locomotor studies. CPT (8-cyclopentyltheophylline), CPX (8-cyclopentyl-1,3-dipropylxanthine), 8-PST (8-p-sulfophenyltheophylline), and XAC (xanthine amine congener) were examined. At 10 min post-injection (IP), CPT and CPX (25 mg/kg and 0.25 mg/kg, respectively) both had penetrated into brain substantially: 49 and 17 % of theoretical levels (assuming free penetration throughout the body), respectively. Brain levels of CPT decreased rapidly, declining to undetectable levels by 30 min post-injection, whereas levels of CPX increased to 51% penetration at 20 min, then declined slowly. As expected, no detectable brain levels of 8-PST were found following intraperitoneal injection of 50 mg/kg. XAC (20 mg/kg) penetrated into brain poorly: 1.6% of theoretical after 10 min and 3.2% 20 min post injection. As a control for vascular content of brain tissue, ^{14}C-bovine serum albumin was administered iv., and the level of radioactivity found in the brain was 1% of that found per equal volume of plasma (corresponds to 1.1% penetration). The ability of CPT to stimulate locomotor suppression paralleled the brain levels, ie. it was similar to theophylline at short times, and the effect rapidly diminished. These studies demonstrate the usefulness of ex vivo binding in determining CNS penetration of adenosine receptor ligands.

617.13

PHORBOL ESTERS DIFFERENTIALLY MODULATE PRESYNAPTIC AND POSTSYNAPTIC ACTIONS OF ADENOSINE A1 RECEPTORS. L. Arlinghaus*, N.F. Kassell and K.S. Lee. Dept. of Neurosurgery, Univ. of Virginia, Charlottesville, VA 22908.

Phorbol esters and adenosine are potent modulators of neuronal activity in the central nervous system. In the CA1 region of hippocampus, activation of protein kinase C (PKC) by phorbol esters results in an increase in neuronal activity, whereas activation of adenosine A1 receptors inhibits neuronal activity. Previous studies indicate that these systems can interact; phorbol esters attenuate the inhibitory action of adenosine on population spike responses in the hippocampus (Worley et al., PNAS 84, 1987). The studies presented here examined the influence of phorbol esters on two physiological actions of adenosine mediated by A1-type receptors.

In vitro hippocampal slices from adult gerbils were utilized to record: 1) orthodromically-evoked, synaptic responses in the stratum radiatum of CA1 (fEPSPs) and, 2) antidromically-evoked afterdischarges in the stratum pyramidale of CA1 (recorded in the presence of low calcium medium). The inhibitory effect of adenosine on fEPSPs was diminished by phorbol 12,13 diacetate (PDAc) and phorbol 12,13 dibutyrate (PDBu), but not by the relatively inactive phorbol 12,13 didecanoate (PDD). In contrast, the inhibitory action of adenosine on repetitive neuronal discharges elicited in low calcium was enhanced by PDAc and PDBu while PDD was ineffective. Thus, phorbol esters can differentially modulate presynaptic and postsynaptic actions of adenosine A1 receptors. These observations may reflect fundamental differences in the manner in which the PKC second messenger system interacts with the adenosine modulatory system at presynaptic and postsynaptic sites. (Supported by NSF Grant BNS-8901154)

617.14

SELECTIVE CHANGES OF ADENOSINE RECEPTORS IN RAT BRAIN SLICES FOLLOWING IN VITRO EXPOSURE TO RECEPTOR AGONISTS. M.P. Abbracchio, A.M. Paoletti*, B. Paternieri* and F. Cattabeni. Institute of Pharmacological Sciences, University of Milan, Via Balzaretti 9, 20133 Milan, Italy

Adenosine is known to physiologically inhibit excitatory neurotransmission in the CNS by activation of specific receptor subtypes. This is believed to be at the bases of neuroprotection by adenosine analogues against ischemia- and excitatory amino-acid-induced cerebral damages. However, there are indications that adenosine neuroprotection might be reduced when brain adenosine concentrations are markedly and pathologically increased (e.g., prolonged ischemia and aging). To verify whether such possible losses of adenosine neuroprotective activity might be due to agonist-induced desensitization of cerebral receptors in consequence to the prolonged exposure to adenosine, we have studied the regulation of adenosine receptors in rat brain slices exposed for different time-periods to selective adenosine receptor analogues, such as cyclo-pentyl-adenosine (CPA) and N-ethyl-carboxamido-adenosine (NECA). After exposure of slices to 0.1-10 μM CPA for up to 1 h, the ability of adenosine analogues to inhibit membrane adenylate cyclase activity and cAMP production was dose- and time-dependently reduced, suggesting a loss of A1 receptor function. Desensitization of A1 receptors with NECA was slower in onset and less pronounced, according to the lower A1 receptor selectivity of this adenosine analogue with respect to CPA. Moreover, A2 receptors seemed to be less affected by both agonists in the experimental conditions used, resulting in an umbalance between A1 and A2 receptor function, which might be of functional relevance to ischemic conditions.

617.15

ADENOSINE RELEASE EVOKED BY ISCHEMIC-LIKE CONDITIONS AND ELECTROPHYSIOLOGICAL ACTIVITY IN HIPPOCAMPAL SLICES. F. Pedata*, A.M. Pugliese*, M. Pazzagli* and G. Pepeu. Pharmacol. Dept., Univ. Florence, Italy.

The release of endogenous adenosine (ADO) and inosine (INO) from rat hippocampal slices was investigated by HPLC. Recovery of stable evoked population spikes, recorded from the CA1 region, occurred within 150 min from cut when ADO and INO release were stable and reached 14.3±1.5 and 16.0±2.0 ng/g/min respectively. Ischemia was then produced by superfusing the slices with glucose free Krebs saturated with 95% N2/5% CO2 for 5 min. The ischemia-evoked release of ADO and INO was respectively 132±37 and 106±20 ng/g/min. Electrophysiological responses disappeared within 3 min of ischemia; complete recovery occurred in 4 min after returning to normal oxygenated Krebs solution. Release of ADO and INO evoked by 5 min ischemia was reduced by 62% and 39% respectively in the presence of TTX (0.5 uM) and by 25% and 20% in the presence of the NMDA receptor antagonist D-AP7 (100 uM). The results indicate that ischemia-evoked release of ADO and INO is greatly dependent on influx of Na+ through voltage dependent Na+ channels activity and partly mediated by activation of NMDA receptors. (C.N.R. and University Grants).

617.16

FORSKOLIN-STIMULATED CYCLIC AMP ACCUMULATION IN RAT CORTEX SLICES IS MARKEDLY ENHANCED BY ENDOGENOUS ADENOSINE. N. W. DeLapp and K. Eckols*. Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285.

This study investigated a role for endogenous adenosine in forskolin (F)-stimulated cyclic AMP (cAMP) accumulation in brain slices. cAMP accumulation was measured in adult rat cortex slices prelabeled with [3H] adenine. Adenylate cyclase was assayed in membrane preparations by conversion of [3H] ATP to [3H] cAMP.

Adenosine deaminase (ADA)-treatment of slices caused marked reductions in F-stimulated cAMP accumulation of 50% at 100 uM F up to 96% at 1 uM F. ADA inhibition was completely reversed by 5 uM 2-chloroadenosine (2CA). In ADA-treated slices stimulated with 1-100 uM 2CA, 1 uM F shifted the dose-response curve to the left and increased maximal stimulation 6-fold. Caffeine (200 uM) and 8-phenyl theophylline (10 uM) significantly inhibited 1 uM F stimulation in control slices and 1 uM F + 5 uM 2CA stimulation in ADA-treated slices. 1 uM vasoactive intestinal polypeptide stimulation of cAMP accumulation in slices was unaffected by 200 uM caffeine or ADA. 1 uM F-stimulated adenylate cyclase in cortex membranes was unaffected by ADA, 200 uM caffeine, or 100 uM 2CA. These data demonstrate that stimulation of cAMP accumulation in cortex slices by 1 uM F is primarily due to synergism with endogenous adenosine and that its inhibition by caffeine is largely due to blockade of adenosine receptors.

617.17

ADENOSINE A1-RECEPTOR ACTIVATION DECREASES NEURONAL EXCITABILITY IN RAT BRAINSTEM NEURONS: IN-VITRO INTRACELLULAR STUDIES. J.D. Marks and G.G. Haddad. Dept. of Pediatrics, Section of Respiratory Medicine, Yale Univ. Sch. of Medicine, New Haven, CT 06510.

Adenosine analogue administration into the brainstem decreases blood pressure and breathing in in-vivo preparations. To understand the cellular basis of these adenosine-mediated effects, we recorded intracellularly from vagal motoneurons using superfused rat brainstem slices. Input resistance (R_N), membrane potential (Vm), firing rate, and action potential waveform were recorded from 25 neurons before and after superfusion with a potent and specific A1 receptor agonist (S-ENBA or CPA) at 3 doses (1-100 uM). With A1 agonist superfusion, spontaneous firing ceased, without statistically significant changes in Vm. Both R_N and spike after-hyperpolarization amplitude (AHP) increased in a dose-dependent fashion (ANOVA, $p<.05$). Prior superfusion with a potent and specific A1 receptor antagonist (8-CPT, 800 uM) prevented these effects. Since the increase in AHP suggests an alteration in a Ca-dependent conductance, additional slices were superfused with Ca-free solution containing $CoCl_2$. This treatment blocked the adenosine-induced increases in R_N and AHP. We conclude that A1 receptor activation 1) reduces vagal motoneuron excitability by increasing $gK_{(Ca)}$; 2) most likely decreases other conductances to explain the increase in R_N.

617.18

FUNCTIONAL ADENOSINE A_2 RECEPTORS MAY BE PRESENT ON ENTERIC NERVE ENDINGS. R.M.Broad, T.J.MacDonald* and M.A.Cook. Departments of Pharmacology & Toxicology, and Medicine, University of Western Ontario, London, CANADA, N6G 2J7

Adenosine is a neuromodulator which may play a physiological role in the enteric nervous system to modify gastrointestinal motor function. We have demonstrated the inhibition, by adenosine analogs, of the evoked release of Substance P (SP) and Neurokinin A (NKA) from nerves in the myenteric plexus of the guinea pig ileum as well as inhibition of field-stimulated contraction of guinea pig longitudinal muscle-myenteric plexus (LMMP) preparations. Identification of the subtype of adenosine receptor mediating this response was sought. Inhibition of contraction of field stimulated LMMP was observed for all adenosine analogs; the A_1-selective agonist N^6-cyclopentyl adenosine (CPA) being more potent than the A_2-selective agonists which included CGS-21680. Antagonism of the responses with the A_1-selective antagonist 1,3-dipropyl-8-cyclopentylxanthine (DPCPX) and subsequent Schild analysis revealed, for CPA, a slope of 0.971 and a pA_2 of $1.1x10^{-8}$M. The same analysis for the A_2-selective agonists yielded slopes that were not significantly different from zero. Direct measurement of the inhibition of release of SP and NKA from perifused enteric ganglia yielded similar results. These data provide evidence for the presence of A_1 receptors functionally coupled to the release of excitatory mediators. The consistent micromolar EC_{50}'s obtained for the A_2 agonists suggest either a high threshold activity at A_1 receptors or action at putative distinct A_2 receptors. The lack of antagonism of A_2 agonists by the A_1 antagonist DPCPX suggests that the former possibility may not involve simple competitive interaction. Supported by MRC of Canada.

617.19

N^6-CYCLOPENTYLADENOSINE, A STABLE ADENOSINE ANALOG, EXERTS CONCENTRATION DEPENDENT EFFECTS ON EVOKED DENTATE GRANULE CELL ACTIVITY. T.H. Swanson and L.M.Masukawa. Dept. of Neurology, Graduate Hospital and Institute of Neuroscience, Univ. of Pennsylvania School of Med., Philadelphia, PA 19106.

Adenosine is involved in intercellular signalling in the hippocampus, although in what capacity is not entirely clear. Inhibitory actions of adenosine have been identified in regions CA3 and CA1 , but relatively little is known about its role in the dentate gyrus. Since the dentate is an important "gateway" to the hippocampus, we chose to characterize the role of adenosine in this region. Standard microelectrode field recordings were performed on dentate granule cells during perforant path stimulation in rat hippocampal slices. The amplitudes of the population post synaptic potential (PSP) and population spike (PS) were measured before and after application of N6-cyclopentyladenosine (CPA), a stable, A-1 receptor specific adenosine analog, at several concentrations. CPA at concentrations at and above 10 nanomolar (nM) reversibly inhibited the PSP and PS amplitude shortly after application. Application of 1 nM CPA resulted in little alteration of the PSP, but a marked enhancement of the PS amplitude which was slow to reach its maximal effect, but reversible. These preliminary data indicate that A-1 adenosine receptor activation results in both excitatory and inhibitory effects at different concentrations. The inhibitory effect may be due to a decrease of excitatory transmitter release while the excitatory effect may be a direct effect on the post-synaptic membrane, or a decrease of inhibitory transmitter release. Supported by a grant from the Epilepsy Foundation of America to THS, and NIH #NS23077 to LMM.

617.20

UNITARY EPSPs MEASURED BY WHOLE-CELL RECORDING ARE REDUCED BY ADENOSINE IN RAT HIPPOCAMPAL CA1 PYRAMIDAL NEURONS *IN VITRO*. T.V. Dunwiddie, C.R. Lupica, and W.R. Proctor, Veterans Administration Medical Research Service and University of Colorado Health Sciences Center, Denver, CO 80262.

Adenosine is an inhibitory neuromodulator of synaptic transmission in the central nervous system. Although indirect evidence indicates that adenosine acts at least in part by reducing transmitter release, direct evidence regarding the actual pre- and postsynaptic actions are lacking. In the present experiments, we have characterized the effects of adenosine at excitatory Schaffer collateral and commissural synapses onto hippocampal CA1 pyramidal neurons. Unitary EPSPs generated by low intensity stimulation ("minimal stimulation") showed a high degree of variability, which is most likely the result of quantal release of the excitatory transmitter. The stimulus voltage was set so that in 10 - 30% of the trials there was no apparent response ("failures"), i.e., the response (if any) was smaller than the baseline noise (std. dev. of noise estimates was 50 μV). Superfusion of adenosine (20 μM) resulted in a profound reduction in the mean EPSP amplitude and an increase in the number of failures. The ratio of variance/mean EPSP amplitude for these responses was virtually unchanged by adenosine, and was the same for both the 1st EPSP and the 2nd potentiated EPSP elicited with paired-pulses (50 msec interval). These results are consistent with the hypothesis that the major effect of adenosine in this preparation is to decrease the amount of transmitter released from excitatory presynaptic nerve terminals.

Supported by NIDA grant DA 02702 and the Veterans Admin. Medical Research Service.

617.21

SYNAPTIC INHIBITION BY ADENOSINE IS NOT ABOLISHED BY BARIUM S. Birnstiel, U. Gerber, and R.W. Greene.
Harvard Medical School/VAMC, Brockton, MA 02401

Adenosine-induced inhibition of evoked postsynaptic potentials (PSPs) and epileptiform burst firing was studied with intracellular recordings in the CA1 subfield of rat hippocampal slices. Adenosine (50 μM) hyperpolarized the membrane by 3.7±1.4 mV (n=19) in association with a decrease of the evoked PSP of 75±22% for control PSPs of 10-15 mV amplitude (n=9). During superfusion with 2 mM Ba^{2+}, the hyperpolarization evoked by 50 μM adenosine was abolished. The adenosine-induced inhibition of the PSPs was still evident, although the magnitude of this effect was significantly reduced ($p<.05$, Wilcoxon matched-pairs signed-ranks test). Analysis of the input-output curves showed little dependence of the percent PSP inhibition on the amplitude of the pre-adenosine PSP.

Adenosine also reduced Ba^{2+}-induced burst firing by 32±16% (n=16). Epileptiform activity evoked by 5 mM TEA was more potently inhibited by adenosine (by 88±11%, n=3). The results suggest that adenosine inhibits synaptic transmission and epileptiform activity by at least 2 mechanisms: an postsynaptic increase in g_K and a presynaptic effect independent of the adenosine-evoked outward potassium conductance.

617.22

ADENOSINE MODULATES PURINERGIC MOTOR TRANSMISSION IN RAT URINARY BLADDER. J. Pablo Huidobro-Toro*, C. Gloria Acevedo*, E. Contreras and G. Bustos. Neurohumoral Regulation Unit, Dept. Physiology, Faculty of Biological Sciences, P. Catholic Univ. Chile and Dept. Pharmacology, Faculty of Biological Sciences, Univ. Concepción, CHILE.

To examine whether adenosine (A) modifies the purinergic motor tone of the bladder,experiments were conducted in isolated bladders superfused with buffer containing atropine plus guanethidine; tissues were electrically stimulated. A and related structural analogs blocked the electrically induced twitches (0.1-5 Hz) in a concentration-dependent fashion. The rank order of potency was R-PIA > CHA > NECA > A > S-PIA. A also reduced the ATP-induced contraction (NECA > CADO > R-PIA > A > CHA). The application of 8 phenyl theophylline (8PT) increased the magnitude of the twitch response without modifying the basal activity; 8PT antagonized the A-induced inhibitory effect. The ATP-induced contractions were mimicked by mATP, ADP but not by AMP or A. Preincubation with 5-30 μM 8PT potentiated the ATP but not the mATP-induced contractions. Results support the hypothesis of a physiological regulatory A tone in the rat bladder mediated via A1 and A2 receptors acting at pre and postjunctional sites respectively. FONDECYT Grants #699-90 and 767-90.

617.23

REDUCTION OF EPILEPTIFORM ACTIVITY BY THE ADENOSINE BINDING ENHANCER PD 81,723. C.A. Janusz[1] & R.F. Berman[2]. Dept. [1]Physiol. & [2]Psych., Wayne State Univ., Det. MI.

The potency of exogenous adenosine in reducing orthodromically evoked population spike potentials elicited in area CA1 of rat hippocampal slices was greatly enhanced by the addition of the adenosine binding enhancer, PD 81,723, to the bathing medium. PD 81,723 alone had no effect on hippocampal cell excitability, but required the presence of adenosine. In addition, PD 81,723 significantly enhanced the adenosine-mediated facilitation observed in paired pulse trials. Since the CA2/CA3 pyramidal cells are believed to be primary generators of epileptiform activity in the hippocampus, we studied the actions of PD 81,723 on synaptic bursting in this area. Superfusion of slices with low Mg^{2+} medium while antidromically stimulating the Schaffer collaterals in stratum radiatum of CA3 resulted in the development of spontaneous as well as stimulus-triggered bursts of multiple population spikes. Addition of PD 81,723 to the low Mg^{2+} medium resulted in a dose-dependent reduction in the number and duration of the evoked and spontaneous multiple population spikes. These results suggest that bursting activity seen in the hippocampal slice can be suppressed by the adenosine binding enhancer, PD 81,723, perhaps through an enhancement of the actions of endogenous adenosine, released during synaptic bursting. (Supported by NIH Grant No. RR-08167).

NEUROTRANSMITTER TRANSPORT AND RELEASE

618.1

CHARACTERIZATION OF [^{3}H]DIPYRIDAMOLE BINDING TO RABBIT AND GUINEA PIG CNS NUCLEOSIDE TRANSPORTERS. K.W. Jones*, M.A. Cook, and J.R. Hammond*. Department of Pharmacology and Toxicology, University of Western Ontario, London, Canada, N6A 5B8.

In some species, [^{3}H]dipyridamole ([^{3}H]DYP) may label a subset of nucleoside transporters that the more established probe [^{3}H]nitrobenzylthioinosine ([^{3}H]NBMPR) cannot. This transporter heterogeneity was investigated in both rabbit and guinea pig CNS by comparing the binding of [^{3}H]DYP and [^{3}H]NBMPR to cortical synaptosomes. In initial studies, two "specific" (inhibitor displaceable) binding components were observed for [^{3}H]DYP: the first (50%) being to the cortical membranes, the second being to the glass fibre filters. [^{3}H]DYP binding to the filters was inhibited by 1μM DYP and dilazep (DIL) but not by nitrobenzylthioguanosine (NBTGR), and could be prevented completely by including 0.01% CHAPS in the assay medium. This concentration of CHAPS did not affect [^{3}H]DYP binding to the cortical tissue. In rabbit, using 10 μM DIL to define non-specific tissue binding and 0.01% CHAPS to prevent "specific" filter binding, [^{3}H]DYP bound to a single class of high affinity sites (K_D=1.4nM, B_{max}=153 fmole/mg, n_H=0.99) and was inhibited in a monophasic manner by the recognized transport inhibitors NBTGR (K_i=0.78nM), DIL (K_i=0.082nM), and R75 231 (K_i=0.65nM). Almost identical results (B_{max} and K_i) were found using [^{3}H]NBMPR as the probe. [^{3}H]NBMPR and [^{3}H]DYP (+0.01% CHAPS) were also found to label a similar number of binding sites (B_{max} ≈ 175 fmole/mg) in guinea pig cortical synaptosomes. After correcting for the displaceable binding of [^{3}H]DYP to glass fibre filters, no evidence was found for specific NBMPR-resistant [^{3}H]DYP binding sites, nor DYP-resistant [^{3}H]NBMPR binding sites. These results suggest that [^{3}H]DYP and [^{3}H]NBMPR bind to the same subset of nucleoside transport proteins in rabbit, and possibly guinea pig, cortical membranes. Supported by the MRC of Canada.

618.2

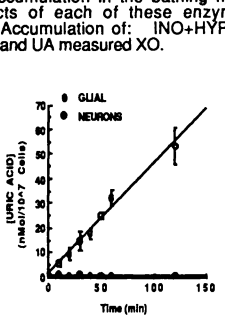
EVIDENCE FOR AN ESSENTIAL HISTIDINE IN CYTOCHROME b561 FROM SECRETORY VESICLES. P.M. Kelley and D. Njus*, Dept. Biol. Sci., Wayne State Univ., Detroit MI 48202

The biosynthesis of catecholamines, such as epinephrine and norepinephrine, as well as peptide hormones, such as vasopressin and oxytocin, within secretory vesicles requires reducing equivalents from ascorbic acid. To maintain the levels of ascorbic acid within these vesicles, reducing equivalents are shuttled in to the ascorbate via a trans-membrane protein, cytochrome b561. We have recently analyzed the kinetics of the individual reactions between ascorbate and the cytochrome both on the internal and external surfaces of the vesicle membrane and hypothesized that the reaction between cytochrome b561 and ascorbate involves the concerted transfer of both a proton and an electron (J. Biol. Chem. 266, 6878 (1991)). Since histidine has a pK near neutrality and might participate in proton transfer, we tested compounds which react with histidine for effects on the electron transfer properties of the cytochrome. We now report that diethylpyrocarbonate, a chemical modifier of histidine residues, dramatically slows the rate of electron transfer from ascorbate to oxidized cytochrome b561. The inhibition is greater at higher pH, and the electron transfer is completely blocked at pH 8.5. This argues that at least one of the seven histidines in cytochrome b561 participates in the electron transfer process and supports the hypothesis of concerted electron/proton transfer. Supported by NIH grant GM 33849 and the American Heart Association.

618.3

DIFFERENTIAL DISTRIBUTION OF PURINE DEGRADATIVE PATHWAYS BETWEEN GLIAL (G) AND NEURONAL (N) CELLS IN CULTURE. G. CEBALLOS*, R. RUBIO*, and J.TUTTLE. Univ. of Virginia, Charlottesville, VA., 22908.

Degradation of adenosine (ADO) in nervous tissues appears to occur in G but not in N. To demonstrate that is the case we performed studies in cultured embryonic chick G and N cells from ciliary ganglia. Cells were incubated in Krebs-Henseleit solution containing saturating concentrations (10^{-4} M) of the exogenous substrate for each degradative enzyme activity: ADO for adenosine deaminase (ADA), inosine (INO) for nucleoside phosphorylase (NP) and hypoxanthine (HYP) for xanthine oxidase (XO). Accumulation in the bathing media (nMoles/10^7 cells) of the products of each of these enzymatic reactions were evaluated by HPLC. Accumulation of: INO+HYP+UA measured ADA, HYP+UA measured NP and UA measured XO. As far as XO, the accumulation of UA rises with time and is greater in G than in N (fig). These results show that XO is preferentially found in G cells and is almost absent in N. The same patterns was found for ADA and for NP.



These results demonstrate that there is a preferential distribution of purine degradative pathways in G. In addition our results also demonstrate, for the first time, the presence of XO in G. Supported by Nat. Inst. of Cardiol.Méx., IPN. School of Med., Méx. and focusing giving grant Johnson & Johnson .

618.4

RELEASE OF AN ENDOGENOUS LIGAND FOR THE CANNABINOID RECEPTOR FROM RAT BRAIN SLICES. D.M. Evans, M.R. Johnson* and A.C. Howlett. Dept. Pharmacological and Physiological Science, Saint Louis University, 1402 S. Grand Blvd., St. Louis, MO 63104

Although marijuana and cannabinoid drugs have been used for medicinal purposes for centuries, only recently has the cellular mechanism of action - namely inhibition of adenylate cyclase via a Gi-coupled receptor - been elucidated. Binding of the synthetic ligand [^{3}H]CP55940 allowed the characterization of the cannabinoid receptor. This receptor has been shown to exist in brains of many animal species and has recently been cloned. However, to date, an endogenous ligand for this receptor has not been identified. The localization of receptors on neurons may suggest a role in modulation of responses and hence would indicate a possible neurotransmitter-like molecule as the endogenous ligand. In order to investigate whether an endogenous ligand is present in rat brain and can behave in a similar fashion to "classical" neurotransmitters we examined the ability of the calcium ionophore A23187 to release a compound which could subsequently interact with the cannabinoid receptor on rat brain membranes. We report that release of inhibitory activity was dependent on both concentration of A23187 and time of exposure to the ionophore. Because many classical transmitters as well as eicosanoid metabolites are released in response to such treatment, a large number of these compounds were analyzed for their abilities to inhibit [^{3}H]CP55940 binding. Results from these studies are reported.

Supported by NIDA grants DAO3690, DAO3612 and Glaxo, Inc.

618.5

METHIONINE INCORPORATION AND TAURINE CONTENT DURING DEVELOPMENT. P.-L. Lleu* and R.J. Huxtable. Department of Pharmacology, University of Arizona Health Sciences Center, Tucson, Arizona 85724.

Huxtable *et al.* (*Neurochem. Int.*, 15:233; 1989) found an inverse correlation between the increase of the phosphatidylethanolamine/phosphatidylcholine ratio and the decrease of the taurine concentration in rat brain P_2B fraction during development. However, Schaffer *et al.* (*FASEB Abs.*, A591; 1991) found that, in the heart taurine inhibits phospholipid methyltransferase, the enzyme converting phosphatidylethanolamine to phosphatidylcholine. These observations led us to study the incorporation of [^{3}H-methyl]methionine in rat hemisphere P_2B fraction during development to determine if a relationship exists between taurine concentration and the incorporation of [^{3}H-methyl]methionine into phospholipid. Seven, 14, 21 and 28 days-old rat were injected i.p. with 300 μCi/Kg [^{3}H-methyl]methionine. At 9 h, the amount of radioactivity was determined in the P_2B fraction (protein, supernatant and phospholipid). Compared to younger rats, 28 days-old rats have significantly increased incorporation of [^{3}H-methyl]methionine into phospholipid, with no change in incorporation into protein. In general, incorporation is inversely correlated with taurine concentration. These results suggest that taurine alters the phospholipid membrane composition which in turn may affect taurine modulation of Ca^{2+} binding to membranes.

618.6

RELEASE OF CHOLECYSTOKININ AND GASTRIN-RELEASING PEPTIDE, AND THEIR PRECURSORS FROM THE HUMAN NEUROEPITHELIOMA CELL LINE SK-N-MCIXC P.N.M. Konings, M.C.Beinfeld, T.W. Moody and T.P. Davis. Dept. of Pharmacol, Univ. of Arizona, Tucson, 85724, Univ. Med. Center, St. Louis, Dept. of Biochem., The George Washington Univ. Med. Center, Washington D.C..

A variety of neuropeptides are involved in the central control of gastrointestinal (GI) functions. Amongst these peptides are cholecystokinin (CCK) and gastrin-releasing peptide (GRP). A large number of studies indicate that these two peptides function as neurotransmitters or neuromodulators in the brain. Several of the CCK and GRP central effects relate directly to GI tract functions and control, such as effects on gastric acid secretion, satiety and feeding behavior. The role which these peptides play is linked to the mechanisms which regulate their biosynthesis and metabolism. The cholinergic human neuroeptihelioma cell line SK-N-MCIXC expresses high levels of mRNA for CCK and GRP (Verbeeck M.A.E. and Burbach, J.P.H.;FEBS 268:88-90 (1990). This cell line provides a useful model to study regulation of gene expression and metabolism of CCK and GRP. The secretion of CCK and GRP was studied to further characterize the cell line. Spontaneous release (s) versus 75 mM KCl stimulated release (KCl) experiments were performed. CCK/GRP were detected by region specific RIA following chromatography. The cell line mainly releases nonamidated CCK-precursor fragments (all data in pg/million cells/10 min: CCK-58-like, 270(s) /330(KCl); CCK-39/33, 280(s)/518(KCl); Gly extended CCK, 314(s)/536(KCl)), and a relatively small portion of amidated CCK, 24(s)/ 40(KCl)/amidated GRP, 369(s). Therefore, spontaneous release versus KCl depolarization release of CCK/GRP demonstrate regulated secretion in this cell line. (Supported by NIH grants DK-36289 and NS-18667).

BIOGENIC AMINES: UPTAKE AND RELEASE

619.1

ANALYSIS OF SINGLE ADRENAL MEDULLARY CELLS BY REVERSED PHASE MICROCOLUMN LIQUID CHROMATOGRAPHY. B.R. Cooper, J.A. Jankowski, D.J. Leszczyszyn, R.M. Wightman, J.W. Jorgenson*. Dept. of Chemistry, Univ. of North Carolina, Chapel Hill, NC 27599-3290.

The increasing need to analyze small sample volumes has prompted the development of miniaturized liquid chromatography designs. Micropacked liquid chromatography columns not only are capable of handling low nL injection volumes, but they offer higher separation efficiencies than conventional sized columns. A micropacked liquid chromatography column with electrochemical detection has been used to quantitatively determine the total amount of epinephrine (EPI) and norepinephrine (NE) in single cultured adrenomedullary cells. The percent release of EPI and NE by a single cell due to a chemical stimulation has also been determined. A single cell was exposed to acetylcholine or carbachol, and an aliquot of the cellular bathing solution was removed and analyzed. Then the stimulated cell was lysed, and the remaining solution was analyzed.

619.2

INTRICACIES OF HANDLING [^{3}H]RESERPINE AND DETERMINING RESERPINE BINDING PARAMETERS. J.D. Deupree and R. Zielinski*, Dept. of Pharmacology, Univ. Nebraska Medical Center, 600 S. 42nd Street, Omaha, NE 68198-6260.

Reserpine has been used extensively for both therapeutic purposes and as a research tool for depleting catecholamine stores and to identify the catecholamine transporter in the membranes of storage vesicles found in adrenergic nerve endings and in the chromaffin cells of the adrenal gland. One of the difficulties of working with reserpine is that it sticks extensively to both glass and plastic surfaces. In the presence of less than 20 μg of protein, over 70% of the [^{3}H]reserpine in a binding assay bound to glass test tubes in a time dependent fashion with steady state conditions being reached in one hour. Stock solutions of [^{3}H]reserpine, prepared in either 1 mM HCl or 10 mM acetic acid, also bound to glass test tubes to the extent of 25 and 45% respectively. Much of the variation in apparent Kd values reported for [^{3}H]reserpine binding to the catecholamine transporter appears to be due to depletion of free [^{3}H]reserpine due to binding to both the test tube and the vesicle membranes. Both of these problems can be overcome by using assay volumes in the 4 to 6 ml range with protein concentrations of less than 10 μg/assay tube. Using these improved assay conditions the concentration of [^{3}H]reserpine required to occupy 50% of the catecholamine displacable binding sites on storage granule membranes (the apparent Kd) dropped from 1 nM to 0.03 nM. These results point out that quite often reserpine concentrations are up to 90% less than expected and large assay volumes with small protein concentrations are essential for obtaining reasonable estimates of the apparent Kd. (Support by NIH grant #NS 15187)

619.3

ION DEPENDENCES OF SEROTONIN TRANSPORT IN A TRANSFECTED CELL MODEL. Albert S. Chang* and Dominic Man-Kit Lam. Center for Biotechnology, Baylor College of Medicine, The Woodlands, TX 77381.

A clonal, transfectant cell line, L-S1, which stably manifests high-affinity serotonin transport properties, was produced by transfection of human genomic DNA into mouse L-M fibroblasts. Using this cell line, we analyzed imipramine-sensitive, 5-[^{3}H]HT binding to the transport system. This binding activity was not attributable to the presence of 5-HT receptor subtypes in L-S1 cells, and was Na^+ independent but Cl^- dependent. In contrast, 5-[^{3}H]HT transport was both Na^+ and Cl^- dependent for these cells. The apparent K_m, but not the apparent V_{max}, varied with extracellular concentrations of either ion.The coupling ratio for 5-HT, Na^+ and Cl^- was deduced to be 1:1:1 per transport cycle.

By postulating that both Na^+ and Cl^- ions facilitate 5-HT transport by directly interacting with, or binding to, the transport system along with 5-HT , we used kinetic modelling to account for the involvement of each ion in this transport process. Both binding and transport results supported an ordered sequence of Cl^- and 5-HT binding to the transporter (Cl^- first, and may facilitate 5-HT binding); Na+ binding is not ordered relative to 5-HT binding, but is necessary for membrane translocation of 5-HT.

619.4

KINETIC AND ALLOSTERIC MODEL FOR THE ACETYLCHOLINE TRANSPORTER-VESAMICOL RECEPTOR. E.D. Clarkson, B.A. Bahr, G.A. Rogers and S.M. Parsons. Neuroscience Research Institute, Univ. of California, Santa Barbara , California 93106

The ligand binding relationship between the acetylcholine transporter (AcChT) and the vesamicol receptor (VR) in synaptic vesicles purified from the Torpedo electric organ was studied using analogs of AcCh and vesamicol. AcCh inhibits binding of [^{3}H]vesamicol with an affinity much lower (200-fold ratio) than its affinity for active transport (K_m= 0.3 mM). Three analogs of AcCh that are up to 57% larger in volume than AcCh also exhibit large ratios for the macroscopic competitive dissociation constants measured by inhibition of vesamicol binding and active transport of AcCh. AcCh analog 15 (N,N-dimethyl-3-pyrrolidinium benzoate iodide), that exhibits a ratio of 20, was synthesized in tritiated form and shown to be actively transported with the same V_{max} as AcCh itself. Trans-6-methoxyvesamicol was shown to be a noncompetitive inhibitor of AcCh and analog 15 active transport. The data demonstrate that vesamicol binds to an allosteric site in the AcChT. A specific model is proposed, and values for some of the microscopic constants in the model are estimated. The model posits very loose binding of AcCh to the AcChT (K_{AO}= 50 mM) that is followed by rapid transport. Little selectivity for the structure of the transported ion is exhibited.

619.5

EFFECTS OF LOCAL ANESTHETICS/COCAINE ON CHOLINE UPTAKE. Merouane Bencherif and Ronald J. Lukas. Division of Neurobiology, Barrow Neurol. Institute, Phoenix, AZ 85013.

Cells of the TE671/RD human clone and the PC12 rat pheochromocytoma exhibit a number of neuron-like features including the expression of high affinity choline uptake (HACU). IC_{50} values for blockade of HACU in the presence of 10nM ^{3}H-choline at 20°C are 1uM for native choline and 2uM for hemicholinium-3. By contrast, IC_{50} values are 100 and 1000uM, for nicotine and carbamylcholine, respectively. HACU is also sensitive to blockade by local anesthetics dimethisoquin (5uM), tetracaine (50uM) and procaine (500uM) at concentrations comparable to those that inhibit ion flux through nicotinic receptors expressed by TE671/RD or PC12 cells (IC_{50} values of about 3, 3, and 40 uM, respectively). These observations suggest that local anesthetics at therapeutic doses may not only affect ligand-gated and voltage-gated ion channel function, but may also affect HACU, which is the principal regulatory factor in acetylcholine synthesis. Furthermore, the local anesthetic cocaine inhibits HACU by TE671/RD and PC12 cells with IC_{50} values of about 500uM, suggesting that anomalous parasympathetic effects of cocaine and the observation that behaviorally-relevant and lethal doses of cocaine exceed those known to inhibit high affinity catecholamine uptake may both be explained by heretofore unrecognized effects of cocaine on acetylcholine metabolism and HACU.

619.6

Endogenous Sodium-dependent High Affinity Choline Uptake in *Xenopus Laevis* Oocytes. R.S. Fiore, S. Shimada, G. Uhl and J.T. Coyle. Depts. of Neurosci., Psych., and Neurol., Johns Hopkins Sch. of Med., Balto., MD 21205 and Lab. Mol. Neurobiol., ARC/NIDA, Balto., MD 21224.

Sodium-dependent high affinity choline uptake (SDHACU) is believed to be the rate-limiting mechanism in the production of acetylcholine by cholinergic neurons. We have discovered that *Xenopus laevis* oocytes possess endogenous high affinity choline uptake which is pharmacologically and kinetically similar to the SDHACU of cholinergic synaptosomal preparations.

Defolliculated *Xenopus laevis* oocytes were incubated with 100nM[^{3}H]choline chloride and examined for choline uptake under varying conditions. Competition against [^{3}H]choline uptake by unlabeled choline resulted in a complex displacement curve. Computer analysis indicated two components of uptake with high(Kt=3.7μM) and low affinities(Kt=148μM). Hemicholinium-3(HCh-3), a competitive inhibitor of SDHACU, selectively inhibited high affinity [^{3}H]choline uptake with an apparent Ki of 278nM. At much higher concentrations, HCh-3 also inhibited low affinity uptake with an apparent Ki of 36μM. Similar to the SDHACU of cholinergic preparations, the uptake of 100nM choline chloride by *Xenopus* oocytes was both sodium-, time- and temperature-dependent.

619.7

INHIBITION OF THE REUPTAKE OF NOREPINEPHRINE AND SEROTONIN. CONFORMATIONALLY CONSTRAINED ANALOGUES OF ANTIDEPRESSANT DRUGS AS SELECTIVE MONOAMINE REUPTAKE INHIBITORS. Gary L. Grunewald, David B. Lewis*, and Sheila M. Zipfel*. Department of Medicinal Chemistry, University of Kansas, Lawrence, KS 66045.

While some antidepressant drugs show selectivity in the inhibition of reuptake of either serotonin (5-HT) or norepinephrine (NE), no clear relationship between the chemical structure of the antidepressant drug and the neurotransmitter selectivity has been shown. Previous molecular mechanics calculations in our laboratory (*J. Med. Chem.* **1988**, *31*, 913-919) have suggested a correlation between the neurotransmitter selectivity and the conformation of key portions of flexible amine reuptake inhibitors. To probe this point, we have synthesized several conformationally constrained monoamine reuptake inhibitors which have been designed to mimic calculated low energy conformations of known antidepressant drugs selective for either NE or 5-HT reuptake.

The new conformationally constrained analogues have been evaluated for selectivity for the inhibition of [^{3}*H*]NE *vs.* [^{3}*H*]5-HT into chopped rat cortex. The selectivity ratio (ratio of IC_{50} values for inhibition of the uptake of NE compared to 5-HT) was determined for each compound and compared to the conformationally flexible parent drug. We will discuss our preliminary results, which show that the synthesis of conformationally constrained analogues may be of value to probe the effects of conformation on the selectivity of neurotransmitter reuptake by antidepressant drugs. Supported by NIH training grant GM 07775 and by NSF REU grant CHE 9100476.

619.8

INTERACTION BETWEEN OUABAIN AND THE DOPAMINE TRANSPORT RECEPTORS IN THE HUMAN FRONTAL CORTEX. J. Clardy*, A. Hitri, R.J. Wyatt and J.E.Kleinman. NIMH Neuroscience Center at St. Elizabeths, Washington, D.C. 20032.

Dopamine uptake (DAU) and the binding of DAU blockers to the transport receptors is a Na^+ and K^+ dependent process that may be linked to the Na^+,K^+-ATPase. Ouabain as an inhibitor of the Na^+,K^+-ATPase causes accumulation of Na^+ ions inside of the cell and competitively inhibits DAU in the striatal synaptosomes (Holz and Coyle 1974). We hypothesize that the effect of ouabain on DAU results from its direct inhibition of DA binding to the transporter receptor. To test this hypothesis, we labeled the DA transport receptors with [^{3}H]GBR 12935 and studied the binding inhibition by inhibitors of Na^+,K^+-ATPase in human brain. Aliquots of membrane preparations were incubated with 10 nM [^{3}H]GBR 12935 in the presence of increasing concentrations of ouabain analogue strophanthidine. The results indicate a 40% reduction in [^{3}H]GBR 12935 binding, which is concentration independent as the 40% reduction remained constant throughout nano and micromolar concentrations. In contrast the DAU blocker GBR 12909 inhibited [^{3}H]ouabain binding in a concentration dependent fashion with a shallow inhibition cur(IC_{50} of $1x10^{-5}$M). The decrease in saturable [^{3}H]GBR 12935 binding by a ouabain analogue is suggestive of receptor deactivation, and contrasts the nondestructive nature of inhibition of [^{3}H]ouabain binding by GBR 12909.

619.9

DORSOVENTRAL STRIATAL DIFFERENCES IN STIMULATED DOPAMINE (DA) OVERFLOW: RELATIONSHIP TO NOMIFENSINE EFFECTS. D. Ruskin and J.F. Marshall, Dept. of Psychobiology, University of California, Irvine, DA 92717

Relative to the caudate-putamen (CPu), the nucleus accumbens septi (NAS) has a paucity of high-affinity DA uptake sites, as evidenced by a lower *Vmax* for [^{3}H]DA uptake and a lower *Bmax* for [^{3}H]mazindol binding to the associated recognition sites. We investigated possible effects of these differences on stimulated DA overflow with a brain slice superfusion paradigm. [^{3}H]DA - labelled 350 μm coronal rat CPu and NAS slices (bathed in 10 μM pargyline) were electrically stimulated at 10 Hz to promote ^{3}H release. Calcium-dependent overflow of ^{3}H was demonstrated to be >85% [^{3}H]DA. Stimulated overflow (expressed as % of total radioactivity of the tissue at the start of stimulation) from NAS was 53% greater than that from CPu. This result is apparently due to regional differences in uptake site density, since stimulated overflow was equivalent in both areas in the presence of 10 μM nomifensine. Nomifensine potentiated overflow in both NAS and CPu; however, potentiation of CPu overflow was 1.9 times that of NAS. The results suggest that a given amount of terminal depolarization will cause greater overflow in ventral striatum than in dorsal due to differential transmitter reuptake, while the dorsal striatum may be preferentially affected by DA-reuptake blockers.

619.10

IMMUNOHISTOCHEMICAL EVIDENCE OF AMPHETAMINE INDUCED EXTRACELLULAR DOPAMINE DIFFUSION IN THE RAT NEOSTRIATUM RELEVANCE OF VOLUME TRANSMISSION

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Dept. of Histology & Neurobiology, Karolinska Institutet, Stockholm, Sweden. §Dept. of Pharmacology. Free University, Amsterdam, The Netherlands

Dopamine (DA) may diffuse from DA nerve terminals to reach, via extracellular fluid pathways, D2-receptors on prolactin producing cells transplanted into neostriatum, and modify their activity (Bjelke et al. 91). In order to further analyze DA diffusion we induced DA release by amphetamine administration. Rats (Sprague-Dawley 200g b.w.) were given a high dose of d-amphetamine (10mg/kg i.p.) one hour prior to sacrifice. Perfusion with 5% glutaraldehyde was performed followed by vibratom sectioning. Antisera against DA and DARPP-32 were applied to the sections using a double immunofluorescence protocol. Following d-amphetamine administration increased DA immunoreactivity (IR) was seen in the striatal patches. DA IR was even visualized within the cytoplasm of the DARPP-32 nerve cellbodies especially within the striatal patches. Thus, after amphetamine induced DA release, DA appears to diffuse extra cellularly and to be taken up by DARPP-32 IR cells. On the basis of these findings Volume Transmission appears to be an important mode of communication for amphetamine induced increases of DA neurotransmission.

Bjelke B., Agnati LF., Fuxe K.: Experimental evidence for volume transmission by analysis of host graft interaction using intrastriatal adenohypophyseal transplants in the rat in combination with 3-dimensional reconstruction. In Fuxe K., Agnati L.F (Eds.) Volume Transmission in the Brain: Novel Mechanisms for Neural Transmission. Advances in Neuroscience, Vol 1, Raven Press, 1991, pp. 463-478

619.11

AMMONIUM RELEASES DOPAMINE IN THE NUCLEUS ACCUMBENS: MICRODIALYSIS EVIDENCE FOR A WEAK BASE MODEL OF PSYCHOSTIMULANT ACTION. H. M. Sung*[1], E. Pothos[1], D. Sulzer[2], G.P. Mark[1], S. Rayport[2] and B. G. Hoebel[1]. [1]Dept. of Psychology, Princeton Univ., Princeton, NJ 08544 and [2]Dept. of Psychiatry/NYS Psychiatric Inst., Columbia Univ., New York, NY 10032.

D-amphetamine (AMPH) and phencyclidine (PCP) are membrane-permeable weak bases. Local infusion of AMPH or PCP significantly elevates synaptic dopamine (DA) levels in the rat nucleus accumbens (NAC) by more than 20-fold (Br. Res. Bull. 19: 623, 1987; Life Sci.42: 1713, 1988) and rapidly reduces pH gradients in cultured midbrain DA neurons (Neuron 5: 797, 1990). It has been suggested that reduction of synaptic vesicle pH gradient contributes to elevated DA levels for some psychostimulants. In the present study, the classic weak base ammonium chloride (NH_4Cl) or ammonium sulfate ($(NH_4)_2SO_4$) was dissolved in Ringer's solution and infused in the NAC of freely moving rats through reverse dialysis (30 uL of a 100 mM solution was infused for 30 minutes; an estimated 10%, i.e. 10 mM, diffused into the brain). Both compounds increased extracellular dopamine more than 20-fold ($n=10, p<.05$). DOPAC and HVA also increased ($p<.01$). The same effects were observed when the pH of the infused ammonium solution was adjusted to the original pH of the perfusate (pH=6.2). Other weak base compounds gave similar results. The findings indicate that ammonium, a weak base that changes vesicular proton gradients, releases DA and therefore mimics the effects of AMPH and PCP if applied to the accumbens terminals in vivo. Consequently, the mechanism of action of some psychostimulants may be related to the reduction of intracellular proton gradients.

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619.12

REVERSE ACTION OF THE PLASMA MEMBRANE TRANSPORTER MEDIATES WEAK BASE-INDUCED DOPAMINE RELEASE: IMPLICATIONS FOR AMPHETAMINE ACTION. D. Sulzer[1], N.T. Maidment[2], and S. Rayport.[1] [1]Dept. Psychiatry, Ctr. Neurobiol. & Behav., Columbia Univ.; Dept. Neuropathol., NYS Psychiatric Inst., NY 10032; [2]Dept. Psychiatry, UCLA, Los Angeles 90024

As a lipophilic weak base, amphetamine (AMPH) reduces vesicular pH gradients; this inhibits uptake and increases release of monoamines from storage vesicles (Sulzer & Rayport, 1990). We examined whether the resulting increased cytosolic transmitter reaches the synapse via reverse action of the uptake transporter. Ventral midbrain from P7 rats was dissociated, plated at a density of about 1,000 DA neurons/well, and maintained for >2 weeks. In control incubations, we measured about 100 fmoles of extracellular DA (release *minus* reuptake). Extracellular DA increased over 5-fold with 200 μM AMPH. The plasma membrane DA uptake blocker benztropine (BENZ; 10 μM) significantly increased extracellular DA (release alone). When normalized for the effect of BENZ on reuptake, BENZ attenuated the AMPH-induced increase in DA. BENZ might act by inhibiting uptake of AMPH through the DA transporter, blocking reverse transport of DA, or both. To circumvent effects due to specific AMPH uptake, we mimicked the weak base action of AMPH with ammonia (added as NH_4Cl), which is membrane permeable and partitions according to the pH gradient, and the proton/cation ionophore monensin. NH_4Cl (5 mM) increased extracellular DA 2-fold; BENZ inhibited this increase by 50%. Monensin (50 nM) increased extracellular DA 3-fold, but this was not affected by BENZ, perhaps due to an attenuation of the Na^+ gradient by monensin. These findings support a model whereby AMPH and other weak bases, once accumulated in the cell, increase cytosolic DA through inhibition of vesicular proton gradients; elevated cytosolic DA is then discharged, at least in part, by reverse action of the uptake transporter.

619.13

THE AROMATIC AMINO ACID DECARBOXYLASE INHIBITOR, NSD-1015, INCREASES DOPAMINE RELEASE IN VITRO FROM SUPERFUSED CORPUS STRIATAL TISSUE FRAGMENTS OF MALE RATS. D. E. Dluzen and J. L. McDermott. Departments of Anatomy and Geriatric Medicine, N.E.O.U.C.O.M., Rootstown, OH 44272, and University Hospitals, Cleveland, Ohio.

Addition of NSD-1015 (10μM) to Krebs-Ringer Phosphate (KRP) superfusion medium significantly increased potassium stimulated (K^+ - 30mM) dopamine (DA) release in vitro from superfused corpus striatal tissue fragments (areas under K^+ - stimulated curves: KRP = 134 ± 23, N=6 vs. KRP + NSD-1015 = 203 ± 22, N=10, pg/70 min). This increased K^+ - stimulated release appears attributable to an increase in the spontaneous output of DA since basal (pre - K^+) DA release of superfusions with KRP + NSD-1015 (17 ± 6 pg/mg/min) were significantly greater than those with KRP alone (6 ± 3 pg/mg/min). To test the effect of NSD-1015 upon spontaneous DA release, superfusions with KRP medium were either continued with KRP or changed to KRP + NSD-1015. The change to KRP + NSD-1015 produced a significant increase in DA release (areas under DA release curves: KRP = 19 ± 8, N=4 vs. KRP + NSD-1015 = 107 ± 37, N=4, pg/70 min). Moreover, spontaneous DA release showed a dose dependent response as a function of NSD-1015 concentration (areas under DA curves: 0.1μM = 20 ± 3, N=4; 1.0μM = 103 ± 23, N=4; 10μM = 104 ± 21, N=6; 100μM = 181 ± 17, N=3; 1000μM = 419 ± 27, N=4, pg/70 min). Supported by grants from the Parkinson's Disease Foundation and United Way of Stark County.

619.14

ONTOGENETIC STUDIES ON BINDING SITES FOR DOPAMINE UPTAKE BLOCKERS IN NIGRO-STRIATAL DOPAMINERGIC NEURONS. M. Valchar*, A. G. Wright, Jr.* and I. Hanbauer. Lab. of Chemical Pharmacology, National Heart, Lung, and Blood Institute, Bethesda, MD 20892.

The expression of dopamine uptake sites that are sensitive to dopamine uptake blockers is developmentally linked to neuritic outgrowth. Our results show that in embryos and in newborn rats the binding sites for dopamine uptake blockers appear not to be colocalized with the dopamine transporter in plasma membranes. In 19-day-old embryos and in newly born rats specific binding sites for ^{3}H-GBR-12935 can be measured in intact striatal synaptosomes, but are not detectable in washed synaptosomal membrane preparations. Permeabilization of striatal synaptosomes prepared from these age groups with streptolysin-O (0.2 units/ml) causes leakage of cytosolic proteins into the incubation medium and reduces ^{3}H-GBR-12935 binding by 70%. In contrast, six days after birth 100% of the specific binding sites for ^{3}H-GBR-12935 and ^{3}H-WIN-35428 are expressed in neuronal membranes; no loss of binding sites for these radioligands occurs in lysed and washed synaptosomes. This is similar to the distribution of binding sites found in striatal synaptosomes of adult rats. The cytosolic binding protein for ^{3}H-WIN-35428 that leaks out of streptolysin-O-treated synaptosomes is presently being purified. (M. Valchar is supported by a grant from ICI Pharmaceuticals, Wilmington, DE, and the Scottish Rite, N.M.J., Lexington, MA.)

619.15

AGONISTIC AND ANTAGONISTIC INTERACTIONS OF DOPAMINE UPTAKE BLOCKERS IN THE HUMAN FRONTAL CORTEX. Ana Hitri Ph.D., Manuel E. Casanova M.D., Joel E. Kleinman M.D. Ph.D. and Richard Jed Wyatt M.D. NIMH, Neuroscience Center at St. Elizabeths, Washington, D.C. 20032.

The pharmacology of the high affinity site of [^{3}H]GBR 12935 binding in the human frontal cortex is consistent with that of the dopamine transporter in the striatum. Of the structurally unrelated dopamine uptake blockers the most potent inhibitor of [^{3}H]GBR 12935 binding in the frontal cortex is benztropine (BZT) whereas amfonelic acid is the weakest (Hitri et al 1991). BZT inhibition of [^{3}H]GBR 12935 binding in the human frontal cortex involves multiple binding sites. To determine the pharmacological profile of the BZT inhibited binding sites we studied the effects of the known dopamine (DA) blockers on BZT inhibition. Aliquots of human frontal cortex homogenate were incubated with [^{3}H]GBR 12935 and increasing concentrations of BZT in the presence and absence of DA uptake blockers at their uptake IC50 concentrations. GBR 12909 potentiated nanomolar BZT inhibition from 20 to 80%. In contrast amfonelic acid prevented the inhibition of BZT in the nano and micromolar range. Nomifensine, cocaine, and DA moderatly potentiated BZT inhibition without altering the shape of the curve. The data indicate that GBR 12909 exerts a synergistic agonistic effect, whereas amfonelic acid exerts an antagonistic effect on BZT sensitive [^{3}H]GBR 12935 binding sites in the human frontal cortex.

619.16

^{125}I-PIA (*p*-IODOAMPHETAMINE) MAY LABEL MONOAMINE OXIDASE A (MAO_A) IN CRUDE RAT FRONTAL CORTEX HOMOGENATE. Xuemei Huang*, Michael P. Jonhson, David E. Nichols and Chester A. Mathis. Depts. of Pharmacology and Toxicology, and Medicinal Chemistry and Pharmacognosy, Purdue Univ., W. Lafayette, IN 47907. Donner Laboratory, Lawrence Berkeley Laboratory, Univ. of California, Berkeley, CA 94720.

In attempts to develop a novel label for the serotonin uptake carrier, ^{125}I-*p*-iodoamphetamine (^{125}I-PIA) was synthesized. A specific binding site for ^{125}I-PIA was subsequently identified in rat frontal cortex homogenate. Scatchard analysis yielded a K_D of 421 ± 16 nM, a Hill coefficient of 0.991 ± 0.003, and B_{max} of 32.1 ± 1.9 pmol/mg protein. Certain amphetamine derivatives were tested but did not displace ^{125}I-PIA with very high affinity. The K_I values of *p*-chloroamphetamine (PCA), d-amphetamine (*d*-AMP), and *S*-3,4-methylenedioxymethamphetamine (*S*-MDMA) were 603 ± 53 nM, 2045 ± 238 nM, and 4758 ± 603 nM repectively. Surprisingly, the K_I value for displacement of ^{125}I-PIA by the 5-HT uptake inhibitor paroxetine was 6654 ± 965 nM. The NE and DA uptake inhibitors desipramine and GBR-12909 did not displace ^{125}I-PIA ($K_I > 10$ μM). Serotonin and dopamine also had K_Is > 10 μM. One week after treating rats with a neurotoxic regimen of PCA (10 mg/kg i.p. twice), neither the K_D nor B_{max} of ^{125}I-PIA binding were significantly different from saline treated animals. However, the B_{max} of [^{3}H]-paroxetine binding was decreased almost 80%. This suggested that the ^{125}I-PIA binding site was not the serotonin uptake carrier and was also not located on the 5-HT axon terminal. It seemed possible that membrane-bound monoamine oxidase might therefore be the site labeled by ^{125}I-PIA. The MAO_B inhibitor deprenyl did not displace ^{125}I-PIA ($K_I > 10$ μM). However the MAO_A inhibitor clorgyline displaced ^{125}I-PIA binding with a K_I of 95 ± 14 nM. The results suggest that ^{125}I-PIA may bind to membrane bound MAO_A. The possible use of tritiated clorgyline to label membrane bound MAO_A is suggested.

619.17

BW 1370U87 - A SELECTIVE, COMPETITIVE INHIBITOR OF MONOAMINE OXIDASE-A H.L. White and P.W. Scates*. Division of Pharmacology, Wellcome Research Laboratories, Research Triangle Park, NC 27709.

BW 1370U87, a potent, selective inhibitor of rat and human brain monoamine oxidase-A (MAO-A) is being developed as a potential antidepressant/antiphobic/antipanic agent. Kinetic experiments demonstrate a competitive mechanism of action for this compound, with $K_i = 0.01$ µM using either serotonin or tyramine as substrate. After preincubation of BW 1370U87 with mitochondrial MAO, full activity was restored by dialysis, indicating complete reversibility of the inhibition. Following oral administration to rats, BW 1370U87 inhibited brain MAO-A in a dose-dependent manner, with an ED_{50} = 8 mg/kg p o and a duration greater than 7 hr, but less than 24 hr. No significant inhibition of MAO-B by BW 1370U87 was observed either in vitro or ex vivo. Ex vivo inhibition was also reversed by extensive dialysis of brain homogenates from pretreated rats. The selectivity, reversibility, and competitive kinetics of the inhibition by BW 1370U87 may contribute to an improved safety profile with this novel MAO-A inhibitor.

619.18

EFFECT OF 1370U87, BROFAROMINE, AND MOCLOBEMIDE ON RAT BRAIN BIOGENIC AMINES AND THEIR ACID METABOLITES. R. Norton*, S. Jones-Humble*, G. Pollard, H.L. White, B. Cooper. Division of Pharmacology, Wellcome Research Labs., Research Triangle Pk., NC 27709.

Monoamine oxidase (MAO) inhibitors have been shown to increase brain concentrations of biogenic amines while decreasing concentrations of their acid metabolites. It has been suggested that the magnitude of this effect is an indicator of MAO inhibition. Oral doses of 1370U87, a novel and reversible selective MAO-A inhibitor, moclobemide and brofaromine were given to rats at doses shown to inhibit brain MAO-A by 80%. The effects on brain biogenic amines and their metabolites were quantified 2 and 4 hours after injection. Moclobemide (10mg/kg) and 1370U87 (20mg/kg) induced larger increases in 5HT, NE, and DA and larger decreases in DOPAC, 5HIAA, and HVA than did brofaromine (3mg/kg). The effect of 1370U87 (20mg/kg po) on biogenic amines lasted 6 hours significantly ($p <= 0.05$) increasing NE, 5HT, and DA while decreasing DOPAC, HVA, and 5HIAA. Similar results were found in a dose response study when 1370U87 was given orally at 10, 20, or 40mg/kg.

619.19

BLOOD PRESSURE EFFECTS OF MONOAMINE OXIDASE INHIBITORS IN RESPONSE TO ORALLY ADMINISTERED TYRAMINE IN THE RAT. M. S. Carroll*, O. G. Beek*, and B. R. Cooper. Division of Pharmacology, Burroughs Wellcome Co., Research Triangle Park, NC 27709.

The reversible monoamine oxidase A inhibitors 1370U87, 616U76, brofaromine, and moclobemide, and the irreversible nonselective monoamine oxidase inhibitor phenelzine were compared for potentiation of the pressor response to oral tyramine. Conscious rats were pretreated with doses of the monoamine oxidase inhibitors sufficient to produce 80% inhibition of brain monoamine oxidase, and then were challenged with orally-administered tyramine. Blood pressure was monitored prior to and after tyramine, and peak pressor responses were compared. At a dose of 15 mg/kg tyramine, the pressor response of 1370U87 was statistically similar to the vehicle control response. 616U76, brofaromine, and moclobemide elicited mild tyramine pressor effects, whereas phenelzine resulted in a marked elevation of blood pressure. Higher doses of tyramine elicited blood pressure elevations from all of the monoamine oxidase inhibitors.

619.20

CNS PHARMACOLOGY OF 1370U87: A NOVEL REVERSIBLE SELECTIVE MAO-A INHIBITOR. B. Cooper, H.L. White, G. Rigdon, O. Beek*, R. Norton*, G. Kraemer*, R. Ferris. Division of Pharmacology, Wellcome Research Labs., Research Triangle Pk., NC 27709.

1370U87 is a potent reversible selective inhibitor of rat and human brain MAO-A having a competitive mechanism of action and an oral ED_{50} (ex vivo) of 8 mg/kg. The duration of action exceeds eight hours. The ED_{80} dose for inhibition of MAO-A (20 mg/kg) elevates NE, DA, and 5HT levels in brain without significantly potentiating the blood pressure effects of orally administered tyramine. No inhibition of MAO-B with 1370U87 has been observed. 1370U87 is effective in the 5-HTP potentiation test and Porsolt test. The compound also has effects on abnormal behavior produced by early mother/infant separation in monkeys. Autonomic testing with 1370U87 revealed that there are no adverse cardiovascular symptoms in dogs or rats. No pharmacologically significant effects were observed on various isolated tissues, receptors or uptake systems. 1370U87 is representative of a new class of safer reversible MAO-A inhibitors which are expected to create a new generation of agents to use for depression, anxiety conditions, phobias, obsessive compulsive behaviors and borderline personality disorders.

619.21

AUTORADIOGRAPHIC LOCALIZATION OF NOREPINEPHRINE UPTAKE SITES IN RAT BRAIN USING [^{125}I]-IODOTOMOXETINE. S.L. Gackenheimer, E.S. Littlefield*, D. E. Mais*, D.W. Robertson and D.R. Gehlert. CNS and Cardiovascular Pharmacology, Lilly Research Labs., Indianapolis, IN 46285.

The norepinephrine (NE) uptake transporter is a primary mechanism involved in the termination of the effects of NE. These sites were originally studied using radioligands which lacked specificity for this site or had high levels of nonspecific binding. Therefore, localization using autoradiography has been difficult. We have developed a method for localization of NE uptake sites using a novel radioiodinated analog of the potent and specific NE uptake inhibitor, tomoxetine.

A series of biochemical studies were conducted to determine the appropriate conditions for labeling slide mounted sections of rat forebrain. Once optimal conditions were determined, labeled sections were exposed against a sheet of Hyperfilm-B max for 24-48 hours. Quantitation of the binding was accomplished using [^{125}I]-Microscales standards (Amersham) which were placed against the film along with the labeled sections. Following film development, the images were analyzed by desitometry using a MCID (Canada) image analysis system.

[^{125}I]-iodotomoxetine (N-methyl-3-[2-[^{125}I]-iodophenoxy] benzene propanamine, 2200 Ci/mmol) bound to tissue sections with an apparent K_d value of 50 pM and a B_{max} of 26 fmol/mg tissue. Specific [^{125}I]-iodotomoxetine binding was inhibited by low nanomolar concentrations of desipramine or tomoxetine while higher concentrations of paroxetine (5HT uptake inhibitor) or GBR 12909 (DA uptake inhibitor) were required to displace binding. The distribution of binding sites correlated well with the known location of NE containing cell bodies and terminals. High densities of binding were found in the locus coeruleus, zona incerta, nucleus of the solitary tract, dorsal raphe nucleus, anterior thalamic nuclei, hypoglossal nucleus and ventral tegmental area. Moderate levels of binding were seen in the paraventricular nucleus of the hypothalamus. Low densities were found in the caudate-putamen, globus pallidus and cerebral cortex. There appeared to be no laminar distinction within the cerebral cortex.

These results indicate that [^{125}I]-iodotomoxetine exhibits high affinity and specificity for NE uptake sites and provides a useful tool for autoradiographic studies by providing rapid exposure of autoradiograms.

619.22

PHORBOL ESTERS CAUSE PREFERENTIAL SECRETION OF NOREPINEPHRINE FROM BOVINE CHROMAFFIN CELLS. A.L. Cahill and R.L. Perlman, Depts. of Pediatrics and Pharmacol. and Physiol. Sciences, The University of Chicago, Chicago, IL 60637

Phorbol esters stimulate the secretion of [^{3}H]norepinephrine from intact bovine chromaffin cells and potentiate Ca^{2+}-induced secretion from permeabilized chromaffin cells (Pocotte et al., PNAS 82, 930-934, 1985).To examine the effects of phorbol esters on the secretion of the individual catecholamines, we have assayed catecholamine secretion by HPLC with electrochemical detection. We find that phorbol dibutyrate (PDBu) causes a much greater release of norepinephrine (NE) than of epinephrine (E):

Secretagogue	% NE Secreted	% E Secreted	%NE/%E
None	0.29 ± 0.03	0.27 ± 0.03	1.07
55 mM K^+	22.21 ± 0.67	13.77 ± 0.61	1.61
1 µM PDBu	11.26 ± 0.53	2.44 ± 0.23	4.61

In efforts to understand this differential effect of phorbol esters, we have measured secretion in primary cultures of bovine chromaffin cells which are highly enriched in either epinephrine- or norepinephrine-containing cells (Moro et al., Anal. Biochem. 185, 243-248, 1990). Phorbol dibutyrate was a much more effective secretagogue in norepinephrine- as opposed to epinephrine-containing cells. These studies reveal an unsuspected difference in the regulation of epinephrine and norepinephrine secretion. (Supported by research grants from NIH and NSF)

619.23

REAL-TIME IDENTIFICATION OF EPINEPHRINE AND NOREPINEPHRINE COSECRETION FROM INDIVIDUAL BOVINE ADRENAL MEDULLARY CELLS. E.L. Ciolkowski, B.R. Cooper, J.W. Jorgenson*, and R.M. Wightman, Dept. of Chemistry, University of North Carolina, Chapel Hill, N.C. 27599-3290.

Carbon-fiber microelectrodes placed adjacent to individual bovine adrenal medullary cells have been used to monitor the nicotine-evoked release of catecholamine and to distinguish between epinephrine (E) and norepinephrine (NE). The identity of released catecholamine was determined using cyclic voltammetry at electrodes coated with a thick (>350 nm) Nafion film. The identification is based on the difference in the rates of the intra-cyclization reaction of the oxidized forms of the catecholamines (1). Three types of cells were identified: those which secreted primarily E; those which secreted primarily NE; and those which secreted mixtures of E and NE. Liquid chromatographic analysis of single cells shows that the relative proportions of E and NE in the individual cells is the same as the proportions observed during secretion of those same cells. These findings show that although individual adrenal cells in culture primarily contain either E or NE, they are capable of storing and secreting both catecholamines simultaneously.

(1) Hawley, M.D., et.al. *J. Am. Chem. Soc.* **1967**, *89*, 447-450.

NEUROTRANSMITTER AND HORMONE RECEPTORS

620.1

STEROIDAL REGULATION OF TYPE II CORTICOSTERIOD RECEPTORS IN PRIMARY CULTURES OF DISPERSED HIPPOCAMPAL NEURONS. D. O'Donnell and M.J. Meaney. McGill University, Dept. of Neurology & Neurosurgery, Douglas Hosp. Res. Ctr., Montreal, Que. H4H 1R3, Canada.

The existence of two corticosteroid receptor systems in the rat brain is well established, however regulation of their expression by corticosteroids remains controversial. The present study examined the effects of RU 28362, corticosterone (CORT) and aldosterone (ALDO) on type II (^{3}H-dexamethasone ± RU 28362) corticosteroid receptor binding in dispersed hippocampal cell cultures derived from animals sacrificed at E19-20 days of gestation. Four days of exposure to 10 nM RU 28362 resulted in a robust down-regulation of type II receptors (~70%) whereas similar exposure with 10 nM of either CORT or ALDO produced a more moderate (40-45%) decrease in type II receptor binding capacity. Scatchard analysis revealed that diminished type II receptor level was due to a decrease in total number of binding sites (Bmax) with no differences in Kd (~0.8nM). Given that both CORT and ALDO decrease type II binding to the same extent, it is possible that this effect is mediated via type I corticosteroid receptors. Furthermore, the calculated EC_{50} for ALDO in the hippocampal cell cultures is low (EC_{50} = 7-8 nM) and thus strongly suggests the involvement of type I receptors. In order to address this question, we treated cultured hippocampal cells with 10 nM ALDO ± 20 nM of either type I receptor antagonist (RU 26752) or type II recepotor antagonsist (RU 38486). Neither antagonist was successful in blocking the type II corticosteroid receptor down-regulation by ALDO. Surprisingly, incubation with either RU 26752 or RU 38486 on their own produced a decrease in type II binding. Studies are currently underway to further characterize these agonist-like effects on type II receptors via type I and/or type II receptors.

620.2

DIURNAL RHYTHMS OF GLUCOCORTICOID AND MINERALOCORTICOID mRNA EXPRESSION IN THE HIPPOCAMPAL FORMATION: REGIONAL SPECIFICITY AND STEROID DEPENDENCE. J.P. Herman, B.S. McEwen, H.M. Chao, H Coirini, and S.J. Watson, Mental Health Research Institute, U. of Michigan, Ann Arbor, MI 48109, and the Rockefeller University, New York, NY 10021.

Brain mineralocorticoid (MR) and glucocorticoid (GR) receptor subtypes are exposed to a wide range of diurnal glucocorticoid (GC) concentrations. The availability of MR and GR proteins is clearly essential to integration of GC signals across the circadian cycle. To assess this availability, we have employed semi-quantitative in situ hybridization histochemical analysis of MR and GR mRNA expression across the circadian cycle (4 h intervals) in intact rats and rats deficient of endogenous GCs (adrenalectomized (ADX) rats). MR mRNA showed a significant, bimodal diurnal rhythm in all hippocampal subfields. ADX resulted in a small (25%) but significant overall increase in MR mRNA in all subfields. GR mRNA, on the other hand, showed a monotonic diurnal rhythm restricted to CA1 and dentate gyrus (DG). Effects of ADX were subfield dependent: pronounced round-the-clock increases were observed in CA1 and CA3 following ADX, whereas in the DG ADX served to eliminate the circadian trough in GR mRNA expression. No circadian or ADX-induced changes in GR or MR mRNA were observed in the frontoparietal cortex or in the posterior thalamic nuclei. These data indicate that MR and GR mRNA exhibit hippocampus-specific diurnal rhythms of expression which interact with, but cannot be totally explained by, circulating GCs. We hypothesize that hippocampal steroid receptor gene expression is synchronized by both steroid and neuronal input across the daily light-dark cycle.

Supported by MH422251, DA02265 and MH41256.

620.3

HOMOLOGOUS REGULATION OF GnRH RECEPTOR mRNA IN A GONADOTROPE CELL LINE. Tsutsumi, M., S.C. Laws*#, P. L. Mellon##, J. L. Roberts, and S. C. Sealfon. Fishberg Center in Neurobiology, Mount Sinai School of Medicine, New York, NY 10029, US EPA, Research Triangle Park, NC 27711#, and The Salk Institute, La Jolla, CA 92037##.

Using the *Xenopus* oocyte system as a bioassay for GnRH receptor (GnRH-R) mRNA level, we have previously demonstrated that regulation of the GnRH-R in ovine pituitary cultures by gonadal hormones is associated with concomitant changes in receptor mRNA levels (Mol. Endo. 4:119,1990). The transgenic mouse-derived cell line, αT3-1 cells, express a GnRH-R similar to those found in rat and mouse pituitary (Mol. Endo.5:347, 91), making this cell line an excellent model system for the study of the regulatory mechanisms of the GnRH-R by GnRH.

Exposure of αT3-1 cells to GnRH induces alterations of GnRH-R mRNA levels, as assayed in oocytes. The effect of GnRH is bimodal and both time and concentration dependent. Exposure to physiological concentrations of GnRH for 20 minutes causes a significant increase in GnRH-R mRNA at 24 h, but after a continous 24 h exposure, the mRNA level is unchanged. In contrast, a 20 minute exposure to 1μM GnRH does not alter GnRH-R mRNA at 24 h, whereas 24 h exposure leads to a marked decrease. Preliminary binding data suggest that up- and down-regulation of the GnRH-R mRNA is accompanied by similar changes in receptor number. In rat pituitary, similar effects on GnRH-R biosynthetic rate have been reported (Endo. 126:2577,90). Thus, both homologous and heterologous regulation of the GnRH-R involve modulation of biosynthetic rate. (NIH Grant K11 DK01854)

620.4

REGULATION OF SUBSTANCE P RECEPTOR SYSTEMS BY NALTREXONE. O.J. Igwe. Division of Pharmacology, Schools of Pharmacy/Medicine, UMKC, Kansas City, MO 64108.

Opiate antagonist, naltrexone (NALT), increases opioid peptides in the brain and up-regulates brain opioid receptors resulting in functional supersensitivity, i.e., an enhanced morphine-induced analgesia. SP causes the release of met-enkephalin. Recent evidence strongly suggests that SP system is regulated by endogenous opioid peptides. Here, NALT was used to explore the functional link between SP and endogenous opioid receptor systems. Male Sprague-Dawley rats under ether anesthesia were implanted subcutaneously with Alzet® miniosmotic pumps, filled with either NALT HCl (100mg/ml) or vehicle (control), for 7 days. Animals were decapitated on day 8, whole brains rapidly removed and homogenized in ice-cold buffer for use in in vitro binding assays in order to explore the regulation of SP and intracellular inositol 1,4,5-trisphosphate (IP_3) receptors. Using tritiated SP and IP_3 as ligands, the affinities (K_D) for SP and IP_3 receptors were unaffected by chronic NALT treatment. However, the densities (B_{max}) for both SP and IP_3 were significantly increased by 35% and 52%, respectively. NALT appears to up-regulate SP receptor. It also appears that IP_3 receptor, a calcium mobilizing receptor, is positively modulated by stimulation of other classes of cell-surface receptors.

620.5

UP-REGULATION OF δ OPIOID RECEPTORS IN NEUROHYBRID CELLS IS ACCOMPANIED BY LOSSES IN LYSOSOMAL ENZYME ACTIVITIES M. M. Belcheva#*, J. Barg#*, R. J. McHale#*, X-M. Gao†*, D-M. Chuang† and C. J. Coscia# # Dept. of Biochem. and Mol. Biol., St. Louis Univ. Sch. Med. St.Louis, MO 63104, and the †Biological Psychiatry Branch, NIMH, Bethesda, MD 20892

Opioid receptor binding affinities and densities in subcellular fractions from neurohybrid cells were assessed using 2 models of up-regulation. Na-butyrate was added to NCB-20 cell cultures to achieve heterologous up-regulation, whereas δ sites in NG108-15 cells were up-regulated by chronic treatment with the opioid antagonist naltrexone (homologous). In both paradigms, sites in light (LM) and heavy membranes (HM) (resolved by density centrifugation of Con A pretreated cells) displayed increases in ^{3}H-DADLE and ^{3}H-diprenorphine B_{max} values without changes in affinity. In contrast to 48 h antagonist treatment, 5 min exposure to naltrexone down-regulated HM δ sites. β-Glucuronidase and β-hexoseaminidase activities were reduced in naltrexone-treated cells in a time and dose-dependent manner suggesting opioid-induced alteration in lysosomal enzyme trafficking. Comparable down- and up-regulation and attenuation of lysosomal enzyme activity was observed following treatment with the δ-selective opioid peptide antagonist ICI174864. These results suggest that homologous up-regulation entails down-regulation followed by an inhibition of receptor degradation.

620.6

IMIPRAMINE REDUCED BINDING NUMBER OF SIGMA SITES IN RAT STRIATUM AND HIPPOCAMPUS. Y.Shirayama*, T.Nishikawa*, M.Watanabe and K.Takahashi Natl. Inst. Neurosci., NCNP, Tokyo and Natl. Defence Med. College, Saitama, Japan

Repeated treatment for 14days with imipramine (IMI; 10mg/kg, i.p., once a day) induced a decrease in the Bmax, without affecting the Kd, of [3H]DTG (1,3-di-o-tolylguanidine) binding to the haloperidol-sensitive sigma sites in the striatum and hippocampus of the rat. This reduction dose not seem to be due to the residual IMI in the brain tissues, since luM of IMI in the assay medium of cortical [3H]DTG binding augmented the Kd value, in the absence of change in the Bmax value, of the binding. Finally, repeated treatment with IMI in combination with p-chlorophenylalanine (a serotonin synthesis inhibitor) no longer altered the striatal and hippocampal [3H]DTG binding. These results indicate that depletion of the brain serotonin may antagonize the ability of subchronic administration of IMI to reduce [3H]DTG binding sites in the striatum and hippocampus, suggesting that serotonergic system could be involved in the regulation of sigma binding sites in these brain areas.

620.7

CO-ADMINISTRATION OF THE PERIPHERAL BENZODIAZEPINE ANTAGONIST PK11195 ATTENUATES LORAZEPAM-INDUCED TOLERANCE AND RECEPTOR DOWNREGULATION. W.R. Galpern, L.G. Miller, D.J. Greenblatt* and R.I. Shader*. Div. of Clinical Pharmacology, Depts. of Pharmacology and Psychiatry, Tufts Univ. School of Medicine, Boston, MA 02111

Some evidence indicates that ligands at the peripheral benzodiazepine site can influence effects at the central $GABA_A$ receptor site. The peripheral antagonist PK11195 (PK) was co-administered with the benzodiazepine agonist lorazepam (LRZ) to evaluate interactions during chronic administration in a mouse model. PK alone (5 mg/kg/d) did not alter motor activity, benzodiazepine binding, or GABA-dependent chloride uptake over 14 days. LRZ alone (2 mg/kg/d) produced tolerance at 7 days, with accompanying benzodiazepine receptor downregulation in cortex (CX) and hippocampus (HI) and decreased GABA-dependent chloride uptake in CX. Animals receiving PK/LRZ in combination had a reduction in the development of tolerance. Benzodiazepine binding in vivo was reduced in cortex but not hippocampus at days 7 and 14. GABA-dependent chloride uptake was decreased, but not significantly ($p<0.15$), in cortex at 7 days. Thus, PK appears to attenuate effects of chronic LRZ on behavior and $GABA_A$ receptor binding and function. This might be due to interactions at peripheral benzodiazepine sites on neurons or to indirect effects of compounds such as neurosteroids.

620.8

CHOLINERGIC INVOLVEMENT IN RENAL PERIPHERAL BENZODIAZEPINE RECEPTOR RESPONSE TO STRESS. P.V. Holmes & R.C. Drugan. Schrier Research Lab., Dept. of Psychology, Brown University, Providence, R.I. 02912

Rats exposed to inescapable shock stress exhibit a rapid reduction in the density (B_{max}) of renal peripheral benzodiazepine receptors (PBR). Prior investigations have revealed that neither adrenalectomy, hypophysectomy, nor 6-hydroxydopamine lesions reverse the stress-induced alteration in renal PBR suggesting that the effect is independent of pituitary and adrenal hormones or catecholaminergic activity. We presently report a dose-dependent attenuation of the stress-induced reduction in renal PBR with methyl-scopolamine pretreatment. Though the previous studies discount a role of noradrenergic mechanisms in the PBR response to stress, peripheral cholinergic receptors appear to be involved. We tested the hypothesis that direct cholinergic innervation of the kidney mediates the stress-induced reduction in PBR by surgically denervating the kidney. PBR in denervated kidneys continued to exhibit a reduction in [^{3}H]-Ro5-4864 binding similar to that seen in sham control kidneys in stressed rats. Denervation had no effect in non-stressed rats. Cholinergic effects on renal PBR appear not to be mediated by direct cholinergic renal innervation. We are currently investigating the role of cholinergic cardiovascular systems in the etiology of the stress-induced alterations in renal PBR. Research supported by NIMH grant #MH44034 and an Alfred P. Sloan Research Fellowship #BR2852 to R.C.D.

620.9

MITOGENICALLY-INDUCED ALTERATION OF PERIPHERAL-TYPE BENZODIAZEPINE (τ) RECEPTOR DENSITY ON THE NB2-11C CELL CLONE. J.M. Evans-Shields* and H.E. Laird II. College of Pharmacy, Dept. of Pharmacology & Toxicology, Univ. of Arizona 85721.

The affinity and density of the peripheral-type benzodiazepine (τ) receptors expressed in Nb2-11c clonal cells were compared under different stages of mitogenesis. Previous work from this laboratory (Laird et al. **Eur. J. Pharmacol.** 171:25, 1989.) has shown that Nb 2 cells possess a τ receptor and that ligands acting on this site modulate prolactin-stimulated mitogenesis. Therefore, this receptor has a functional role in Nb 2 cellular physiology. The Nb2 node lymphoma cells are undifferentiated T cells and are dependent on prolactin for mitogenesis. For this work we chose the Nb2-11c, the cloned cell line due to its genetic homogeneity. When prolactin is removed from the culture medium the cells enter the quiescent or stationary state. Both stationary and log growing cells were collected and processed for binding. Saturation experiments using PK11195, a ligand specific for the τ receptor yielded a Kd value of 1.93 ± .003 nM and a Bmax of 2.19 ± .001 pM for the stationary cells. Log cells yielded a Kd value of 1.54 ± .03 nM which is similar to that of the stationary cells. However, the Bmax was 3 fold lower than the value obtained for the stationary cells (Bmax=879 ± .12 *f*M). These data suggest that the τ receptor may play a significant role in the ability of the cells to undergo mitogenesis. Further studies are underway to kinetically and pharmacologically characterize these changes in the receptor. (Supported by Ariz. Dis. Con. Res. Comm. #82-1685, HEL)

620.10

Hippocampal sympathetic ingrowth alters the kinetics of Phorbol Ester receptor binding. V. Ayyagari and L.E. Harrell. Departments of Neurology and Psychology, VA Medical Center and University of Alabama, Birmingham, AL 35294.

Fibers from the superior cervical ganglion (SCG) replace the cholinergic input to the hippocampus following medial septum (MS) lesions. Hippocampal sympathetic ingrowth (HSI) has been shown to induce an upregulation of the carbachol-stimulated PI hydrolysis. We now report ^{3}H-Phorbol-12,13-dibutyrate (PDBu) binding kinetics in the dorsal and ventral hippocampus. Adult male Sprague-Dawley rats were divided into 3 surgical groups: sham surgery; MS lesions; or MS lesions + SCGx. Receptor binding studies were performed 4 to 6 weeks post-surgery, using cold PDBu to measure non-specific binding. Preliminary results indicate that MS lesions increase the B_{max}, while HSI appears to normalize K_d. Both the K_d and B_{max} of ^{3}H PDBu were consistently higher in the dorsal compared with the ventral part of the hippocampus. These results indicate that HSI, which causes an upregulation of the products of PI hydrolysis, may also activate protein kinase C (PKC), which in turn phosphorylates the cellular proteins involved in learning/memory processes.

621.1

EFFECT OF SOCIAL STRESS AND HOUSING CONDITIONS ON NEUROENDOCRINE MEASURES. R.R. Sakai, S.M. Weiss*, C.D. Blanchard, R.J. Blanchard, R.L. Spencer and B.S. McEwen. The Rockefeller University, 1230 York Ave., NY., NY 10021 and The University of Hawaii, Manoa, 2430 Campus Rd., Honolulu, HI 96822.

Analysis of social and agonistic interactions in rat groups in semi-natural burrowing situations have revealed complex patterns of behavioral changes for subordinate males. These behaviors include reduced social, sexual and aggressive activity, changes in sleep cycles, weight loss associated with lower food intake and other behaviors consonant with the symptoms of clinical depression.

In the current study we examined 8 groups, each with 4-5 males and 2 females in visible burrow systems. Dominant hierarchies were monitored and documented during the 2 week colony period, after which a basal blood sample was collected for determination of plasma corticosterone (CORT) levels and the males were adrenalectomized. Brains (hippocampus and cortex), pituitary, spleen, thymus and adrenal glands were removed on the following day. Plasma CORT levels of dominant male rats were lower than those of male subordinates (6.37μg% vs 16.15μg%) but higher than singly-housed control males (1.4 μg%). Adrenal and spleen weights were increased in both subordinates and dominants compared to controls. In contrast, there was no difference between the three groups in Type I or Type II glucocorticoid binding in the brain areas examined.

These data suggest that both subordinate and dominant male rats in a burrow system habitat may provide useful models for analysis of the behavioral and neuroendocrine effects of prolonged social stess. [Supported by AA06220(RJB), MH42803(DCB) and MH41256(BSM)].

621.2

EFFECT OF SWIM STRESS ON THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS: A TIME COURSE STUDY. J. F. López, D. M. Vázquez, H. Akil and Stanley J. Watson . Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109.

The anterior pituitary corticotroph uses different biochemichal strategies to cope with acute vs chronic stimulation. It has been shown that chronic foot shock stress causes a significant increase in both Proopiomelanocortin (POMC) peptide content and POMC mRNA in the anterior lobe, when compared to acute stress (Endocrinology 119:1793). In contrast, chronic swim stress does not cause a significant increase of POMC peptide content in the anterior lobe (Neuroendocrinology 52:405). To further characterize the effect of swim on the hypothalamic-pituitary-adrenal axis, we performed a time course study in which rats underwent daily swim stress sessions for 1, 3, 7, 10, 14, 17 and 21 days. Rats were sacrificed 24 hours after the last swim session. One group sacrificed immediately after one swim session (acute swim) and one control group were also included. Corticosterone levels were significantly elevated after an acute swim session (ANOVA $p < 0.05$) but returned to baseline after 24 hours. Corticosterone was again slightly elevated after 3 days of swim and slowly decreased after 7 and 10 days of swim. From days 14 to 21, corticosterone levels began to rise again. Plasma ACTH levels closely resembled corticosterone levels. No statistically significant changes in POMC peptide content or POMC mRNA levels were detected in the anterior lobe at any of the time points studied. The relationship of this findings to changes observed in the intermediate lobe of the pituitary will be presented. Supported by The Robert Wood Johnson Minority Medical Faculty Development Program (J.F.L.) and by MH422251 (H.A., S.J.W.)

621.3

HPA RESPONSES TO ACUTE RESTRAINT STRESS FOLLOWING CHRONIC EXPOSURE TO COLD IN HANDLED AND NONHANDLED RATS. S. Bhatnagar, V. Viau, and M.J. Meaney. Douglas Hospital Res. Ctr. Depts. of Psychiatry, and Neurology and Neurosurgery, McGill Univ., Montréal, Canada H4H 1R3

Neonatal handling results in altered HPA responses to stress. As adults, handled (H) animals show reduced plasma ACTH and corticosterone (B) levels both during and following the termination of stress. These effects appear to be, in part, related to differences in the negative-feedback sensitivity to elevated glucocorticoid levels. In the present study we examined differences in response to acute exposure to a 20-min restraint stress in H and NH animals following exposure to 3-weeks to cold stress. Chronic stress has previously been shown to enhance HPA responses to novel stressors. In the present study we found an overall effect of handling, such that H animals, regardless of pretreatment, showed lower plasma B levels than NH animals during and following stress. Chronic stress had little or no effect on the plasma B response to restraint in H animals. In contrast, chronically-stressed, NH animals exhibited significantly elevated plasma B levels following the termination of stress, compared with NH controls. Thus, the effect of previous exposure to chronic stress on HPA responses to a novel stressor were significantly greater in the NH animals. These findings extend previous data on differences in delayed negative-feedback between H and NH animals. Such differences appear to be selective to the delayed forms of negative-feedback. In a second study we examined plasma ACTH responses to restraint in H and NH rats treated 5-min prior to stress with 30 µg B or saline. Plasma ACTH responses to stress were greater in NH animals, but the response to pretreatment with B was similar in both groups of animals.

621.4

ENDOCRINE RESPONSE OF INFANT RATS TO HANDLING. C. Kuhn, J. Eck* and R. Francis*. Dep't of Pharmacology, Duke Medical Center, Durham, NC 27710.

"Handling"(removal from the nest) of infant rats influences adult responsivity to stress, an effect that may be mediated by the altered ontogeny of glucocorticoid receptors that results. The neural/hormonal mediators of this response are not clear. In the present study, we evaluated the effect of a single handling episode on several potential endocrine mediators of handling effects. Corticosterone (CS), growth hormone (GH), T_3 and T_4 secretion were measured throughout the developmental period that handling maximally influences glucocorticoid receptors and behavior.

Five, 10 and 15 day old rats were placed in a room-temperature container for 15 min. Animals were decapitated, and serum collected for hormone assay at the end of this time, or were returned to the dam, and killed 15, 45 min or 2 hrs later. Controls remained with the dam until decapitation. All hormones were measured by radioimmunoassay. CS was elevated significantly at the end of treatment at every age, but the response was larger and more prolonged in 15 d.o. pups than in younger pups. GH rose transiently on day 10 and 15 but not on day 5. Neither T_3 nor T_4 changed at any age. These results best support a role for CS as a mediator of biochemical and behavioral changes, as handling produces robust changes only in CS throughout the critical period. Supported by NIH DK 41777

621.5

ANDROGEN INHIBITS THE NEUROENDOCRINE AND NEUROCHEMICAL RESPONSE TO NOVELTY STRESS. R.J.Handa, S.A.Lorens, K.R.Nunley, M.George and G.Hejna. Depts. of Cell Biology, Neurobiology and Anatomy and Pharmacology. Loyola University, Chicago. Maywood, IL 60153

To determine the effects of androgen in modifying the neuroendocrine and neurochemical response to novelty stress, we gonadectomized (gx'd) male rats and implanted a subcutaneous Silastic capsule containing either dihydrotestosterone (DHT; 2cm length), or estradiol (E; 0.5 cm length). Control groups were either left intact or gx'd and given a blank capsule. After 28 days of treatment, animals were placed into an open field (OF) and sacrificed after 20 min. Control animals within each treatment group were killed upon removal from their home cage (HC). Increases in plasma corticosterone (CORT) were seen following OF in all treatment groups ($p<0.01$ vs. respective HC controls). OF CORT levels in gx'd males were greater than levels in intact rats ($p<0.01$). DHT treatment of gx'd rats **decreased** OF CORT levels to the level of the intact animal whereas treatment with E **increased** OF CORT levels ($p<0.02$) above that of gx'd males. Dopamine (DA) turnover in the medial frontal cortex was elevated in response to OF in gx'd, intact and E treated groups but **not** in DHT treated animals (OF DOPAC/DA ratio = 2.0$\pm$ 0.1 GX'D; 1.8$\pm$ 0.1 intact; 0.7$\pm$0.1 DHT; 2.4$\pm$0.2 E; $p<0.01$). DHT treatment also decreased activity in the OF during the first 5 minutes ($p<0.02$) but not during later periods. These data demonstrate that androgen treated rats respond differently to stress. Such differences may underlie androgen dependent non-reproductive behavioral changes.

621.6

INTERACTIONS OF NOVELTY STRESS AND TREATMENT WITH GONADAL STEROIDS ON CIRCULATING CORTISOL. J. A. Czaja, Department of Psychology, Miami University, Oxford, OH 45056

Individual and combined effects of novelty stress and gonadal steroids on circulating cortisol levels were studied in guinea pigs. Forty eight gonadectomized adults (24 females and 24 males) were injected for 7 days with either sesame oil (0.2 cc per day), testosterone propionate (TP, 1 mg/kg BW), or estradiol benzoate (EB, 10 µg/kg BW). On the last day of injections, subjects assigned to the Novelty Groups were individually moved from their home cage into a novel environment (open-topped glass container). After 30 minutes, they were placed into a similar sized methoxyflurane-filled container with a cover. Two minutes later they were sacrificed by decapitation and trunk blood collected. Subjects in the Control Groups were moved from their home cages immediately into the methoxyflurane-filled container and sacrificed 2 minutes later in the same manner. Plasma cortisol was estimated by radioimmunoassay. The gonadal hormone treatments had an effect ($F[2,36] = 3.85$, $p < .04$). Estradiol elevated cortisol levels relative to treatments with oil ($t[14] = 2.667$, $p < .02$) or TP ($t[14] = 4.471$, $p < .001$). Exposure to the novel environment also had a significant impact on cortisol levels ($F[1,36] = 30.691$, $p < .0001$). There were no significant overall gender differences in these cortisol responses ($F[1,36] = .158$, $p > .10$). The novel experience elevated cortisol levels in the oil and TP treated subjects ($t[14] = 4.508$, $p < .0001$ and $t[14] = 6.488$, $p < .0001$). In EB treated subjects, this increase was not significant compared to the already elevated levels produced by this hormone treatment ($t[14]=0.863$, $p > .10$).

621.7

MALE AND FEMALE DIFFERENCES IN RESPONSE TO RESTRAINT STRESS IN RATS. W.T.Gallucci*, L. Schneiderman,* M.A. Smith, H. J. Whitfield and P.W. Gold*. Clinical Neuroendocrinology Branch, NIMH, Bethesda MD 20892

Clinical data suggest that stress may precipitate the onset of major psychiatric illness such as depression and eating disorders. Manifestations of these illnesses may be attributed to perturbations in stress-responsive neurohormonal systems such as the hypothalamic-pituitary-adrenal axis (HPA). Epidemiological studies have shown that these illnesses are much more common in women than in men. Previous studies in animals have shown that female rats are more responsive than male rats to restraint stress as indicated by elevated levels of ACTH, corticosterone and AVP in peripheral blood during a single 2 hour restraint period. In this study, we investigated the secretion of these stress responsive hormones into the blood after 0, 15, 45, or 180 minutes of restraint stress. After the restraint period, the animals were sacrificed and trunk blood collected. The results indicate that female rats had significantly higher ($p<.001$), corticosterone levels than male rats over the total restraint period. In addition, female rats had higher peak ($p<.002$) and basal corticosterone levels ($p<.001$) than males. The AVP response showed marked differences between the sexes ($p<.0001$). Female AVP levels were elevated after the 15, 45, and 180 minute periods of restraint and showed a 44% increase over males at 45 and 180 minutes. On the other hand, males were not significantly different from baseline at 15, 45 and 180 minutes of restraint. The ACTH response also showed a significant sex difference ($p<.0005$). This response is in contrast to the males whose response is not significantly different from baseline at the 180 minute time period. These findings suggest that female rats produced more ACTH, AVP and corticosterone in response to similar stress conditions. Central factors involving corticotropin-releasing activity may differ between male and female rats which may account for the increased hormonal responsiveness in females. Whether these results are related to behavioral vulnerabilities between the sexes and whether they reflect excessive stress system responsiveness of the female sex to stress, remains to be studied.

621.8

EVIDENCE FOR ENHANCED TRANSLOCATION OF LYMPHOID GLUCOCORTICOID RECEPTORS IN PTSD. R. Yehuda, E.L. Giller, D. Boisoneau, S.M. Southwick, M.T. Lowy and John W. Mason. Psychiatry Department, University of Connecticut Health Center, Farmington, CT 06032.

Our previous finding of a significantly lower mean 24-hr urinary cortisol excretion and a greater number of lymphocyte glucocorticoid receptors (GR) in combat veterans with posttraumatic stress disorder (PTSD) has suggested an enhanced negative feedback sensitivity of the hypothalamic-pituitary-adrenal axis in this disorder. To further explore this possibility we examined 8:00 a.m. cortisol levels and lymphocyte GR number in 10 PTSD and 10 normal controls at baseline and following the 11:00 p.m. administration of 0.5 and 0.25 mg doses of dexamethasone (DEX). Consistent with our earlier report, the PTSD group had a significantly larger number of 8:00 a.m. GR at baseline compared to normal controls (mean GR/cell = 8716 ± 1212 and 5128 ± 1284 for PTSD and normals, respectively). Nine hours following DEX, the PTSD group showed a significantly greater decline in cortisol (i.e., a 90% vs. 76% suppression to 0.5 mg DEX, and 58% vs. 30% suppression to 0.25 mg DEX), and a decrease in the number of cytosolic GR (i.e., 40% vs. 14% fewer receptors following 0.5 mg and 19% vs. 3% following .25 mg DEX in PTSD and normals, respectively). Percent cortisol suppression at 8:00 a.m. postDEX was significantly correlated with decreases in GR in both groups at the 0.50 mg dose. The greater decline in cytosolic GR following DEX in the PTSD likely represents an enhanced translocation of the steroid-GR complex into the cell nucleus, and provides further support for HPA dysfunction in this disorder.

621.9

GENETIC DETERMINANTS OF HYPOTHALAMUS-PITUITARY-ADRENAL AXIS RESPONSIVENESS IN HUMANS. C. Kirschbaum*, S. Wüst*, H.-G. Faig*, J. Buchtal*, C. Strasburger*, H. Lehnert and D. Hellhammer. University of Trier, Trier, F.R.G.

Hypothalamus-pituitary-adrenal (HPA) axis responses are characterized by large interindividual differences. The present study was designed to clarify if a specific individual sensitivity of the HPA-axis exists and to which extent it is genetically determined.

Twenty-five monozygotic and dizygotic twin pairs (15-33 yrs) performed physical exercise (ergometry until exhaustion) and were exposed to psychological stress (public speaking and mental arithmetics in front of an audience and a video camera). Additionally, 20 of these twin pairs received an injection of 100 μg h-CRH i.v. HPA activity was indexed by salivary cortisol measures with saliva samples being obtained in 10-min intervals. On each occasion twins were studied together with their siblings and tests were performed between 5 p.m. and 6 p.m. Moreover, each twin pair spent one day together in their natural environment and saliva samples were collected for analysis of circadian cortisol rhythm at 20-min intervals from 8 a.m. to 10 p.m.

Under all experimental conditions, significant increases in salivary cortisol levels were observed. Since analysis of zygosity has not been completed results on intra-pair correlations with respect to HPA axis responses in twins will be presented on the meeting.

621.10

DIFFERENTIAL DEVELOPMENT OF THE STRESS RESPONSE IN CONGENITAL LEARNED HELPLESSNESS. J.A. King, D. Campbell, F.A. Henn and E. Edwards.
Dept. of Psychology, Emory University, Atlanta GA 30022.
Dept. of Psychiatry, SUNY, Stonybrook, NY 11794-8101.

Early in the development of the hypothalamic-pituitary-adrenal (HPA) axis the rat undergoes a stress hyporesponsive period of blunted responses to several stressors including cold exposure (CE) and maternal deprivation (MD). We examined the development of the axis by monitoring adrenocorticotropic hormone (ACTH) plasma levels in an animal model of depression and/or anxiety characterized by learned helpless (LH) behavior and a dysfunctional HPA axis in adult life (Biol. Psychiatry 26:530, 1989). On postnatal day 7 there was no significant difference between basal plasma ACTH levels among LH rats and controls, but LH animals showed a blunted response to CE ($p<0.05$). By postnatal day 15 there was a 98% increase in ACTH response to CE. On postnatal day 21 baseline ACTH and response to CE were significantly suppressed in LH rats. Stress response to MD was present in all groups but was insignificantly different for all ages of development between groups. These findings suggest that rats with congenital learned helplessness undergo a differential response in the development of the HPA axis in that the axis was hypersensitive at postnatal day 15 and hyporesponsive by day 21 and this may in part account for the dysfunctional stress response observed during adulthood.

621.11

CORTICOSTERONE RESPONSE TO RESTRAINT STRESS IN RATS BRED FOR LEARNED HELPLESSNESS. F.A.Henn, E. Edwards, R.L. Spencer, Y. Watanabe and B.S. McEwen. SUNY at Stony Brook & Rockefeller University.

We have measured the corticosterone (CORT) response to a 1hr restraint stress of rats bred for learned helplessness (LH).

Basal CORT levels in the morning (AM) were lower in LH strain rats compared to rats bred for non-learned helplessness (NLH), and basal cort levels of LH rats in the evening (PM) were lower than those of outbred Sprague Dawley controls.

Outbred Sprague Dawley rats, NLH and LH strain rats could be differentiated by their overall CORT response to the restraint stress paradigm. Plasma CORT of LH rats immediately after restraint stress was significantly reduced as compared to NLH and outbred controls (μg/100ml: 10.4 ± 1.5, LH vs 32.8 ± 1.7, NLH & 32.1 ± 4.0, outbred controls; n=8/group). Plasma Cort levels of LH strain rats after a 1hr recovery from restraint stress were lower than NLH in the AM (but not PM) and lower than outbred controls in the PM. There was no significant differences in Type I nor Type II steroid receptors in the hippocampus & the hypothalamus of LH strain rats as compared to outbred control and NLH strain rats. CRF and ACTH mechanisms of LH strain rats are under investigation.

621.12

PHYSIOLOGIC, BIOCHEMICAL AND BEHAVIORAL RESPONSES TO UNCONTROLLABLE STRESS IN HUMAN SUBJECTS J. Fertig, R. Peters*, J. Leu, G. Mueller, T. Bottegal.* Walter Reed Army Institute of Research, Washington, D.C. 20307.

In an attempt to characterize the physiologic, biochemical and cognitive responses to uncontrollable stress, 40 healthy male subjects were exposed to bursts of 95 dB noise while attempting to solve a visual-spatial task under either controllable stress (CS) or uncontrollable stress conditions (UCS). CS subjects could terminate both stressors while their yoked UCS partners could not. Measures of physiologic reactivity, biochemical response, cognitive function, and mood were made before and after stress induction. Both CS and UCS stress produced a rise in diastolic and systolic blood pressure during the stress induction period. However, these rises were significantly attenuated in the UCS condition. Several biochemical markers of stress accompanied these physiologic changes and differentiated the UCS from the CS subjects. Cortisol and immunoreactive beta endorphin were significantly elevated by UCS. Subjects in the UCS condition also reported higher ratings of helplessness, lack of control, confusion and total mood disturbance. Cognitive performance on a delayed memory task was impaired by both stress manipulations. Results suggest that lack of control over psychological stressors can produce alterations in mood as well as physiologic and neuroendocrine changes in healthy subjects.

621.13

DIFFERENTIAL ENDOCRINE AND AUTONOMIC REGULATION OF RESTRAINT-INDUCED HSP70 EXPRESSION IN THE ADRENAL GLAND AND VASCULATURE. M.J. Blake, R. Udelsman*, and N.J. Holbrook*. Lab. Mol. Genet., NIA, Gerontol. Res. Cntr., Baltimore, MD 21224.

Restraint is known to illicit a physiologic stress response characterized by sympathetic nervous system stimulation and activation of the hypothalamic-pituitary-adrenal (HPA) axis. We have shown that restraint induces the selective expression of HSP70 mRNA in the adrenal cortex and blood vessels of the rat and have proposed that their induction in mammalian tissues plays an important role in physiologic stress response mechanisms. In this report we have used perturbations of the HPA axis and sympathetic adrenergic receptor blockade to assess the respective contribution of neuroendocrine and sympathetic activity to restraint-induced HSP70 expression *in vivo*. The influence of the HPA axis was investigated by restraining hypophysectomized and control rats for 60 min then determining HSP70 expression in adrenals and aorta by northern blot analysis and *in situ* hybridization. Hypophysectomy resulted in a 5-fold decline in HSP70 expression in the adrenals but did not significantly alter expression in the aorta. Treatment of normal rats with chronic release pellets containing the glucocorticoid receptor antagonist RU486 (5 mg) for two weeks prior to restraint also significantly reduced HSP70 expression in the adrenal but had less effect in the aorta. To determine if the reduced responsiveness of the aorta to HPA manipulation was due to alternative regulation in this tissue by sympathetic nervous system activity, rats were pretreated with either propranolol (30 mg/kg i.p.) or prazosin (1 mg/kg i.p.) prior to restraint. Prazosin virtually eliminated aortic HSP70 induction but did not significantly alter its induction in the adrenal. Propranolol was less effective than prazosin in reducing restraint-induced expression in the aorta (3-fold decline) and also did not affect induction in the adrenal. Thus, restraint-induced HSP70 mRNA expression in the adrenals is modulated by the HPA axis whereas expression in the aorta is controlled by adrenergic sympathetic activity, each involving distinct receptor mediated events.

PAIN MODULATION: CNS

622.1

Afferent projections from the anterior pretectal nucleus to the ventral medulla oblongata: anterograde tract tracing with Phaseolus vulgaris leuco-agglutinin in rats. M.G.Terenzi*, A.Zagon[1], M.H.T.Roberts*, Department of Physiology, UWCC, Cardiff and [1]University Department of Pharmacology, Oxford U.K.

Recent electrophysiological data have shown that the anterior part of the anterior pretectal nucleus (APTA) plays a role in processing noxious stimuli while other nuclei of the pretectal complex are engaged in control of visual system reflexes. The ventral medulla oblongata is known to be involved in central cardiovascular and nociceptive regulation and medullo-spinal pathways can transmit descending impulses from several higher centres. In the present study the possibility of a direct projection from the APTA into the ventral medulla was investigated. Ionophoretic injections of the anterograde tract tracer Phaseolus vulgaris leuco-agglutinin were placed into the APTA and control injections into the surrounding posterior thalamic cell groups.

Fibres descending from the APTA innervated almost exclusively the ipsilateral side of the medulla oblongata. A high density of varicose labelled fibres was observed in the rostral part of the ventral gigantocellular reticular nucleus, in the gigantocellular reticular nucleus pars alpha. Labelled terminals were present in the rostral part of the raphe obscurus and within the raphe magnus. Labelled fibres in the inferior olivary complex were most numerous in the ventral aspects of the dorsal olive. From control injections the majority of labelling in the ventral medulla appeared as non-varicose fibres in the region of the pyramidal tract and the dorsal inferior olive.

A direct APTA-medullary connection might be involved in descending control of nociception.

622.2

RESPONSES OF RAPHESPINAL NEURONS TO VAGAL AFFERENT NERVE STIMULATION. **A.R. Evans and R.W. Blair. Dept. Physiol. & Biophysics, Univ. Okla. Health Sci. Ctr., Oklahoma City, OK, 73190.**

Stimulation of vagal afferent nerve fibers has been shown to modulate nociceptive transmission in the spinal cord. It has been proposed that vagal afferent fibers engage medullary raphespinal (RAS) neurons to modulate nociception. The goal of this study was to determine response characteristics of medullary raphespinal (RAS) neurons to electrical and chemical stimulation of vagal afferent fibers. Six cats were anesthetized with sodium pentobarbital (35 mg/kg). Extracellular potentials were recorded from 10 RAS neurons whose axons were antidromically activated from the T_2-T_3 segments of the spinal cord. Seven cells were inhibited and 3 cells were excited by electrical stimulation of vagal afferent fibers. The inhibitory response to electrical stimulation was intensity and frequency dependent. Left atrial injections of veratridine and phenylbiguanide were used to chemically stimulate vagal afferent fibers. Four of 7 cells inhibited by electrical stimulation were also inhibited by chemical stimulation. Two of 3 cells excited by electrical stimulation were inhibited by chemical stimulation. No cells were excited by chemical stimulation. We conclude that vagal afferent fibers can modulate the activity of RAS neurons and, therefore, can affect RAS influences in the spinal cord. (Supported by NIH grant HL29618 and OCAST grant HR9-089).

622.3

C-FOS GENE EXPRESSION IN PONS AND MEDULLA DURING PERIPHERAL INFLAMMATION. G. Draisci, D.G. Quarta*, M.E. Dell'Anna*, D. Camaioni*, M.J. Iadarola & M. Molinari. Istituto di Anestesiologia e Rianimazione e Istituto di Neurologia, U.C.S.C., 00168 Roma, Italy

In the CNS, c-fos is an useful marker of neuronal activity that can be used to map functionally related neural pathways. Our previous studies showed an increase of c-fos gene expression in the spinal cord and in the PAG during experimentally induced peripheral inflammation in rats. In the present study we analyze immunocytochemically c-fos gene expression in pons and medulla.

Carrageenan was injected in the left foot of 4 male Sprague Dawley rats, 3 non-injected rats were used as controls. 24 hrs after injection, the rats were perfused transcardially with 10% formalin. Serial 40 µm criostate coronal sections were obtained from pons and medulla and processed for ABC immunocytochemistry and for cresyl violet staining. Fos-immunoreactive cells were identified with an affinty purified antiserum to a conserved sequence in the c-fos DNA binding region.

Quantitative analysis evidences a clear increse of the number of c-fos IR cells in the treated animals with respect to the controls in the pontine and medullary reticular formation and in the raphe magnus.

These results in line with our previous findings, indicate that c-fos expression is enhanced after inflammation in pontine and bulbar structures that have been related to nociception.

622.4

THE RELATIONSHIP OF SUBSTANCE P VARICOSITIES TO PERIAQUEDUCTAL GRAY-RAPHE MAGNUS PROJECTION NEURONS: ANALYSIS USING INTRACELLULAR INJECTION AND LASER CONFOCAL MICROSCOPY D. R. Onstott and A. J. Beitz, Department of Veterinary Biology, Univ. of Minnesota, St. Paul, MN 55108.

The descending projection from the periaqueductal gray (PAG) to the raphe magnus (NRM) has been identified as an important component in the pain modulation system. Substance P (SP) has been shown to produce analgesia when injected into the PAG, and SP-immunoreactive (SP-IR) terminals make synaptic contacts within the PAG. The present study was undertaken to develop a method of quantifying possible sites of interaction between SP-IR axonal varicosities and PAG-NRM projection neurons, including sites on distal dendrites not normally filled by retrograde tracers. After injection of Fluoro-Gold into the NRM of rats, retrogradely labeled cells in the PAG were visualized in 150 µm vibratome sections with epifluorescence illumination and injected iontophoretically with Lucifer yellow (LY). SP-like immunoreactivity was detected in the same sections using Fluoroprobe 670-labeled secondary antiserum (Jackson ImmunoResearch). Confocal scanning laser microscopy (BioRad MRC-600 Imaging System) was used to produce two separate series of 1 µm "optical sections" of injected cells and of SP-IR varicosities. Individual images from the same plane in each series were merged to reveal varicosities apposed to neuronal elements of LY-filled cells. Complete LY filling of the soma and dendritic tree was accomplished in several cells of varying morphology in each section. Occasionally dye-coupling between PAG neurons was noted, suggesting the presence of gap junctions. Preliminary examination has indicated that a small number of SP-IR varicosities come into close contact with the somas and the proximal and distal dendrites of PAG-NRM projection neurons. This study suggests that retrograde labeling coupled with LY intracellular injection and confocal microscopy may be a useful procedure for elucidating the interactions of peptide-containing varicosities with identified projection neurons. Supported by DA06687, DE06682, DC10806 and NS19208.

622.5

DOMINANCE OF 5HT-1A OR 5HT-2 AND CHOLINERGIC RESPONSE IN RAPHE MAGNUS CELLS VARIES WITH INHIBITORY, EXCITATORY OR MIXED EFFECT OF NOXIOUS STIMULI. K. Taguchi, M. Andresen*, T. Shibuya, and I. D. Hentall. Univ. of Ill. Coll. of Med., Rockford, IL 61107.

Neurons of the nucleus raphe magnus (NRM) are known to be responsive to serotonin (5HT), possibly via autoreceptors, recurrent axonal branching, or remote inputs. In pentobarbital-anesthetized rats, we examined responses to 5HT, to 5HT-1A agonist buspirone, to 5HT-1A antagonists propranolol and methiothepin, to 5HT-2 antagonist ketanserin, to mixed antagonist methysergide, and to acetylcholine (ACh). Neuronal responses to skin pinching were also tested. As well as the previously known on-cells and off-cells, we found at ventral sites neurons with biphasic responses to prolonged (10s) pinching; the excitatory and inhibitory responses became prominent, respectively, at low and high spontaneous activity.

Iontophoresis of 5HT (5-60 nA for 10s) caused excitation (*E*), inhibition (*I*), inhibition followed by excitation (*IE*), or no effect (n=76). In off-cells most responses were *I*; in biphasic-cells they were about equally *I* and *IE*; in on-cells they were about equally *I*, *IE*, and *E* (chi-square: P=0.026, 4x3 table). A similar distribution of responses was obtained with ACh (n=36). Iontophoresis of ketanserin prevented *E* from 5HT or unmasked *I*; methysergide prevented *I*. Alone, buspirone or ketanserin caused *I*, methysergide either *I* or *E*, and methiothepin *E*. Propranolol caused *I* (probably a ß- adrenergic effect).

In conclusion, NRM cells which tend to have inhibitory nociceptive responses are more likely to be inhibited by 5HT-1A action and by ACh. (Supported by NINDS grant NS 26116.)

622.6

LAMINA II CELLS PROJECT STRONGLY TO THE VENTROLATERAL RETICULAR FORMATION OF THE MEDULLA OBLONGATA IN THE RAT D. Lima* and A. Coimbra* (Spon: European Neuroscience Association) Institute of Histology and Embryology of the Faculty of Medicine of Oporto, 4200 Porto, Portugal

The substantia gelatinosa Rolandi (lamina II) is considered a pain modulatory system acting locally upon the transmission of nociceptive input through dorsal horn neurons. Several retrograde tracing studies accordingly showed no significant numbers of lamina II cells projecting supraspinally. Stereotaxic injections of 1.5% cholera toxin subunit B (CTb) in the ventrolateral reticular formation intermediate between the lateral reticular nucleus and the spinal trigeminal nucleus, pars caudalis, of adult, chloralose anaesthetized rats, resulted in retrograde labelling of spinal neurons in laminae I-III and the lateral spinal nucleus. Labelled lamina II cells were on average 430 in the cervical and 210 in the lumbar enlargement, representing, respectively, 28% and 21% of the labelled spinal cells. Most cells had dendritic arbors spread in the parasagittal plane making up a circular dendritic field across lamina II. The ventrolateral reticular formation thus appears to be a specific target of lamina II cells. It is suggested that these neurons exert a modulatory action upon the descending, probably noradrenergic antinociceptive input generated in that medullary region. (Supported by JNICT, project nº PMCT/C/SAU/31/90)

622.7

HYBRIDIZATION HISTOCHEMICAL ANALYSIS OF GLUTAMATE RECEPTOR mRNAs IN THE VENTRAL MEDULLA IN A RAT MODEL OF PERIPHERAL NEUROPATHY. E.M. Jansen[1], F.G. Williams[2] and A.J. Beitz[1,2,1]Grad. Prog. in Neurosci., Univ. of Minnesota, Minneapolis, MN 55455, [2]Dept. of Vet. Biology, Univ. of Minnesota, St. Paul, MN 55108.

Nuclei in the ventral caudal medulla (VCM) are involved in the modulation of nociception. However, the distribution of neurons that express the mRNAs for glutamate receptors within the VCM nuclei is not known. The purpose of this study was to compare the number and distribution of neurons expressing GluR-A flip and flop mRNAs in VCM nuclei from control animals and from animals with a unilateral peripheral neuropathy (according to the model of Bennett and Xie, Pain, 33:87, 1988). cDNA oligonucleotide probes for the GluR-A flip and flop receptors were synthesized according to the sequences published by Sommer, et al. (Science, 249:1580, 1990). Hybridization histochemistry was performed using biotinylated oligonucleotides followed by FITC-avidin detection. The resulting labeling was quantified using image analysis of digitized video micrographs. Neurons containing GluR-A flip mRNA were observed in the following VCM nuclei: raphe magnus, gigantocellular reticular, lateral paragigantocellular and gigantocellular pars alpha. There was considerably less hybridization signal in the VCM following application of the GluR-A flop probe. The raphe magnus in lesioned animals contained significantly higher levels of GluR-A flip mRNA compared to unlesioned controls (p<.01, ANOVA and Fisher PLSD). The frequencies of raphe magnus neurons expressing this mRNA were increased, but did not achieve statistical significance. These changes were less pronounced in other nuclei in the VCM. These data suggest that peripheral neuropathy activates glutamate receptor biosynthesis in nuclei in the VCM and imply that peripheral neuropathy affects excitatory amino acid transmission in medullary nuclei involved in antinociception. Supported by NIH grants NS28016, DA06687, DE06682 and NS19208.

622.8

NOCICEPTIVE MODULATORY NEURONS IN THE ROSTRAL VENTROMEDIAL MEDULLA ARE FOUND IN BOTH HALOTHANE- AND BARBITURATE-ANESTHETIZED RATS. M.M. Morgan, M.M. Heinricher and H. L. Fields. Depts. of Neurology and Physiology, Univ. of California, San Francisco, CA 94143.

The physiological and pharmacological properties of two classes of putative nociceptive modulatory neurons in the rostral ventromedial medulla (RVM) have been extensively characterized in rats lightly anesthetized with barbiturates. "On-cells" show a burst of activity, and "off-cells" a sudden pause immediately preceding nocifensive reflexes. We have recently shown that the reflex-related pause of off-cells is mediated by GABA, and since barbiturates are known to potentiate GABAergic influences, it is important to demonstrate that these neurons can be characterized in non-barbiturate preparations.

Activity of RVM neurons was recorded in rats maintained under halothane anesthesia (0.6-1.0% in oxygen, 0.3 l/min). RVM cells that showed an increase in activity (on-cells) or a decrease in activity (off-cells) that immediately preceded the occurrence of the tail flick were found in this preparation. On-cells were found more frequently, but this seemed to result at least in part from using somatic stimulation as a search stimulus for cells of this class. When noxious inputs were limited by testing only one cell per animal, similar numbers of on- and off-cells were found.

Thus, both on- and off-cells can be identified in rats anesthetized with halothane, indicating that neurons with these properties are not an epiphenomenon of barbiturate anesthesia. Moreover, the reported inability to demonstrate off-cells in the awake rat (Oliveras et al., 1989; 1990) may be due to the lack of an appropriate search stimulus for cells of this class.

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622.9

ACTIVATION OF THE RAPHE-SPINAL PAIN INHIBITORY SYSTEM BY MEDIAL PREOPTIC NUCLEUS(MPO). M. Jiang*, M.M. Behbehani, S.D. Chandler, T. Rizvi and M.T. Shipley. Depts. of Physiology and Anatomy. U. of Cincinnati. Cincinnati, OH 45267-0576.

Anatomical studies (See Rizivi et al.) have shown a strong projection from the MPO to the nucleus raphe magnus(NRM). In this study we examined the functional interaction between these sites. In rats, recordings were made from NRM neurons that were identified by their response to peripheral stimulation and designated as "E" and "I" if they were excited or inhibited by pinch. In addition cells were identified by the ability to be antidromically activated by DLF stimulation at the lumbar level. A total of 100 neurons were recorded. Stimulation of MPO at a frequency of 100 Hz excited 17/43 and inhibited 12/43 of the E cells. The majority (20/30) of the I cells were inhibited by train stimulation of MPO and only 5/30 I cells were excited. Injection of 5 mM homosysteic acid (HA) into the MPO excited 10/31 E and inhibited 8/13 I cells. The response to HA lasted for an average of 106± 143 seconds. Electrical stimulation of MPO at 1 Hz excited 23%, inhibited 5% and had no effect on 70% of NRM cells. The mean latency to peak excitation was 9.6 ± 6.6 msec. Antidromic activation of MPO neurons by NRM stimulation showed an average latency of 6.3 ± 3.4 msec. Iontophoresis or micropressure injection of proglumide, naloxone and kynurenic acid next to the recording electrode suggested that a glutaminergic pathway may play a role in this system but the involvement of either a cholecystokinin or an enkephalinergic pathway in the NRM-MPO interaction is unlikely. It is concluded that 1) MPO inhibits the I cells and excites the majority of E cells, 2) simultaneous activity of many synapses is required for activation of MPO-NRM pathway and 3) MPO-NRM interaction is mediated by fibers with a conduction velocity of less than 1m/sec. Supported by PHS grant NS20643.

622.10

A CONFOCAL MICROSCOPIC STUDY OF ENKEPHALIN IMMUNOREACTIVE APPOSITIONS ONTO PHYSIOLOGICALLY IDENTIFIED NEURONS IN THE ROSTRAL VENTROMEDIAL MEDULLA (RVM) OF THE RAT. P. Mason, S.A. Back and H.L. Fields. Department of Neurology, UCSF, San Francisco, CA 94143-0114.

The RVM contains neurons that are inhibited (on-cells), excited (off-cells) or unaffected (neutral cells) by systemic morphine. Iontophoretic application of morphine, in contrast, while inhibiting on-cells, does not alter the spontaneous or evoked activity of off- or neutral cells (Heinricher et al., Soc. Neurosci. Abstract 1991), indicating that opioid receptors may be differentially distributed upon RVM neurons of different physiological classes. The present study, therefore, investigates the distribution of enkephalin-immunoreactive appositions onto physiologically identified RVM cells.

RVM neurons were characterized by their response during a nociceptive withdrawal reflex in lightly anesthetized rats. Cells were then injected intracellularly with Neurobiotin and subsequently visualized with a Texas Red fluorophore. Sections that contained labeled neurons were processed for enkephalin immunoreactivity using a FITC fluorophore. These sections were optically sectioned at 1 m intervals using a dual channel confocal laser scanning microscope (MRC-600). Appositions between enkephalin immunoreactive varicosities and intracellularly labeled neurons were mapped onto reconstructions of labeled on-, off- and neutral cells.

Enkephalin appositions were found on the somata and dendrites of all on-cells. Although enkephalin varicosities were also apposed to some off-cells, their density was significantly less than onto on-cells. These results support the idea that on-cells are directly inhibited by opioid peptides and that the opioid excitation of off-cells is produced indirectly, possibly by opioid blockade of an on-cell mediated inhibition.

622.11

Comparing the effects of barbiturate and Ketamine anesthesia on the neuronal output in the rostral ventromedial medulla of the rat.
S. McGaraughty*, S. Reinis, & J. Tsoukatos. Div. of Biopsychology, U. of Waterloo, Waterloo, Ont., Canada N2L 3G1.

Using a novel technique developed in our laboratory we simultaneously record the activity of both a single neuron and the cells immediately surrounding it. Then, correlograms illustrating the commonality of interspike intervals are calculated giving information about the neuronal output at the single cell, multi-unit, and interactional levels. While recording in the rostral ventromedial medulla (RVM) we have previously shown that a majority of neurons in Ketamine-Rompun anesthetized rats have about a 30 ms delay in their output which is independent of nociceptive input. These findings were replicated with Ketamine (80 mg/kg, IP) and Rompun (8 mg/kg, IM). Similar findings have been seen with Pentobarbitol (40 mg/kg, IP). The rats were in a deep state of anesthesia throughout recording under both anesthetics. The barbiturate anesthetized rat had a greater quantity of cells exhibiting output delay, the delay in most cases was longer than 30 ms. In the multi-unit records of Pentobarbitol rats there was almost a complete absence of any other activity other than the delay which is in strong contrast to the Ketamine rats. This suggests that under anesthesia the RVM is producing lower output probably due to the direct actions of the anesthetics. Pentobarbitol decreases the RVM output more than Ketamine.

622.12

The Location of Noradrenergic Neurons that Innervate the Ventromedial Medulla and Modulate Nociception: Anatomical Studies Using Retrograde Tracers and Immunocytochemistry.
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Noradrenergic neurons modify the activity of neurons in the ventromedial medulla (VMM) that modulate nociception. We determined the location of noradrenergic neurons that project to the VMM using retrograde tracers combined with tyrosine hydroxylase immunocytochemistry. Each of three retrograde tracers were injected into the VMM: horseradish peroxidase, Fluoro-Gold or rhodamine labeled latex microspheres. The A5 cell group contained about 80% of the total number of tyrosine hydroxylase-immunoreactive (TH-ir) neurons in the brainstem that were retrogradely labeled by rhodamine microspheres. In contrast, the other two retrograde tracers labeled only a few TH-ir neurons. These results demonstrate that noradrenergic neurons in the A5 cell group are the principal source of noradrenergic neurons that innervate the VMM. In addition, the retrograde tracers horseradish peroxidase and Fluoro-Gold do not appear to be transported by the axons of noradrenergic A5 neurons. Supported by USPHS Grant DA03980.

622.13

AN ANIMAL MODEL FOR INVESTIGATION OF THE NEUROPHYSIOLOGIC MECHANISMS OF TOURNIQUET PAIN
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Tourniquet pain is a clinical problem resulting from the maintenance of inflation of a pneumatic tourniquet on an extremity to provide a bloodless operating field during orthopedic surgical procedures. This severe, dull, aching, pain sensation complicates regional or general anesthetic procedures despite otherwise adequate anesthesia for the surgical procedure. Treatment of tourniquet pain is nonspecific and involves increasing anesthetic depth to maintain patient comfort and hemodynamic stability. Investigation into the etiology of this pain has been limited due to the lack of an animal model for study. This study utilized an anesthetized rodent model for detection and monitoring of nociceptive activity in medullary nuclei in response to tourniquet inflation. Male rats were prepared for single unit recording of cell firing rate (CFR) of medullary neurons in nucleus raphe magnus (NRM). Anesthesia was maintained by IV infusion of pentobarbital at 20mg/kg/hr. BP was monitored continuously. A 1x9mm tourniquet was inflated on the (R) lower extremity. Changes in BP and CFR were analyzed by "t" test, with $p<0.05$ as the minimum level of statistical significance. The animals demonstrated an increase in BP and a nociceptive response to maintenance of tourniquet inflation manifested as a decrease in CFR of medullary OFF cells. After a 60 min inflation period the tourniquet was deflated; BP and CFR returned to preinflation baseline values following a brief period of hyperexcitability. These results indicate that this rodent model demonstrates a reproducible nociceptive response to maintenance of tourniquet inflation and is suitable for use in the investigation of the neurophysiologic and neuropharmacologic mechanisms of tourniquet pain.

622.14

STUDY OF NOCICEPTIVE AND OTHER NEURONAL SYSTEMS IN THE VENTROPOSTERIOR THALAMIC COMPLEX OF ANAESTHETIZED RATS.
J. Tsoukatos, S. Reinis, and S. McGaraughty*. Department of Psychology, University of Waterloo, Waterloo Ont., Canada.

We have used auto-, cross-, and mass-correlograms to identify the types of cell response in the ventroposterior thalamic complex. We have recognized three different types of cell activity, under spontaneous conditions and during noxious tail heating. The first type is characterized by constant levels of activity both in the spontaneous and the stimulated condition. In the second type, the spontaneous activity of the leading cell decreases in the stimulated condition. In the third type, leading cell activity increases in the stimulated condition. The mass-correlograms of the background activity for the second and third types showed that the background cells interact forming regular sinusoids, at a frequency of approximately 70/sec. Other cells and systems showed a self-inhibitory period of about 40 ms, a replication of similar findings in the ventromedial medulla. We hypothesize that the cells of the first type may represent networks unrelated to analgesia (e.g. proprioception). The cells of the second type might be related to the transmission of the stimulation in the areas of the ventroposterior thalamus. The cells of the third type might be implicated in the production of analgesia, at the level of the thalamus.

622.15

BURSTING ACTIVITY OF THALAMIC NEURONS RECORDED FROM CHRONIC PAIN PATIENTS IS MODIFIED BY ELECTRICAL STIMULATION IN PVG.
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In chronic pain patients electrical stimulation of the PAG or PVG (periaqueductal or periventricular gray) and somatosensory relay nuclei Vc (Ventralis caudalis of Hassler or VPL & VPM, ventralis posterolateral & posteromedial) via permanently implanted electrodes has been employed over the past 15 years to treat intractable pain. The mechanisms for PVG/PAG pain relief are thought to involve descending systems associated with opioid, noradrenergic and serotonergic mediation. However, little is known about the role of PVG/PAG modulation of somatosensory thalamic activity.

The present work is part of an ongoing study to assess the effect of PVG stimulation on neurons in Vc in patients suffering from intractable pain. Electrical activity of single cells in Vc was recorded while the patient was undergoing stereotactic surgery under local anesthetics for implantation of chronic stimulating electrodes in PVG and Vc. The chronic electrode in PVG, implanted first, provided electrical stimulation (single pulses & trains) controlled with a Grass stimulator and isolation unit. A custom designed microelectrode introduced for localization of the appropriate target for the Vc chronic electrode served for recording single cells in that region and also allowed for microstimulation to determine fields for a specific cell. Activity of single neurons was recorded and taped for later analysis.

Computer analysis consisted of determination of interval histograms and detailed intraburst analysis for pre- versus post-stimulus time periods. Stimulation of PVG produced pronounced inhibition of activity of neurons recorded from Vc, the length of the inhibition varying with the amount of stimulation. Intraburst intervals as well as rhythmicity of Vc cells were also altered following return of activity after PVG stimulation. This modification of somatosensory activity may play a role in pain relief elicited by PVG stimulation.

622.16

THE FORMALIN TEST FOR TONIC PAIN AFFECTS LIMBIC, BASAL GANGLIA AND THALAMIC REGIONS IN RAT BRAIN.
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The [^{14}C]-2-deoxyglucose method was used to identify regions in the rat central nervous system activated during the rat paw formalin test, a continuous, inflammatory pain stimulus. Animals were awake but partially restrained. [^{14}C] -2-deoxyglucose was injected 20 minutes after the formalin (5%, 0.05 ml s.c.) or saline (0.05 ml s.c.) injection. Bilateral increases in local cerebral glucose utilization in the formalin-treated group as compared with the saline-treated control group included the hippocampus (granular layer, +34%), lateral and medial habenula (+51, +73%, respectively), anterior accumbens (+30%), subthalamic nucleus (+41%), entopeduncular nucleus (+70%) and ventromedial thalamic nuclei. Unilateral changes were found in areas of the hippocampus, amygdala, and in medullary reticular nuclei implicated in pain and analgesia. The results demonstrate the involvement of many structures in the rat CNS which represent associated functions activated during a painful stimulus. Of special interest is the specific limbic structures involved.

622.17

NEURONAL RESPONSES IN CINGULATE CORTEX TO NOXIOUS STIMULATION MAY ORIGINATE IN MEDIAL THALAMUS
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Anterior cingulate cortex has been implicated in affective responses to noxious stimuli. This study investigates the neuronal basis for such responses. Single-unit recordings were made in area 24 of Dutch-belted rabbits anesthetized with halothane. Stimuli included transcutaneous electrical, mechanical and thermal stimulation. About 25% of cingulate neurons responded to noxious levels of these stimuli and did not respond to innocuous levels. Nearly 84% of the units that responded to noxious stimulation were located in layers II and III. Excitatory units were located mainly in layer III while most inhibitory units were in layer II. Only about 16% of layer V units responded to these stimuli.

To identify the source of this input, cortical lesions were made separating area 24 from adjacent cortical areas. In other animals, lidocaine injections were made to reversibly block neuronal activity in the medial thalamus. Responses to noxious stimuli occurred after complete cortical lesions while lidocaine injections reversibly blocked the responses of all units tested.

In conclusion, neurons in cingulate cortex respond to noxious stimulation and this signal may originate in the medial thalamus.

622.18

NEURONAL RESPONSES TO STIMULATION OF REPRODUCTIVE AND OTHER PELVIC ORGANS IN THE THALAMUS OF FEMALE RATS.
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Recent studies indicate the existence of CNS input from the uterus and other pelvic organs which gives rise to conscious sensations, including pain, suggesting that neurons in somatosensory relay nuclei receive such input (1). The present study examined responses of 61 neurons in and near the thalamic ventrobasal nucleus (VB) of 14 estrous female rats anesthetized with nitrous oxide/halothane to mechanical stimulation of uterus, cervix, vagina, colon and somatic structures (skin, joints). Neurons responsive to uterus (26%) or uterus and vaginocervix stimuli (28%) were located throughout VB, preferentially dorsally and rostrally (as were neurons responsive to colon stimuli ; 18%). Of 42 viscera-responsive neurons, half also had somatic receptive fields, most on the hindlimb (86%), but also on forelimb or face (14%). Many (62%) required noxious levels of somatic stimulation. While these neurons (38% of sample) were located equally within VB and its dorsal or ventral borders, neurons responsive only to somatic stimulation (30% of sample) were located primarily within VB and those responsive only to visceral stimulation (28%) were all located at VB's dorsal (88%) or ventral (12%) borders. These results reveal a surprisingly large, well-organized representation of internal female reproductive organs in and near VPL.

(Supported by MRT-France and R01 NS11892-NIH, USA.)

(1) Berkley et al., 1988; 1990a;1990b; Berkley, 1990.

PAIN MODULATION: MONOAMINES

623.1

RECEPTOR MEDIATION OF 5-HT-INDUCED INFLAMMATORY NOCICEPTION IN RATS.
K. J. Sufka, F. Schomburg* and J. Giordano. Neuropharmacology Lab., COPHS, Drake Univ., Des Moines, IA 50311.

The present study examined the effects of intraplantar (ipl) injection of 5-HT and the ability of selective 5-HT receptor antagonists to differentially attenuate 5-HT-induced edema and nociception. Ipl 5-HT (0.05-1 µmol) produced dose-dependent hindpaw edema and nociceptive responses (lifts/licks of affected limb). The 5-HT_1 antagonist, methysergide (MS: 1-10 nmol; ipl), 5-HT_2 antagonist, ketanserin (KT:30-300 nmol; ipl) and 5-HT_3 antagonist, odansetron (OD:100 nmol - 1 µmole; ipl) differentially affected the nociceptive and edematous response produced by a fixed algesic dose of 5-HT (0.25 µmol; ipl). Ranked by magnitude of inhibition of 5-HT-induced nociception, MS > KT > OD. These results demonstrate the differential involvement of heterogeneous 5-HT receptors in 5-HT-induced inflammatory nociception.

623.2

PATTERNS OF ANALGESIA PRODUCED BY S AND R ISOMERS OF THE NOVEL 5-HT3 RECEPTOR ANTAGONISTS ADR-851 AND ADR-882 IN RATS.
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The present study examined the antalgic efficacy of S and R isomers (0.1-10 mg/kg; sc) of the novel 5-HT_3 receptor antagonists, ADR-851 and ADR-882 against acute thermal, mechanical and formalin-induced inflammatory pain in male rats. No motoric, overt behavioral or physiologic effects were produced by any dose of S and R isomers of ADR-851 and ADR-882. Neither isomer of ADR-851 or ADR-882 was analgesic in the thermal or mechanical pain test. In the formalin test, ADR-851R produced significant antalgic effects at 3 mg/kg and 10 mg/kg doses. ADR-851S produced analgesia only at 1 mg/kg. Neither isomer of ADR-882 was effective in this assay. These results suggest that by antagonizing peripheral 5-HT_3 receptors, ADR-851 may be useful in the therapy of inflammatory pain.

623.3

EFFECTS OF THREE ALPHA-2 ADRENERGIC AGONISTS GIVEN INTRATHECALLY IN RATS.
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Systemically given alpha-2 adrenergic agonists can produce analgesia, marked sedation and significant cardiovascular changes. Intrathecal (IT) administration produced more intense analgesia with fewer systemic effects. This study examined the analgesic and sedative properties of 3 alpha-2 agonists given IT.

Forty male Sprague Dawley rats had IT catheters placed via the cisterna magna with the tip lying at the thoracolumbar junction. At least 2 weeks after surgery rats were randomly assigned to one of 4 groups to receive 10 ul IT injections of saline, xylazine (30,60,120 ug), detomidine (DET; 20,40,100 ug) or dexmedetomidine (DEX; 2.5,5,10 ug). Drug dose order was randomized. Tail flick latency and motor function were assessed 5,15,30,45,60,90 and 120 min following IT drug injection.

IT DEX and DET produced significant, dose-dependent analgesia. Sedation accompanied analgesia at higher doses but was usually of shorter duration. Analgesia and sedation were prevented by pre-administration, and reversed by post-administration, of the alpha-2 antagonist atipamezole (1 mg/kg). Xylazine produced analgesia and sedation at higher doses with antagonism by atipamezole.

Thus, appropriate doses of DEX or DET may afford analgesia with less accompanying sedation.

623.4

DIFFERENTIAL EFFECTS OF MONOAMINE UPTAKE BLOCKADE ON MU, KAPPA$_1$ AND KAPPA$_3$ ANALGESIA.
D. Paul. Department of Pharmacology, Louisiana State University Medical Center, New Orleans, LA 70112.

Selective stimulation of mu, kappa$_1$ or kappa$_3$ opioid receptors produces analgesia through neuroanatomically and neuropharmacologically distinct mechanisms. Blockade of monoamine uptake potentiates morphine analgesia. To determine whether monoamine uptake blockade will potentiate kappa$_1$ and kappa$_3$ analgesia, as well as mu analgesia, we assesed the analgesic effects of U50,488H, NalBzoH and morphine in mice treated with clomipramine or saline using the tail-flick assay. We first determined that 1 mg/kg of clomipramine, given 2 hr before testing, produced a 10-fold shift to the left of the analgesic dose-response curve for intrathecal NE without affecting intrathecal 5-HT analgesia. This dose by itself did not produce analgesia. The clomipramine treatment potentiated the analgesic effects of morphine (2 mg/kg, s.c.) and NalBzoH (10 mg/kg, s.c.), but had no significant effect on U50,488H analgesia (2 mg/kg, s.c.). These results indicate that a monoamine may modulate kappa$_3$ analgesia as well as mu analgesia.

623.5

ENKEPHALINERGIC ANTINOCICEPTION INDUCED BY ELECTRICAL STIMULATION AND SUBSTANCE P MICROINJECTION INTO THE A7 NORADRENERGIC CELL GROUP D. C. Yeomans and H. K. Proudfit
Dept. of Pharmacology, Univ. of Ill. at Chicago, Chicago, Ill. 60612.

We have recently demonstrated that the A7 catecholamine cell group in Sprague-Dawley rats (Sasco, Inc.) provides a dense noradrenergic innervation of the dorsal horn of the spinal cord. We have also demonstrated that activation of A7 cells by either electrical stimulation or microinjection of substance P induces antinociception which can be attenuated by intrathecal injections of noradrenergic antagonists. In addition, we have used neuroanatomical tract-tracers combined with immunocytochemistry, to show that some met-enkephalin immunoreactive cells in the A7 area project to and terminate in the dorsal horn. To investigate the possibility that these cells might be involved in the antinociception induced by stimulation of the A7 area, we determined whether such antinociception could be blocked by intrathecal injection of an opioid antagonist using lightly anaesthetized (urethane 1.0 g/kg) rats. The latency to foot withdrawal in response to a strong thermal stimulus was used as a measure of nociception. Electrical stimulation of the A7 area induced antinciception that was reduced by intrathecal injection of the opioid antagonist naltrexone (30 μg in 15 μl saline). Similarly, microinjection of 3.7 pmoles of substance P into the A7 area produced antinociception which was also partially antagonized by intrathecal administration the same dose of naltrexone. These results support the proposal that spinally-projectiong enkephalin-containing neurons near the A7 cell group can modulate nociception. Supported by USPHS Grants DA03980 to HKP and DA05406 to DCY.

623.6

EVIDENCE THAT THE ANTINOCICEPTION PRODUCED BY STIMULATION OF NEURONS IN THE DORSOLATERAL PONTINE TEGMENTUM IS MEDIATED BY RAPHE-SPINAL SEROTONIN NEURONS. J.A. Paice and H.K. Proudfit. Department of Pharmacology, University of Illinois at Chicago, Chicago, IL 60680.

Anatomic evidence indicates that neurons originating in the dorsolateral pontine tegmentum (DLPT) project to the nucleus raphe magnus (RMg). These neurons may contribute to the modulation of nociception by activating the spinally projecting serotonergic neurons in the NRM. To test this hypothesis, the DLPT, specifically the region near the A7 noradrenergic cell group was stimulated in partially anesthetized female Sprague Dawley rats. The latency to elicit a reflex paw-withdrawal to noxious heat was determined before, during and after electrical stimulation. Stimulation of the A7 region resulted in antinociception as measured by increased paw-withdrawal latencies. This effect was blocked by the intrathecal administration of the serotonergic antagonists methysergide and propranolol, but not by saline. These findings provide evidence for the existence of projections from the DLPT to the RMg, which are involved in modulating nociception. (Supported by USPHS grant DA03980.)

623.7

ANTINOCICEPTION INDUCED BY ELECTRICAL STIMULATION OF NORADRENERGIC NEURONS IN THE A7 CATECHOLAMINE CELL GROUP OF THE RAT. F.M. Clark, D.C. Yeomans, J.A. Paice and H.K. Proudfit, Pharmacology Dept., Univ. of Illinois at Chicago, Chicago, Illinois 60612.

We have shown that noradrenergic neurons in the A7 catecholamine cell group innervate the spinal cord dorsal horn in rats. This observation suggests that these neurons modulate nociception by inhibiting nociceptive transmission in the spinal cord. This hypothesis was tested by determining whether electrical stimulation at various sites in the dorsolateral pontine tegmentum in lightly anesthetized rats could modify nociception. The results indicated that stimulation at sites near the A7 cell group produced antinociception that was reduced by intrathecal injection of the α-noradrenergic antagonists phentolamine and yohimbine. The results of these experiments provide evidence that pontospinal noradrenergic neurons located in the A7 cell group are important components of the descending neuronal system that inhibits nociception. Supported by USPHS Grant DA03980.

623.8

CHARACTERIZATION OF α-ADRENERGIC RECEPTORS IN RAT BRAINSTEM. G.C. Newsom, J.I. Choca, and H.K. Proudfit. Pharmacology Dept., Univ. of Illinois, Chicago 60612.

Previous studies provide evidence for the existence of α_1 and α_2 noradrenergic receptors in the ventromedial medulla that are involved in the modulation of nociception. The present studies characterized the adrenoceptor subtypes in this area using radioligand binding assays. α_1 receptors were characterized using [^{3}H]prazosin. Ligand analysis yielded a K_d of 89 pM and a B_{max} of 48 fmoles/mg protein. Displacement studies were performed using WB4101 and phentolamine. Ligand analysis of these data best fit a one site model. Treatment with chloroethyl clonidine, which selectively alkylates α_{1b} receptors, reduced specific binding by 70 to 80%. These data suggest that [^{3}H]prazosin binds to a single site with characteristics of the α_{1b} receptor. α_2 receptors were studied using [^{3}H]rauwolscine. Ligand analysis yielded a K_d of 16 nM and a B_{max} of 58 fmoles/mg protein. Displacement studies were done using prazosin, yohimbine, and idazoxan; these data were best fit by a one site model. The K_i values were 1.5 nM, 200 nM, and 140 nM, respectively. The high affinity of prazosin and the low affinities for yohimbine and idazoxan suggest that the majority of the rauwolscine binding sites appear to correspond to the α_{2b} subtype. Supported by USPHS Grant DA03980.

623.9

RELATIVE INTRINSIC ACTIVITY OF INTRATHECALLY ADMINISTERED ALPHA 2 AGONISTS, DEXMEDETOMIDINE AND CLONIDINE, AS DETERMINED BY THE USE OF EEDQ, AN IRREVERSIBLE ANTAGONIST Y. TAKANO,* M. GRAFE, T. L. YAKSH Department of Anesthesiology, University of California, San Diego, La Jolla, CA 92093

Intrathecal dexmedetomidine and clonidine produce a dose dependent antinociception, which, based on the similar relative antagonist potency of intrathecal α_2 adrenergic antagonists (atipamezole = idazoxan > yohimbine >> prazosin) suggests an action on an α_2 adrenergic receptor site. To estimate the relative intrinsic activity of these spinally administered α_2 adrenergic agonists in vivo, dose response curves for dexmedetomidine and clonidine were carried out on the 52.5°C hot plate test with Sprague-Dawley rats using chronically implanted intrathecal catheter. EEDQ (N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline), an irreversible α_2 adrenergic antagonist was administered in dose of 8.1, 81 or 810 nmol, 24 hours before the spinal administration of one of the two agonists. Each animal was used once with a single dose of a single agonist. EEDQ alone resulted in an acute mild hyperalgesic effect. Intrathecally injected dexmedetomidine and clonidine resulted in a dose dependent increase in the hot plate response latency: ED50 of the percent of maximal possible effect (%MPE) = 10 (7.3-15) nmol and 118 (86-164) nmol, respectively. Pretreatment with intrathecal EEDQ caused a rightward shift of dose-response curve and reduction of the maximal effect of both dexmedetomidine and clonidine. Analysis of double reciprocal plot indicated that the fractional receptor occupancy required to produce the ED50 was 10 % and 36 % for dexmedetomidine and clonidine, respectively. These results suggest that dexmedetomidine has a higher intrinsic activity than clonidine to produce an antinociceptive effect when injected intrathecally. These experiments were supported partly by Dokkyo University, School of Medicine(YT) and DA02110(TLY).

623.10

AN IN VIVO MICRODIALYSIS STUDY OF THE EFFECTS OF KAPPA AGONISTS ON THE RELEASE OF SEROTONIN IN THE SPINAL CORD DORSAL HORN. J.L. Brown*, F.F. Matos[1], A.I. Basbaum, Division of Neurosciences and Depts. Anatomy and Physiology, University of California San Francisco, CA 94143 and [1]CNS Pharmacology, Bristol-Myers Squibb Company, Wallingford, CT 06492.

Several studies indicate that kappa opioid agonists exert an analgesic effect by activating inhibitory serotonergic mechanisms that operate at the spinal level. Ho and Takemori (1990) reported that kappa agonists evoke the release of newly incorporated ^{3}5-HT from spinal cord slices. To develop an in vivo model of kappa agonist effects on spinal cord 5-HT, we have begun in vivo microdialysis recordings in the awake rat. A loop-shaped dialysis probe was placed transversely across the dorsal horn of the spinal cord according to Skilling et al (1988). Two selective kappa agonists, U50488H (10mM) and CI-977 (1.0mM), were perfused through the dialysis probe for 15 minutes while extracellular release of 5-HT and its major metabolite, 5-HIAA, were monitored by HPLC and electrochemical detection. Infusion of either agonist produced at least a twofold increase of serotonin above basal levels, while 5-HIAA levels decreased. These kappa mediated effects were delayed, occurring one hour after the onset of agonist infusion, and were reversible; both 5-HT and 5-HIAA returned to basal levels within 2 hours. At this time a second injection of the kappa agonist again increased 5-HT release. The high concentration of kappa agonist required and the delayed time course suggest that there is either poor transfer of the compounds across the membrane or that the agonists do not act locally to produce this effect. We are presently examining the effects of intrathecal or supraspinal administration of U50488H and CI-977 on spinal cord 5-HT levels and evaluating the effects of selective opioid antagonists. Regardless of the site of action of these compounds, these results provide the first *in vivo* evidence demonstrating that activation of kappa receptors evokes 5-HT release in the dorsal horn. Supported by NS 14627, DE/NIDA08973.

623.11

THE SIMULTANEOUS MEASUREMENT OF EXTRACELLULAR MORPHINE AND SEROTONIN (5-HT) IN BRAIN TISSUE AND CSF BY MICRODIALYSIS IN AWAKE RATS. F.F. Matos, J. Brown, H. Rollema,* Y. Taiwo, J.D. Levine and A.I. Basbaum. Depts. Anatomy, Physiology, Medicine and Dentistry, UCSF, 94143 and *Univ. Center for Pharmacy, Groningen, The Netherlands.

A systemic injection of morphine produces its analgesic effect through interactions with opioid receptors at diverse supraspinal and spinal targets. Since simultaneous action of opiates at different sites exerts synergistic analgesic effects (Yeung and Rudy, 1980), it is of interest to simultaneously monitor behavior and the levels of morphine in different brain sites and in CSF. Furthermore, since morphine is proposed to exert its analgesic effect in part through release of 5-HT in the spinal or trigeminal dorsal horns (Yaksh and Tyce, 1979; Rivot et al, 1988), concurrent measurement of indolamines would also be valuable. To this end, we have combined *in vivo* microdialysis and HPLC with electrochemical detection to measure the extracellular levels of morphine, 5-HT and 5-HIAA in brain and CSF of the awake rat, while evaluating analgesia with the tail flick and paw withdrawal methods. Rats were implanted with dialysis probes (U- or loop-shaped) in the periaqueductal gray, lateral hypothalamus, cerebellum, spinal cord and cisterna magna.

Extracellular morphine could be detected in brain with doses as low as 1.0mg/kg, ip. After 10mg/kg, morphine was detected within 15min; peak levels were reached 45-65min after injection. Maximal levels varied with the probe used and with the area sampled. The most efficient recovery was in the cisternal CSF, presumably because of minimal tissue interference with the probe membrane. Although there was a high correlation between the time course of morphine recovery in the dialysate and behavioral analgesia and although morphine markedly increased cisternal CSF levels of 5-HIAA, we found no significant effect of morphine on extracellular levels of 5-HT or 5-HIAA in the spinal cord or in any of the brain areas sampled. These studies emphasize the utility of measuring levels of morphine and neurotransmitters in brain while simultaneously monitoring behavior and suggest that spinal cord release of 5-HT is not necessary for morphine to exert its analgesic action.

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623.12

AFFERENT PROJECTIONS FROM THE NUCLEUS RAPHE MAGNUS TO THE LATERAL RETICULAR NUCLEUS AND THEIR ROLE IN THE DESCENDING ANALGESIA SYSTEM. A.Z. Murphy, M.M. Behbehani, and F.P. Zemlan. Dept. of Physiology, Univ. of Cincinnati College of Medicine, Cinti., OH 45267-0559.

Recent anatomical data have shown that the nucleus raphe magnus (NRM) sends direct projections to the lateral reticular nucleus (LRN). We have recorded from 98 cells in the LRN of the rat, determining: (1) response to electrical and chemical stimulation of the NRM, (2) response to noxious and innocuous peripheral stimulation, and (3) the role of glutamic acid and norepinephrine in the NRM-LRN interaction. Of the LRN cells tested, 41/49 (86%) were responsive to both train stimulation of the NRM (100 Hz, 50-200 uA, 200 usec pulses) and microinjection of HA (5 mM). Of these cells, 69% responded in the same direction to both chemical and electrical NRM stimulation, with 41% inhibited and 41% excited. Kynurenic acid was effective in blocking the excitatory effects of HA-induced excitation, suggesting the involvement of glutamate in this pathway. The majority of these cells were classified as either nocispecific or wide-dynamic range. Of the cells that were inhibited by peripheral stimulation, 56% were also inhibited by NRM stimulation. Response to 1 Hz single pulse stimulation showed that 24/50 cells were responsive: 75% were excited and 25% were inhibited. A post-excitation inhibitory response indicative of a collateral inhibition circuitry was noted in 33% of the cells. Phentolamine was effective in blocking this response suggesting the involvement of norepinephrine in this system. Supported by PHS grant NS20643.

623.13

THE EFFECT OF 5-HT_{1A} AGONISTS, SEROTONIN AND RAPHE STIMULATION ON SPINAL DORSAL HORN NOCICEPTIVE NEURONS IN THE RAT. R. Maureen Murphy, M.M. Behbehani, A.Z. Murphy, and F.P. Zemlan. Depts. of Psychiatry and Physiology, Univ. of Cincinnati College of Medicine, Cinti., OH 45267-0559.

The effect of iontophoretic application of serotonin (5-HT), 5-HT_{1A} selective agonists and electical stimulation of 5-HT cells of origin in nucleus raphe magnus (NRM) on lumbar spinal cord dorsal horn nociceptive neurons was determined in the rat. NRM stimulation significantly inhibited 48% of the 78 units tested while 13% were excited. Iontophoretic 5-HT administration inhibited 10 of 15 cells also inhibited by NRM stimulation while iontophoretic administration of the 5-HT_{1A} agonists, 8-OH-DPAT and buspirone, inhibited the response of 19 of 34 cells also inhibited by NRM stimulation. Comparison of the effect of 5-HT versus 5-HT_{1A} agonist iontophoresis on the same dorsal horn cells revealed that 5-HT_{1A} agonist administration inhibited 31 of 46 cells also inhibited by 5-HT iontophoresis. The inhibitory effect of 5-HT_{1A} agonists on unit activity elicited by noxious stimulation, glutamic acid or baseline firing rate could be blocked by the 5-HT_{1A} receptor blocker spiperone. Iontophoretic administration of 5-HT_{1A} agonists increased the firing rate of 7 of 20 cells also facilitated by 5-HT iontophoresis. These data suggest that 5-HT_{1A} receptors mediate a major portion of the inhibitory effect of NRM-induced release of serotonin on dorsal horn nociceptive neurons. The present study, in conjunction with parallel analgesiometric studies, suggests that 5-HT_{1A} receptors differentially affect two anatomically distinct groups of dorsal horn nociceptive neurons. Supported by PHS grant NS20643.

623.14

THE ANTINOCICEPTIVE EFFECTS OF I.V. CLONIDINE ARE ENHANCED IN ARTHRITIC COMPARED TO NORMAL RATS V. Kayser*, G. Guilbaud and J.M Besson U 161 INSERM, 2, rue d'Alésia, 75014 Paris, France.

The antinociceptive effects produced by an acute i.v. injection of the prototypic alpha-2 (α2)-adrenergic agonist clonidine (0.03-0.1 mg/kg) were investigated in normal and in Freund's adjuvant-induced arthritic rats used as a model of clinical pain. As a nociceptive test, we used the measure of the vocalization threshold (VT) elicited by foot pressure. Antagonism of clonidine's effects by the α-2 adrenoceptor antagonists yohimbine and idazoxan (0.25-1 mg/kg i.v., administered 30 min before clonidine) was also tested.

In normal rats, clonidine produced significant and dose dependent effects (mean VT increased by 24, 32 and 35 % of the control at 10 min after the injection, respectively for 0.03, 0.05 and 0.1 mg/kg i.v. clonidine), an effect roughly comparable to that of the μ-opioid agonist morphine (0.1, 0.3 and 1 mg/kg i.v.) in these animals.

In arthritic rats, clonidine displayed a more potent and prolonged analgesic effect than in normal rats (mean VT increased by 60, 63 and 86 % of the control, respectively for 0.03, 0.05 and 0.1 mg/kg i.v. clonidine). For the highest dose used (0.1 mg/kg), the antinociceptive effect of clonidine lasted for up to 110 min. in these animals.

In both groups of rats, yohimbine or idazoxan (0.25 to 1 mg/kg i.v.) significantly antagonized clonidine-induced antinociception in a dose-dependent manner.

These results suggest that α-2 adrenoceptors are involved in clonidine-induced antinociception in both normal rats and this model of inflammatory pain.

623.15

THE ROLE OF NOREPINEPHRINE IN DAMPGO-INDUCED ANTINOCICEPTION. U.D. Sivaskandan*, J.L. Stafinsky* and T. Crisp. Dept. of Pharmacology, N.E. Ohio Univs. Coll. of Med., Rootstown, OH 44272.

The present study was designed to determine if spinal norepinephrine (NE) contributes to supraspinal and/or spinal DAMPGO-induced antinociception. Male Sprague-Dawley rats were cannulated with intracerebroventricular (i.c.v.) and intrathecal (i.t.) catheters, and injected with different i.c.v. or i.t. doses of DAMPGO. Opiate-induced effects were measured on the spinally-mediated tail-flick test. The results demonstrated that DAMPGO (0.3 nmol) significantly elevated tail-flick latency (TFL) when administered i.c.v. or i.t. To determine the contribution of NE to DAMPGO-induced antinociception, a separate group of rats received i.t. pretreatments with prazosin (an α_1-adrenoceptor antagonist) or idazoxan (an α_2-adrenoceptor blocker) prior to DAMPGO. The supraspinal effects of DAMPGO were reversed by i.t. idazoxan, but not by prazosin. Apparently, i.c.v. DAMPGO activates a descending noradrenergic pain-inhibitory pathway to produce elevations in TFL. The NE released at the spinal level then interacts with α_2-adrenoceptors to mediate DAMPGO-induced antinociception. Neither prazosin nor idazoxan inhibited the spinal effects of DAMPGO, suggesting that μ agonists produce spinal antinociception in the absence of a noradrenergic component.

623.16

Monaminergic changes induced by morphine in hypoalgesic and hyperalgesic domestic fowl.
R. A. Hughes, Department of Psychology, Iowa State Univ., Ames, IA 50011-3180, K. J. Sufka, J. Giordano, and D. A. Hoganson*, Drake Univ., Des Moines, IA 50311-4505.
In replication of previous research morphine (0.0, 5.0, 10.0 mg/kg i.m.) induced dose and strain dependent hypoalgesia in Rhode Island Red (RIR) and hyperalgesia in White Leghorn (WL) cockerels on the formalin test. Assay (HPLC) of monoamines (NE, D, and 5HT) and metabolites (DOPAC and 5HIAA) in brain, brainstem, and spinal cord 30 min. after morphine (0.0, 5.0, and 10.0 mg/kg i.m.) increased 5HT ($p<.05$; this and subsequent p values refer to 0.0 vs. 10.0 mg/kg) in sp. cord and br. stem for both strains and no consistently reliable change in NE, D, 5HIAA or DOPAC, in these regions for either strain. Thus, monoaminergic correlates of morphine in brainstem and spinal cord monoamines did not differentiate the hypoalgesic from the hyperalgesic strain. On the other hand, morphine decreased brain **NE** ($p<.01$) and 5HIAA ($p<.02$) in the hypoalgesic RIR strain and increased brain 5HT ($p<.001$) and DOPAC ($p<.05$) in the hyperalgesic WL strain. Thus, morphine-induced changes in brain monoamines discriminated between the analgesic and the hyperalgesic strain.

624.1

IMMUNOLOCALIZATION AND CHARACTERIZATION OF SPECTRIN (240/235) AND (240/235E) IN THE MOUSE VISUAL SYSTEM. T. Isayama, S.R. Goodman†, and I.S. Zagon, Penn. State Univ., Hershey PA and †Univ. S. Alabama Coll. Med., Mobile AL.

Spectrin is a major structural component of the mammalian central nervous system, with cellular/dendritic (brain spectrin 240/235E) and cellular/axonal (brain spectrin 240/235) isoforms. We have characterized spectrin isoforms in the mouse visual system using Western blots, and localized spectrins with immunocytochemistry, using polyclonal antibodies directed against two brain spectrin isoforms. Immunoblot analysis of proteins isolated from mouse retinas revealed the presence of two spectrin isoforms; these two isoforms corresponded to brain spectrins (240/235E) and (240/235) on similar preparations of mouse brain. Spectrin (240/235E) was localized in cell bodies found in the inner nuclear, outer nuclear, and ganglion cell layers, and in processes of the inner and outer plexiform layers. Spectrin (240/235) was more diffusely distributed throughout the retina, with light immunostaining of neurons in the ganglion cell, inner nuclear, and outer nuclear layers. Spectrin (240/235E) was apparent in the nerve fiber layer and in the optic nerve; antibodies to spectrin (240/235) detected only diffuse immunoreactivity in the nerve fiber layer and optic nerve. These results indicate that the neuronal cytoskeletal proteins, brain spectrin (240/235) and (240/235E), are present in the visual system. Although the disposition of spectrin isoforms in the central nervous system and visual system have similarities, some important differences were detected.

This work was supported by NIH grants NS-21246 and NS-19357.

624.2

THE ISOLATION OF DIRADYL CHOLINE PHOSPHOGLYCERIDES, INCLUDING THE PRECURSOR OF PLATELET-ACTIVATING FACTOR, FROM RETINA. **J.S. Hurst,* H.E.P. Bazan, and N.G. Bazan. LSU Eye Center and Neuroscience Center, New Orleans, LA 70112.**

Choline phosphoglycerides (PC) constitute an important source of both arachidonic acid (the precursor of eicosanoids), and bioactive glycerolipids, such as platelet-activating factor (PAF), which are significant inflammatory mediators in many tissues. It would be important, therefore, to establish which PC component in the retina is the most prominent contributor of arachidonic acid. Lipids were extracted from retinal homogenates and the PC fraction was purified by thin-layer chromatography (TLC). Diradyl derivatives, i.e. the alkylacetyl, alkenylacetyl, and diacylacetyl, were prepared from the PC isolate by phospholipase C treatment, followed by acetylation. The derivatives, separated by TLC and high-performance liquid chromatography, were subjected to alkaline methanolysis. The fatty acid methyl esters, generated from the diradyl acyl constituents, were extracted and analyzed by capillary gas-liquid chromatography and quantified, using C21:0 as the internal standard. The diacyl molecular species comprised more than 95%, the alkyl less than 5%, and the alkenyl less than 1% of the PC pool. Arachidonic acid and docosahexaenoic acid were the only significant polyunsaturated fatty acids represented in all three diradyl PC components. However, on a mole basis, the alkenyl fraction was the most enriched in arachidonic acid, with 30% of the total fatty acids as arachidonic acid. The PAF precursor, on the other hand, was the most enriched in docosahexaenoic acid. Supported by NEI EY05121.

624.3

DISTRIBUTION OF G-PROTEIN β SUBUNITS IN THE PRIMATE RETINA. Y.-W. Peng*[1,2], C. Emala*[3], J.D. Robishaw*[4], M.A. Levine*[3] and K.-W. Yau[1,2]. Howard Hughes Medical Institute[1]; Departments of Neuroscience[2] and Medicine[3], Johns Hopkins University School of Medicine, Baltimore, MD 21205; Geisinger Clinic[4], Danville, PA 17822.

The guanine nucleotide-binding proteins (G proteins) involved in various signal transduction processes in cells are heterotrimeric proteins composed of α, β and γ subunits. Each G protein possesses a distinctive α subunit, which is thought to confer functional specificity. In contrast, β subunits appear to be highly conserved, and are thought to couple promiscuously with various α subunits. To further examine this question, we have used anti-peptide antibodies against three β subunits that have been molecularly cloned to locate these proteins in the retina with immunocytochemistry. β_1 immunoreactivity is observed in rod photoreceptors as well as some amacrine cells and ganglion cells. β_2 immunoreactivity is predominently present in discrete sublaminae of the inner plexiform layer and some amacrine cell bodies. Most interestingly, β_3 immunoreactivity is present in all cone photoreceptors, as well as selective bipolar cells. The segregation of β_1 and β_3 in rod and cone photoreceptors, which contain related but distinct G-protein (transducin) α subunits, suggests that β subunits may be involved in defining the functional specificities of G proteins.

624.4

STAINING OF THE HUMAN RETINA WITH MONOCLONAL ANTIBODIES TO PROTEIN KINASE C ISOZYMES. Helga Kolb, Li Zhang* & Nicolas Cuenca*. Physiology Dept., University of Utah, Salt Lake City, U.S.A. and Histology Dept., University of Alicante, Spain.

Monoclonal antibodies made to the three isozymes of protein kinase C (Type 1, Type II and Type III, Seikagaku America Inc.) were applied to post-mortem human retina. Immunostaining was done on wholemount, cryostat or Vibratome sectioned retina and visualized after ABC/DAB procedures.

The three isozymes of PKC gave completely different staining patterns. The Type I antibody failed to stain any cells at all. The Type III antibody stained rod bipolar cells in mid-peripheral and peripheral retina. The smallest-field cells with tiny axon terminals in s5 of the IPL appeared first at 1mm from the foveal pit. Peripheral retina appeared covered with an even distribution of beaded, narrow-field rod bipolar axon terminals in s5 of the IPL. Wide-field bipolar axon terminals (over 100 µm spread) stratifying in s5 were also stained.

The Type II isozyme stained cone bipolar cells, amacrine cells and ganglion cells. Cone bipolar staining occurred all the way into the fovea and clearly both midget and diffuse varieties of cone bipolar were included. The IPL was so heavily stained due to amacrine and ganglion cell immunoreactivity that banding contributed by bipolar endings was difficult to discern. However, s2 and s4 appeared to stand out somewhat more heavily than others. Ganglion cells of all soma sizes, their axons and to some degree their dendrites were also immunostained. PKC appears to be a second messenger used in neurotransmission by both rod and cone systems in the human retina.

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624.5

Retinal Protein Substrates For Tyrosine-specific Kinases. D.J. Bare, J.S. Biscardi* and P.F. Maness. Dept. of Biochemistry and Biophysics, Univ. of North Carolina School of Medicine, Chapel Hill, NC 27599.

Tyrosine-specific protein kinases are abundant in the adult vertebrate retina. Recently, it was shown that protein substrates in the synapse-rich plexiform layers of the retina are phosphorylated to a high degree. Subcellular fractionation of retina homogenate analyzed by immunoblotting with Phosphotyrosine (PTyr) antibodies revealed that several PTyr-modified proteins were enriched 5-20 fold in synaptosomes and that some were preferentially localized in a synaptic vesicle fraction. Immunocytochemical labeling with PTyr antibodies has revealed that PTyr proteins are prominent in the cytoplasm of horizontal cells and their processes. The most abundant substrates for Tyr phosphorylation are 106, 100, 78, 60 and 38 kD proteins. Our approach to revealing the function of Tyr kinases is directed towards the identification of substrates from bovine retina. A synaptosomal fraction was obtained from retina homogenates by differential centrifugation. The synaptosomes were lysed and a pellet that contained synaptic plasma membranes was obtained. Detergent solubilized PTyr-modified proteins were fractionated by affinity chromatography using PY20 monoclonal antibody. Through this procedure 10-15 µg of PTyr protein were purified from 25 bovine retinas.

624.6

COMPARATIVE LOCALIZATION OF C-FOS AND C-JUN-LIKE IMMUNOREACTIVITY IN THE TURTLE RETINA. W.D. Eldred and A. Yaqub*. Department of Biology, Boston University, Boston, MA 02215.

C-jun and c-fos are proteins encoded by the c-fos and c-jun proto-oncogenes, which have been reported to be neuronal activity markers. As a preliminary step in the analysis of the role of these proteins in retinal function, we have localized these antigens in the turtle retina using immunocytochemistry. C-fos-like immunoreactivity was widely distributed in the retina. The labeling was primarily somatic and many cells in the inner nuclear and ganglion cell layers were immunoreactive. In isolated cases, there were cells with higher levels of immunoreactivity throughout their cytoplasm, which filled their processes in the inner plexiform layer (IPL). In contrast, the c-jun-like immunoreactivity was much more selectively localized. Only small numbers of amacrine cells and some ganglion cells were immunoreactive. The immunoreactivity in the amacrine cells was found throughout the cell bodies and in the dendritic arborizations. The majority of amacrine cells were monostratified with extensive dendritic arborizations in strata 2 of the IPL. These amacrine cells had elongated, vertically oriented cell bodies located near the middle of the inner nuclear layer. The immunoreactivity in the ganglion cells was primarily confined to the cell bodies, although some immunoreactivity was apparent in the ganglion cell axon layer. It appeared that most ganglion cells with c-jun-like immunoreactivity had small cell bodies. Our results show that c-jun is more specifically localized in the turtle retina than c-fos immunoreactivity, which suggests that c-jun may provide more selective indications of neuronal activation. This research supported by EY04785 to W.D.E.

624.7

DOUBLE-LABELING STUDIES OF THE DISTRIBUTION OF ALPHA-BUNGAROTOXIN BINDING PROTEINS AND NICOTINIC RECEPTORS IN THE CHICK RETINA. D.E. Hamassaki-Britto, L.R.G. Britto, K.T. Keyser, H.J. Karten and J.M. Lindstrom*. Dept.Neurosci., Univ.California, San Diego, La Jolla, CA 92093, and *Inst.Neurol.Sci., Univ. Pennsylvania, Philadelphia, PA 19104.

The present study was undertaken to compare the distribution of α1 and α2 subunits of the α-bungarotoxin binding proteins (BP)-like immunoreactivities (BP-LI) to that of nicotinic receptor-like immunoreactivity (nAChR-LI) in the chick retina. Double-labeling experiments were conducted to test the possibility that the same cell could contain both BP-LI and nAChR-LI. In the inner nuclear layer, displaced ganglion cells contained only nAChR-LI. Bipolar cells stained for α2BP-LI, and their processes could be observed in the outer plexiform layer. Amacrine cells, and cells in the ganglion cell layer, exhibited α1BP-LI, α2BP-LI and nAChR-LI. However, there were differences in the distribution of these immunoreactivities: (1) the α1BP-LI cells tended to be larger than the α2BP-LI and nAChR-LI cells; (2) the density of α2BP-LI cells was higher than the densities of α1BP-LI and nAChR-LI cells; (3) in the ganglion cell layer, the larger cells containing α2BP-LI also contained α1BP-LI; (4) many cells in the ganglion cell layer exhibiting α2BP-LI showed nAChR-LI, whereas no α1BP-LI cell appear to contain nAChR-LI. Although both BPs were distributed in all laminae of the inner plexiform layer, the outer laminae (mainly lamina 2) exhibited a heavier staining for α2BP-LI, and the inner laminae (mainly lamina 4) for α1BP-LI. In turn, nAChR-LI was observed in laminae 1, 2, and 4 of the inner plexiform layer. Our results show that α1BP-LI, α2BP-LI and nAChR-LI are differentially distributed in the chick retina, although co-localization could be observed in some cases.

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624.8

EXPRESSION OF GLUTAMATE DECARBOXYLASE (GAD) mRNAs IN THE ADULT AND DEVELOPING RAT RETINA. Mable Lai*, Martin Humphrey, Catia Sternini and Nicholas Brecha. Depts. of Medicine and Anatomy & Cell Biology and CURE, UCLA School of Medicine and VAMC-West Los Angeles, Los Angeles, CA., Dept. of Psychology, University of Western Australia.

GABA is a prominent inhibitory neurotransmitter, which is synthesized by two structurally different GAD molecules with predicted molecular weights of about 65 and 67 Kd (GAD_{65} and GAD_{67}, respectively). The cellular expression of GAD mRNAs was examined in the adult and developing rat retina using *in situ* hybridization histochemistry with ^{35}S-labeled rat GAD_{65} or GAD_{67} RNA probes. Cryostat sections were incubated with sense or antisense RNAs, washed at high stringency and processed for autoradiography. Specific labeling is not observed in sections incubated in sense RNA probes. In adult retina, GAD_{65} and GAD_{67} mRNAs are expressed in numerous cells distributed to the proximal inner nuclear layer (INL) and to the ganglion cell layer (GCL), and in some cells located in the inner plexiform layer. At early postnatal ages, cells containing both GAD mRNAs are sparse and localized to the proximal INL and GCL. By day 10, the distribution of GAD mRNAs is comparable to that observed in the adult retina. These results demonstrate that both GAD mRNAs are present in the retina and are predominantly expressed in amacrine and displaced amacrine cells.

We thank Dr. A. Tobin for providing the GAD_{65} and GAD_{67} cDNAs.
Supported by NIH grants EY 04067 and VAMC Research Funds.

624.9

EXPRESSION OF GABA NEURONAL TRANSPORTER mRNA IN THE RAT RETINA. Christine Weigmann* and Nicholas Brecha. Depts. of Anatomy & Cell Biology and Medicine, Jules Stein Eye Institute and CURE, UCLA School of Medicine and VAMC-Wadsworth, Los Angeles, CA.

GABA transporters play an important role in synaptic activity in the nervous system. Recently, a cDNA (named GAT-1) encoding a GABA transporter that is pharmacologically similar to neuronal GABA transporters has been cloned. The present study evaluates the cellular localization of this GABA transporter mRNA in the rat retina using *in situ* hybridization histochemistry with a ^{35}S-GAT-1 RNA probe. Retinas were fixed in 4% paraformaldehyde and sectioned perpendicularly to the vitreal surface with a cryostat. Retinal sections were incubated in antisense or sense RNA at 53°C, washed at high stringency, processed for autoradiography and exposed for 11 to 45 days. Only background labeling was seen in sections incubated with the sense RNA probe. In all retinal regions, GAT-1 mRNA is localized to numerous cells distributed to the proximal inner nuclear layer (INL). There is little labeling over the distal INL. In the inner plexiform layer, some discretely labeled cells are present. In the ganglion cell layer, many cells containing GAT-1 mRNA are seen. Labeling is not observed in the outer nuclear layer. The distribution of GAT-1 mRNA closely matches the reported neuronal uptake pattern of GABA. Overall, this labeling pattern suggests that amacrine and displaced amacrine cells are the main cell types expressing this transporter.

We thank Dr. J. Guastella for providing the GAT-1 cDNA.
Supported by NEI grant EY 04067 and VA Medical Research Funds.

624.10

$GABA_A$ RECEPTOR FUNCTION IN RETINAL NEURONS: MODULATION BY CYCLIC AMP. M.L. Veruki and H.H. Yeh, Neuroscience Program, University of Rochester Medical Center, Rochester, NY 14642.

We are investigating the intracellular mechanisms underlying our previously reported phenomenon that vasoactive intestinal polypeptide (VIP) can potentiate $GABA_A$ receptor-mediated whole-cell current responses in bipolar and ganglion cells of the rat retina. Since VIP is a potent elevator of intracellular cyclic AMP, the peptide may work through a cyclic AMP-dependent intracellular cascade to modify $GABA_A$ receptor function. However, a prerequisite for formulating this hypothesis is the demonstration that elevating intracellular cyclic AMP levels mimics the potentiating effect of VIP on GABA.

Freshly isolated bipolar cells and ganglion cells were obtained from the rat retina following treatment with protease. Agonists, dissolved in bath solution, were applied via a micropressure ejection system and the resultant current responses were monitored under voltage clamp using conventional whole-cell patch clamp recording procedures.

Exposure of bipolar cells and ganglion cells to forskolin ($\leq$200 uM), an activator of adenylate cyclase, but not its biologically inactive analog, 1,9-dideoxyforskolin ($\leq$100uM), resulted in a robust and reversible potentiation of the GABA-activated current (I_{GABA}) recorded in both cell types. Extracellular application of 8-Bromo-cyclic AMP ($\leq$500uM), the membrane permeable analog of cyclic AMP, also potentiated the I_{GABA}. However, control tests of specificity indicated that extracellular application of cyclic AMP ($\leq$1mM) also had a similar potentiating effect. We thus are cautioned to interpret our data with care, especially with regard to the potential non-specific effects of membrane-permeable analogs of cyclic AMP. Overall, our results to date are consistent with the notion that increasing intracellular levels of cyclic AMP in bipolar and ganglion cells may ultimately lead to a facilitation in $GABA_A$ receptor efficacy.

Supported by PHS grants NS24830 and NS01340 and an award from the Hellenic University Club of New York.

624.11

EFFECTS OF DOPAMINE D_1 AND D_2 RECEPTOR AGONISTS ON ELECTRICAL COUPLING BETWEEN FISH HORIZONTAL CELLS. Krisztina Harsanyi* and Stuart C. Mangel. Dept. of Ophthalmology, Univ. of Alabama School of Medicine, Birmingham, AL 35294.

In order to characterize the action of dopamine on horizontal cell coupling, SKF38393, a D_1 receptor agonist, LY171555, a D_2 receptor agonist, or dopamine, itself, were superfused onto the isolated intact goldfish retina, while the light responses of L-type (H1) cone horizontal cells were monitored. Either dopamine (2-50 uM) or SKF38393 (2.5-20 uM) uncoupled horizontal cells. That is, responses to small (500 um) centered spot stimuli were increased and annulus and full field responses were decreased. However, LY171555 (0.5-10 uM) increased the coupling of horizontal cells; spot responses decreased and annulus and full field responses increased during application of this drug. Interestingly, at low dopamine concentrations (0.2 uM), horizontal cells were sometimes observed to first uncouple slightly and then to recouple beyond their initial state.

To test whether fish horizontal cells, themselves, possess D_2 receptors that might directly increase horizontal cell coupling at low dopamine concentrations, LY171555 (10 uM) was bath-applied following horizontal cell uncoupling by SKF38393 (2.5-10 uM). However, LY171555 had no effect under these conditions. These results suggest that LY171555 couples horizontal cells by activating D_2 autoreceptors on dopaminergic interplexiform cells, thus decreasing dopamine release onto horizontal cells.

624.12

HIGH EXTRACELLULAR LEVELS OF DOPAMINE IN XENOPUS RETINA DETECTED BY HIGH-SPEED CYCLIC VOLTAMMETRY. P. Witkovsky, M.E. Rice and C. Nicholson. Depts. of Ophthalmol. and Physiol. & Biophys., New York University Medical Center, New York, NY 10016.

We measured extracellular dopamine concentration in superfused eyecups using Nafion-coated carbon fiber electrodes and high-speed cyclic voltammetry (Rice and Nicholson, *Analyt. Chem.*, 61:1805, 1989). The dopamine concentration in or adjacent to the subretinal space was 734 ± 154 nM (mean ±sem, n=13, 4 eyecups). Peak oxidation/reduction potentials of dopamine and DOPAC were identical. However, the electrode selectivity for dopamine/DOPAC was 15:1 and a dopamine/DOPAC ration of 3:1 in the vitreous was determined by HPLC-ED (K. Bohmaker and M. Meller, personal communication). Thus, DOPAC contributed ≤3% to the voltammetric signal. Preliminary measures indicated that dopamine levels increase in steady light and diminish following light extinction, on a time scale of minutes. Our data show that retinal dopamine levels are much higher and DOPAC levels are much lower, compared to their respective concentrations in brain. These differences were interpreted in terms of the distinct geometries of dopaminergic terminals in retina and brain. Supported by grants EY03570 (PW), NS28480 (MER) and NS28642 (CN).

624.13

TYROSINE DEPENDENCE OF DOPAMINE RELEASE IN STIMULATED RETINA. C.J. Gibson. Dept of Pathology, University of Western Ontario, London, CANADA N6A 5C1.

Retinae were removed from male Sprague-Dawley rats and placed in individual perfusion chambers for the measurement of endogenous dopamine (DA) release (Gibson, J.Neurosci.Methods,32:75-79,1990). DA release was stimulated 5 to 7-fold over basal release following a 10 minute pulse of 40 mM potassium (K). In this system, perfusion with a second pulse of 40 mM K resulted in a roughly similar increase in releasable DA. Expressed as a ratio of the fractional release, K2/K1 = 0.96 $\pm$ .07. When 100 μM tyrosine was added throughout the perfusion this ratio increased to 1.42 $\pm$ .20 ($p \leq .05$). Addition of α-methyl-p-tyrosine (50 to 200 μM) progressively decreased the amount of DA released. At 200 μM, DA release during the second K pulse was totally abolished. In this system, in which DA release is dependent on newly synthesized transmitter, the addition of the precursor tyrosine maintains and even increases the amount of DA released.
(Supported by the Canadian MRC).

624.14

MELATONIN RECEPTOR-MEDIATED INHIBITION OF CYCLIC AMP ACCUMULATION IN CHICK RETINAL CELL CULTURES. P.M. Iuvone and J.Gan*. Dept. of Pharmacology, Emory Univ. Sch. of Med., Atlanta, GA 30322.

In addition to its role as a pineal hormone, melatonin is synthesized in vertebrate retina, where it acts locally as a neuromodulator. Melatonin in retina has been implicated in the circadian regulation of photoreceptor outer segment disk shedding and dark-adaptation, and in the regulation of dopamine release from retinal amacrine cells. The cellular transduction mechanism(s) responsible for melatonin's actions in retina are not known. We have examined the effect of melatonin and related indoles on cyclic AMP accumulation in glia-free monolayer cultures of photoreceptors and neurons prepared from embryonic chick retinas. Melatonin had no significant effect on basal cyclic AMP accumulation, but significantly inhibited forskolin-stimulated cyclic AMP formation. Melatonin inhibited cyclic AMP formation in both photoreceptor-enriched and neuron-rich cultures, suggesting that both photoreceptors and multipolar neurons may contain melatonin receptors. Melatonin receptor agonists and related indoles inhibited cyclic AMP accumulation with an order of potency indicative of action at a melatonin receptor: 2-iodomelatonin $\geq$ melatonin $\geq$ 6-chloromelatonin > 6-hydroxymelatonin > 5-methoxytryptamine $\geq$ N-acetylserotonin > serotonin. Melatonin was potent, acting in the picomolar - nanomolar range, and produced a maximal inhibition of approximately 60% of the cyclic AMP accumulation evoked by 1 μM forskolin. The effect of melatonin on cyclic AMP formation was inhibited by the putative melatonin receptor antagonists luzindole (N-0774) and N-acetyltryptamine. Inhibition of cyclic AMP formation by melatonin was blocked by pretreating the cells with pertussis toxin. The results suggest that some of melatonin's actions in the retina may involve receptor-mediated inhibition of cyclic AMP formation.
Supported by NIH RO1-EY04864.

624.15

TISSUE-TYPIC CELL PRODUCTION IN RETINAL SLICE CULTURE
Andreas F. Mack*, Thomas L. Kasten* and Russell D. Fernald, Institute of Neuroscience, University of Oregon, Eugene OR, 97403 and Department of Psychology, Stanford University, Stanford, CA 94305.

Two features distinguish retinas of teleost fish from those of warm blooded vertebrates: 1. Teleost retinas can regenerate after lesioning and 2. they continue to grow after embryongenesis, generating new retinal tissue in the retinal periphery, and new rod photoreceptors throughout the retina. We have developed a retinal slice culture system to study these processes in the African cichlid fish Haplochromis burtoni. We used ^{3}H-thymidine to label newly divided cells and immunocytochemistry with rod and cone specific antibodies to demonstrate differentiation of proliferating cells in culture.

After 2-3 days in culture, double labelled cells were distributed in a tissue-typic pattern. Newly generated cells were found at the margin and in the outer nuclear layer (ONL). Those in the ONL were labelled with a rod specific antibody whereas those near the margin were principally labelled with a cone specific antibody. This demonstrates that tissue-typic division and differentiation continue in the slice preparation. However, some presumptive rod progenitor cells central from the proliferating edge of the retina also stained positively with the cone antibody. This suggests that rod progenitors are possibly multipotent and may be able to differentiate into phenotypes other than rods. For example, rod progenitor cells might get important cues for differentiation from their cellular environment and its alteration could play a role in changing their fate during retinal regeneration. We are now analyzing candidate factors which play a role in proliferation and differentiation of the retinal slice.
Supported by EY 05051.

624.16

CHEMICAL AND ELECTRICAL SYNAPSES FORMED BY CHICK RETINAL NEURONS MAINTAINED IN DISSOCIATED CELL CULTURE. E. Gleason and M. Wilson. Department of Zoology, University of California, Davis CA 95695.

Sparsely cultured retinal neurons derived from 8 day chick embryos form synapses that may be identified ultrastructurally and whose physiology we have begun to investigate. Communication between 103 pairs of large (cell bodies 12-20 μm diameter) multipolar cells has been examined using the perforated patch technique to voltage clamp both neurons. Electrical coupling was present in 61% of the cell pairs and was always ohmic, time-independent and ranged in magnitude from 100 pS to several nS.

Overall, chemical synaptic communication was seen in 55% of cell pairs with 49% of these pairs showing reciprocal connections. The frequency with which chemical synapses were observed increased over time in culture. On day 5 in culture 44% of pairs showed chemical synapses whereas on day 9, 73% of cell pairs showed chemical communication. Presynaptic depolarization positive to -30 mV elicited postsynaptic currents that were always noisy and could often be resolved into discrete events with conductance values as large as 1 nS. Spontaneous discrete events of similar amplitudes could also be seen in 28% of cell pairs. Discrete events peaked within 13 msec, decayed with time constants of about 18 msec, and were reversibly abolished by 3 mM Co^{++}. All postsynaptic currents were outward positive to -60 mV and in all cell pairs examined (n=7), 3-10 μM bicuculline reversibly inhibited the current, suggesting that GABA was the transmitter at these synapses.
This work was supported by NIH EY04112.

624.17

ORGANOTYPIC SLICE CULTURE OF THE MAMMALIAN RETINA.
H. Wässle and A. Feigenspan*. Max-Planck-Institut für Hirnforschung, Frankfurt, Germany.

Vertical slices of 6 day postnatal rat retina were cut at a thickness of 100 μm and cultured using the roller culture technique of Gähwiler (J. Neurosci. Meth. 4, 329-342). After 14-21 days in vitro, the slices showed the typical pattern of layering of mature retina. The following immunocytochemical markers were used to characterize the different cell types: antibodies against protein kinase C (PKC), calcium binding protein (CabP 28 kD), parvalbumin (PV), neurofilaments (NF), gliaspecific antibodies (GFAP, vimentin) and transmitterspecific antibodies (GABA, TH). These markers labelled populations of cells comparable to those in the mature mammalian retina. In particular, antibodies against PKC in the mature retina stain rod bipolar cells (Greferath et al. J. Comp. Neurol. 301, 433-442). In the 6 day postnatal retina no staining was observed. However, after 14-21 days in the slice culture many presumptive rod bipolar cells were labelled.

The experiments show that a mammalian slice culture can be used to study differentiation of retinal cell types.

624.18

VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) IN NORMAL AND DIABETIC HUMAN RETINA.
E.D. McGookin[1*], E.G. Stopa[1], R. Chorsky[1*], G.H. Collins[1], A. Baird[#*], A.M. Gonzalez[#*] and A Hanneken[#*] Depts. of Pathology[1] and Ophthalmology SUNY-HSC Syracuse, New York 13210; The Whittier Institute for Diabetes and Endocrinology[#], LaJolla, California 92037.

The recent identification of vascular endothelial growth factor (VEGF) has indicated that this growth factor is a mitogen for vascular endothelial cells. Studies, using immunocytochemistry, compared the distribution of VEGF-like and basic fibroblast growth factor-like (bFGF) immunoreactivity in human eyes, snap frozen post-mortem from control and diabetic subjects. Ten micron sections were cut and stained with specific polyclonal antibodies directed against the amino terminus of VEGF (Ab#61) and bFGF (Ab#773) respectively. The results indicate that bFGF-like immunoreactivity was present in the ganglion, inner nuclear and outer nuclear cell layers of the retina. VEGF-like immunoreactivity was most noted in the ganglion cell layer and in contrast to bFGF, VEGF was more diffusely distributed within the inner nuclear and outer nuclear layers. In addition to their presence in neurons, both bFGF and VEGF were also seen associated with the neovascularization of diabetic retinopathy. Although VEGF is thought to have a role in angiogenesis, our data provide evidence that VEGF may also be an important growth factor in the retina and central nervous system.
Supported by AG 09301, NS 28121.

624.19

SITES OF BRAIN INVOLVEMENT IN A MURINE MODEL OF HERPETIC RETINITIS. J.C. Hedreen, J.S. Pepose* and J.A. Whittum-Hudson*. Depts. of Pathol. and Ophthalmol., The Johns Hopkins Univ. Sch. of Med., Balto., MD 21205 and the Department of Ophthalmology, Washington University School of Medicine, St. Louis, MO 63110.

When Herpes simplex virus type 1, KOS strain, is inoculated into the anterior chamber of one eye in BALB/c mice, a severe infection develops in the anterior segment, but the retina remains almost normal. In contrast, the opposite uninjected eye develops a delayed necrotizing viral retinitis. To investigate possible paths of interocular transfer, we examined the localization of virus in the brain by immunocytochemistry at 1-14 days postinoculation. At day three, antigen-positive neurons were observed in the ipsilateral Edinger-Westphal nucleus and vicinity and the ipsilateral oculomotor nerve root exit zone. At day four, neurons in the contralateral medial pretectal area, the ipsilateral suprachiasmatic nucleus, and the ipsilateral intergeniculate leaflet of the lateral geniculate body became positive. Necrotizing contralateral retinitis occurred by day ten. The probable route by which virus reaches the opposite eye is: retrograde axonal transport from the ciliary ganglion to the ipsilateral midbrain; from the midbrain to the ipsilateral suprachiasmatic nucleus; and from the suprachiasmatic nucleus to the contralateral retina.

624.20

PATTERN ELECTRORETINOGRAM & VISUAL EVOKED POTENTIAL (VEP) IN RETINITIS PIGMENTOSA (RP).

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RP is a hereditary disease of photoreceptors, progressive in nature and primarily affects the peripheral retina. Since pattern electroretinogram (PERG) is believed to reflect the activity of the ganglion cells and is mostly evoked from the the foveal retina like VEP, we analyzed PERG and VEP in patients with RP who retained only the central vision.

Ten patients (18 eyes) with RP were evaluated using VEP, PERG, FERG (Flash-ERG) and Goldmann visual fields. For VEP and PERG, the visual stimuli were 0.5^0 black and white checks with 98% contrast reversing at 2 and 8 Hz. The central retina (16^0 x 19^0) was stimulated. FERG was performed using a Ganzfeld stimulator. In 14 eyes $5\text{-}10^0$ of the central retina was spared on the visual field. In these eyes the photopic response was abnormal and PERG was extinguished. In 4 eyes, 30^0 of the central retina was spared. The photopic response was normal and PERG was present in these eyes. The scotopic ERG was abnormal in all the eyes. On the other hand, VEP was abnormal in only 5 eyes. VEP gave normal results in 13 eyes, although 9 of the same eyes tested abnormal using the PERG method. From these findings, it appears that: 1) normal PERG response is dependent not only on the foveal but also the parafoveal retina, 2) PERG is more sensitive than VEP in detecting wide-spread disease of the retina. (Partially supported by NIH EY 07024).

624.21

GLIAL RESPONSE TO HETEROLOGOUS INTRAVITREAL RETINAL PIGMENT EPITHELIAL (RPE) CELLS. S.A. Vinores, H.A. Sen*, E.E. Van Niel*, and P.A. Campochiaro*. Dept. Ophthalmol., Univ. of Virginia, Charlottesville, VA 22908.

The pathogenesis of epiretinal membranes (ERMs), which can lead to traction retinal detachments (TRDs) is not clearly understood. To induce ERM formation, human RPE cells were injected into rabbit vitreous and the eyes were examined grossly, ultrastructurally, and immunocytochemically at 7,14,16,21, and 28 days. Early TRDs were seen at 7 days and progressed thereafter. Donor RPE cells stopped expressing keratin(K) intravitreally as in vitreous cultures. By 21 days, some K+ cells were evident and some RPE cells appeared to begin re-acquiring pigment.

At the site of ERM formation, some Müller cells appear to lose GFAP, retract their processes, and develop abundant rough endoplasmic reticulum (RER). These cells, round GFAP+ cells, which also express glutamine synthetase (GS), and unretracted Müller cell processes, break through the inner limiting membrane. The Müller cells with abundant RER (GS+) remain on the inner retinal surface. The round cells appear to flatten out and cover heterologous cells on the inner retinal surface or migrate into the ERM. These results show that Müller cells react to cells in the vitreous, migrate from the retina and contribute to ERM formation.

624.22

AN ISCHEMIA-REPERFUSION INJURY BY HIGH INTRA-OCULAR PRESSURE IN THE RAT RETINA. Z. H. Liu*, Y. P. Chu*, X. G. Luo. Dept. of Neurobiology, Hunan Med. Univ. P. R. C.

The effect of the high intraocular pressure on artificial glaucoma was investigated in 20 albino rats. Isotonic saline was perfused into the anterior chamber of the right eye, as ischemia/reperfusion (I/R) eye, with intraocular arterial perfusion pressure (PP) for 1 hr (ischemia), then decreased the intraocular pressure to be normal for another hr. The left eye, as simple ischemia (SI) eye, was just perfused only with the PP for 1 hr (reperfusion). As comparing these two groups, the malondialdehyde in I/R retina was higher than that in SI retina ($p<o.oo1$). When both of the I/R and SI retinas were incubated in 1% lanthanum nitrate solution, a lot of lanthanum grains were seen inside along the cytoplasmic membrane of the retinal ganglion cells (RGCs) in I/R group, but the grains were accumulated outside the RGCs in SI group. Morphologically, the clumped nuclear chromatin, vacuolization of the cytoplasm in the RGCs could be observed under light microscope. By electron microscope, the thrombosis in the capillaries and the swollen endothelial cells were demonstrated obviously. The changes of the RGCs included increase in electron density, the disruption of cytoplasmic membrane and/or nuclear membrane, the swelling and vacuolation of mitochondria (Mi). In constrat, only a little changes such as the mild dilatation and the cristae disappearance of the Mi were recognized in the RGCs of the SI retina. These results suggest that the virtual injury occurred mostly during the postischemia rather than the ischemia period. It is reasonable to assume that the oxygen free radical plays vital role in the development of some glaucoma. (supported by NSFC 3870621).

624.23

ARTERIOLES AND VENULES OF NEONATE RETINA DILATE IN RESPONSE TO INTRAVITREAL 2-CHLOROADENOSINE.
J.M. Gidday, T.M. Lanius*, A.R. Shah*, E.R. Gonzales*, and T.S. Park. Department of Neurological Surgery, Washington University, St. Louis, MO 63110.

Studies designed to identify metabolic regulators of retinal blood flow (RBF) in the neonate eye are scant. We are investigating the hypothesis that adenosine regulates neonate RBF. The present study was undertaken to determine if retinal vessels of the newborn pig eye respond to an intravitreal injection of 2-chloro-adenosine (2-CADO), an adenosine agonist. Isoflurane-anesthetized, ventilated, pancuronium-paralyzed piglets (<5 days old) were used (n=5). The iris was dilated with 1% tropicamide, and the cornea and lens were removed and replaced with a glass coverslip to permit visualization of the fundus via videomicroscopy and xenon epi-illumination. Under 310x magnification, the diameters of a single arteriole (71±8 μm) and venule (154±29 μm) were measured by videocalipers before, and at 5 min intervals following, an adjacent intravitreal injection (20 μl) of 100 μM 2-CADO. Arterioles and venules maximally dilated to 168±15% and 121±9% of their respective control diameters 15 min after 2-CADO administration. These dilations were sustained for at least an additional 20 min. The significant increases in arteriolar size induced by 2-CADO lend support to the hypothesis that adenosine may regulate neonate RBF.

625.1

COMPUTER SYSTEM FOR MAPPING PHOSPHENES PRODUCED BY INTRACORTICAL MICROSTIMULATION OF VISUAL CORTEX. E.M. Schmidt and S.R. Charagundla*. Lab. of Neural Control, NINDS, NIH, Bethesda, MD 20892.

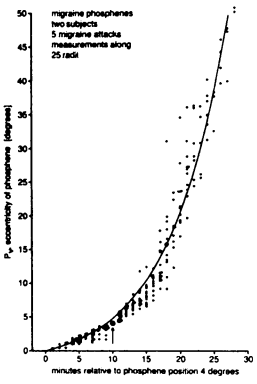
Intracortical microstimulation of the visual cortex in human subjects elicits the sensation of small points of light called phosphenes. Implantation of arrays of intracortical electrodes may provide a means of producing a visual prosthesis for patients with certain types of blindness.

A DEC PDP 11/23 computer has been programmed to provide control of photically isolated stimulators that are connected to an array of intracortical electrodes. Once threshold stimulation currents are established for the electrodes then the position in visual space must be established to provide meaningful information to the subject. The fact that phosphenes move with eye position complicates most methods of mapping the absolute position of phosphenes. Thus a pair-wise mapping procedure was implemented. Stimulation is applied first to a reference electrode and then to the electrode to be mapped. The subject inputs the direction of the vector from the reference electrode to the map electrode by positioning a joystick in one of 16 possible directions. A binary search, regulated by the directional input of the subject, through the phosphenes already mapped determines the neighbors of the phosphene to be mapped. The final position of the phosphene is determined through the use of a weighted average of two directional pairs. As each phosphene is mapped it is displayed on a video monitor so that the investigator can observe the developing map.

625.2

QUANTITATIVE STUDIES IN MIGRAINE PHOSPHENES. O.-J. Grüsser, U. Grüsser-Cornehls* (Spon: European Neuroscience Association) Dept. Physiology, Freie Univ. Berlin, Germany

Scintillating phosphenes are typical aura phenomena of "classical" migraine. We analysed the size ß of the "fortification" particles and the position P_V of the migraine phosphenes within the visual field as a function of time t past since the fortification pattern first appeared in the fovea. The distance P_V could be uniformly described, whereby normalization was attained for 4 degrees distance from the fovea center (fig.):



$$P_V = k\,(e^{bt} - 1)\ [deg] \quad (1)$$

Eq (1) is the solution of :

$$dP_V/dt = a + b\,P_V \quad (2)$$

describing the dynamics of spread of migraine phosphenes. Particle size ß of the pattern was a linear function of P_V:

$$ß = k^{*}\,P_V + c\ [deg] \quad (3)$$

(Supported by a Volkswagen Akademie-Stipendium)

625.3

TEMPORAL ORGANIZATION OF THE VISUAL SYSTEM IN THE AWAKE MACAQUE. C.E. Schroeder & S.J. Givre, Departments of Neuroscience & Neurology, Albert Einstein College of Medicine, Bronx, NY.

We investigated the organization of the primate visual system by comparing the timing of responses to standardized luminance and pattern (in fixating animals) stimuli across LGN, V1, V2, V3a, V4 and inferotemporal (IT) cortex. Timing was measured using laminar profiles of current source density and concomitant multiunit activity, recorded with multicontact electrodes in 9 awake, macaques. Luminance response latencies (range/mean/n of animals sampled) are:

a) LGN, 14-24ms/ 17.5ms/(n=5) b) V1, 20.5-30.5/ 27.6ms/(n=5)
c) V2, 26-32ms/ 28.3ms/(n=2) d) V4, 23-33ms/28.6ms/(n=3)
e) V3a, 42-65ms/ 53.6ms/(n=1) f) IT, 42-60ms/ 52.3ms/(n=3)

Latencies to pattern reversal are 3-16 ms longer than collocated luminance evoked latencies. In cortical areas, responses in superficial laminae lag the initial response by 4-10ms. The direct, luminance response has a minimum duration of about 35 ms in LGN, and 40 ms in V1. We conclude that: 1) The latency differences between LGN and V1, between V1 and V3a and between V4 and IT are consistent with sequential organization of processing; 2) The small latency differences between areas V1, V2 and V4 are inconsistent with purely sequential/hierarchical organization of processing in this portion of the system; 3) Despite the latency offset between early responses in LGN and V1, and later responses in IT, due to the long duration of activity at each point, processing throughout the system is broadly concurrent. Thus, feedback from extrastriate cortices can influence visual processing in V1 and even in LGN, during the time frame of the response to a single stimulus. (supported by MH06723 & by Training Grant TM32GM7288 from NIGMS)

625.4

TIMING AND LAMINAR DISTRIBUTION OF ACTIVITY EVOKED BY WHITE LIGHT VERSUS COLOR IN V1 AND V4 OF THE AWAKE MACAQUE. S.J. Givre[1], C.E. Schroeder[1], R.A. Bazarian*[,3] & J.C. Arezzo[1,2]. Depts. Neurosci.[1], Neurol.[2] & Ophthal.[3], Albert Einstein Coll. Med., Bronx, NY 10461.

Intracortical profiles of visual evoked potentials (VEP), concomitant multiunit activity (MUA) and current source density (CSD) were obtained from V1 (N=1) and V4 (N=2) of awake monkeys. 16-channel electrodes, capable of spanning the cortical laminae, were used for recording. Stimuli were full-field, stroboscopic flashes of white, red, blue and green light. In V1, white flash stimulation predominantly activates Lamina 4, as indexed by current sinks and increased MUA in this layer. Successive decrements in flash intensity reduce the amplitude of the VEP and associated current sinks, and increase the onset latency of the response. Colored stimuli increase the amplitude and peak (not onset) latency of the current sink in Lamina 4cß, but not 4cα, and in addition, produce a pattern of large current sinks of sequential onset in upper Lamina 4 and Lamina 3. Red produces the largest effect, approximately doubling the amplitude of the initial VEP component (N40-50).

In V4, the initial response to white light is a brief, small current sink. As in V1, decreasing the intensity of the flash reduces the amplitude and increases the onset latency of the response. Colored stimuli can prolong the initial sink without increasing its onset latency. In addition, they can prolong MUA or produce MUA bursts which were not resolvable during white light stimulation.

These data illustrate a surprising degree of differential processing of color versus white light full-field flashes both within the laminae of V1 and between V1 and V4. (Supported by MH06723 and T32GM7288-NIGMS).

625.5

SPATIAL DISCRIMINATION OF MULTIPLE NEUROMAGNETIC SOURCES EVOKED BY SEPARATE AND SIMULTANEOUS PRESENTATION OF VISUAL STIMULI. S. Supek, C.J. Aine, and P.A. Medvick*, Los Alamos National Laboratory, MS M715, Los Alamos, NM, 87545.

The objective of this study was to characterize the superposition of human neuromagnetic responses and the resolution of sources when responses evoked by single visual stimuli are compared with responses evoked by the simultaneous presentation of paired stimuli.

Two-dimensional difference of gaussians (DOG) stimuli were presented to three different field positions located 2.5° to the right of the vertical meridian and either 2.5°, 8.5° or 12° below the horizontal meridian. The size of the stimuli was adjusted according to the cortical magnification factor and stimuli were presented either separately or in simultaneous pairs. Neuromagnetic measurements (56-112 sensor locations) were recorded from four right-handed human subjects. Sources were localized by fitting instantaneous neuromagnetic field maps with a multiple-dipole, spherical model. The reduced chi-square statistic was used to determine model order and best-fitting dipole parameter values.

A preliminary analysis of the data suggests that only initial responses (<160 ms) reflect superposition. For separate presentations, two dominant sources were identified from the major waveform deflection at 150ms: (1) a source in the left extrastriate occipital region that varied in location and orientation as a function of stimulus position in the visual field; and (2) a source in left inferior occipito-temporal cortex that was invariant across stimulus position. For simultaneous presentations, a source in left occipito-temporal cortex was observed that was indistinguishable from that seen for separate presentations. In the left extrastriate region two sources predictable from the separate conditions were observed but some of their parameters differed from those in the separate conditions presumably due to their close spatial proximity.

625.6

A 8-12 Hz ("ALPHA") RHYTHM IN CAT VISUAL CORTEX
M. Chatila, C. Milleret, J.J. Bouyer, A. Rougeul, P. Buser. Institut des Neurosciences, Dépt de Neurophysiologie comparée, CNRS-UPMC, 9 quai St Bernard, F-75005 Paris.

In behaving cats, exploration of the visual and adjacent areas reveals several sets of electrocortical rhythms. One of those displays the following characteristics : 1) its frequency lies in the "alpha" 8-12 Hz band. 2) it is interrupted by *visual stimulation*. 3) it is *localized* in area 18, or at the limit between areas 17 and 18, in the projection zone of the central vertical meridian. No such rhythm could be found in the most posterior part of the visual cortex nor in the posterior parietal cortex. 5) it optimally develops while the animal is *waiting* for a significant target to appear; roughly similar conditions were previously shown to favour another set of rhythms, those in the somatic cortex ("mu rhythms"); coherence computation has however indicated that the two rhythms develop independently. 6) concerning its putative *thalamic command zone*, alpha recording electrodes were localized in the medial part of the lateral geniculate body, very close to nucleus lateralis posterior and pulvinar. We thus confirm that an alpha-like rhythm exists in carnivora, but with a restricted cortical localization, and that it is definitely independent of the anterior mu activity whose command zone is situated in n. ventralis posterior of the thalamus. Supported by DRET (Contract 89/069) and Fondation pour la Recherche Médicale.

625.7

INFLUENCE OF PUPIL DIAMETER ON PATTERN-ELICITED VISUAL EVOKED POTENTIALS IN PIGMENTED RATS. W.K. Boyes, D.W. Herr and H.K. Hudnell. US EPA, RTP, NC 27711.

Visual evoked potentials (VEPs) are a useful methodology for studying the neurotoxic and neuroactive properties of chemical compounds. Many compounds, in addition to having CNS actions, may alter pupil diameter and thus effect both the refraction and luminance of the stimulus image on the retina. It is important to differentiate peripheral from CNS changes when using VEPs to study the potential hazards of chemical compounds. Pigmented male rats (Long-Evans) with previously implanted electrodes overlying the left and right sides of visual cortex were given drops of vehicle (n=10), the mydriatic tropicamide (n=7), or the miotic neostigmine (n=4) into either one or both eyes. Stimuli were vertical sinusoidal gratings at 5 spatial frequencies (0.05, 0.1, 0.2, 0.4 or 0.8 cpd), 40% contrast, modulated in an on/off fashion with a 5 Hz temporal sinusoid. VEP waveforms were averaged over 100 trials, spectral analyzed, and spectral amplitudes at 5 (F1) and 10 (F2) Hz were measured. Preliminary data analysis suggested that neostigmine reduced F2 amplitude, and that tropicamide had little effect. A trend across drugs suggested that F1 amplitude was directly related to pupil diameter at certain spatial frequencies. Further studies should increase the available sample sizes and examine the influences of contrast and luminance.

625.8

BACKGROUND WHITE NOISE ALTERS FLASH EVOKED POTENTIALS (FEPs) IN RATS. D.W. Herr[1,2], D. King*[3], W.P. Watkinson[1], W.K. Boyes[1], and R.S. Dyer[1]. [1]US EPA, RTP, NC 27711 and [3]METI, RTP, NC, 27709.

We are examining how test procedures alter FEPs, and have found changes with daily testing, stimulus intensity, and within a test session (Dyer, R.S., Physiol. Behav., 45:355-362, 1989; Herr et. al., Physiol. Behav., 49:355-365, 1991; Herr et al., Soc. Neurosci. Abst., 16:570, 1990). We now report the impact of a non-visual stimulus on FEPs. Long-Evans rats were implanted with epidural electrodes over the visual cortex and subcutaneous ECG electrodes over the dorsal chest areas. Motor activity levels were recorded from the rectified and integrated signal on an exposed headset lead. Sequential 50 trial averages and test days were within-subject factors. On each of 9 days, 350 flashes/day (0.3 Hz) were presented. White noise (80 dB(A)) was presented either over trials 201-350 on day 9, over trials 1-350 on day 9, or over trials 1-350 on days 1-9. The presence of noise increased peak P_{21} and N_{70} amplitudes. When presented over trials 1-350, peak P_{63} and P_{90} amplitudes decreased. Peak N_{160} amplitude decreased initially, then increased, when noise was presented to naive subjects on day 9. Noise presentation over trials 201-350 decreased peak P_{63} latency. Motor activity was briefly increased and heart rate was not altered by noise. The data provide further evidence that non-visual variables play a role in modulating FEPs. Most of these effects appear unrelated to noise-induced effects on motor activity. **[2]DWH was supported by a NRC Research Associateship.**

625.9

CORRELATIONS of EEG and VEP SCOPOLAMINE EFFECTS in HUMANS. W.G.SANNITA.Center for neuroactive drugs, Institute of Neurophysiopathology, University; Center for cerebral neurophysiology, CNR, Genova,16132 Italy.

Acute 0.25-0.75 mg im scopolamine reduced the amplitude of flash-evoked cortical potentials (VEP) (P2-N2 waves) and quantitative background electroencephalogram (EEG) in healthy humans. These effects were parallel in the 0-120 min postdrug time on-going and both related to dose; VEP/EEG correlation was across doses, within-dose correlation was restricted to 0.75 mg administration. Pattern-VEP were unaffected. To remove the EEG effect on flash/pattern-VEP amplitude nonlinear regression vs the EEG total power was computed and VEP residuals from the regression function were re-tested.Flash-VEP residuals replicated the original data as to post-drug timing and relation to dose while pattern-VEP were unchanged by this data processing.The results suggest the indipendence of scopolamine induced flash-VEP changes from those on background EEG signal.The effects of stimulus physical characteristics are to be considered to generalize the results in human neuropharmacology.Quantitative EEG monitoring in VEP drug studies allows inference with respect to the CNS activation levels and assessment of CNS drug effects on VEP and EEG variables unrelated to each other is praticable whenever interferences are excluded.

625.10

BARBITURATE SENSITIVE COMPONENTS OF VISUAL ERPs IN A REPTILE J. C. Prechtl and T.H. Bullock. Dept. of Neurosciences & Neurobiology Unit, Scripps Institution of Oceanography, UCSD, La Jolla, CA 92093-0201.

Barbiturate sensitivity was used to compare different components of visual evoked (VEPs) and omitted stimulus potentials (OSPs) recorded with electrode arrays in the optic tectum (OT), dorsal cortex (DC) and dorsal ventricular ridge (DVR) of pond turtles (*Pseudemys scripta*).

For every 60 s of the baseline and treatment (Na^+ methohexital, 13 mg/kg i-p) periods, 2 s segments of EEG were sampled (pass band 0.1-75 Hz) as well as: the rested VEP to a single flash, the 5 Hz frequency following response (FFR), and the OSP that follows the first missing flash after 10 s conditioning with 10 Hz flashing. The minute-by-minute sampling continued until recovery of the OSP. The diffused flashes were delivered through closed eyelids and under a constant background illumination of ca. 2 lux. Epochs containing movement artifacts were rejected and consecutive 7 min averages were examined for short-lived effects. Longer averages based on 20 epochs were used for comparing baseline and treatment periods.

The OSP (OT, DVR and DC) is particularly sensitive to barbiturate and is reversibly abolished while the first wave of the VEPs in the DC or DVR (110-120 ms) were reduced by only 34 ± 9%. At the same time (OSP abolished) the earliest wave of the tectal VEP (55-65 ms) was increased by 79 ± 21% and its larger wave at 110-120 ms was unchanged or slightly reduced (-15 ± 6%). The abolition of the OSP correlates with the loss of late VEP components in both the forebrain (200-500 ms) and tectum (140-350 ms), and with a decline or loss of the 5 Hz FFR. Notable is the quicker loss and slower recovery of the tectal than the cerebral OSP.

The barbiturate sensitivity of the late VEP components is similar to that reported in the mammalian literature and the sensitivity of the OSP underscores its endogenous nature and its independence in the tectum and the cerebrum.

CHEMICAL SENSES: CENTRAL PATHWAYS III

626.1

INTRACEREBRAL LHRH RELIEVES MATING BEHAVIOR DEFICITS CAUSED BY VOMERONASAL LESIONS: LHRH ANALOGS MAY EXERT AN EXTRA-PITUITARY EFFECT ON MATING BEHAVIOR IN MALE HAMSTERS. G.Fernandez*, G. Howard* and M. Meredith. Program in Psychobiology & Neuroscience., Dept. Biol. Sci., Florida State Univ., Tallahassee, FL.

Vomeronasal organ (VNO) sensory input is followed by the release of LHRH, which facilitates mating behavior in rodents of both sexes. Previous results (Meredith et al, Chem Sens 15:619) suggest that exogenous intracerebral LHRH might "substitute" for VNO input in sexually inexperienced male hamsters with mating behavior deficits following VNO removal (VNX). In the present experiments we used prepubertal VNO removal (17 days) and explored other routes of peptide delivery. Behavioral restoration by intracerebral LHRH was confirmed in VNX animals. LHRH, saline or an LHRH analog (NAL- GLU or Ac-LHRH 5-10) was injected (2ul) intracerebrally into the lateral ventricles of VNX or intact (CON) animals. Mating behavior was tested 30 min after injection and investigation time, mounts, thrusts and intromissions were recorded. VNX animals, compared to controls significantly increased mating behavior ($p<0.05$) after LHRH (50ng). Subcutaneous (500ng) or intranasal (10ug) injection had no significant effect. NAL- GLU, a potent antagonist of pituitary LH release and Ac-LHRH 5-10, a fragment of LHRH that has no effect on pituitary LH release, both appeared to significantly facilitate mating behavior in both VNX and CON animals ($p<0.02$) suggesting that analogs effective in facilitating mating behavior may act via receptors other than the pituitary type.

Supported by Grants from NSF, BNS-8615159 & NIH, DC00906.

626.2

SPATIO-TEMPORAL CHARACTERIZATION OF FACILITATED OPTICAL RESPONSES DURING PAIRED ORTHODROMIC STIMULI IN THE SALAMANDER OLFACTORY BULB. A.R. Cinelli and J.S. Kauer, Neuroscience Program, Tufts/NEMC, Boston, MA 02111.

Response suppression and facilitation have been reported in orthodromic optical responses using paired stimuli. The presence of suppression or facilitation depends in the stimulus intensity as well as in the condition/test (c/t) intervals. In the present study, the spatio/temporal properties of the signals were studied by video imaging voltage-sensitive dye responses, after orthodromic electric stimulation of the olfactory nerve or of peripherial nerve fascicles. Low intensity stimulation which submaximally activates the olfactory bulb was necessary for the response facilitation. In addition, low temperature could enhance the size, duration and spatial distribution of these facilitated test responses. Depending on the intensity and c/t interval, different sectors in the same layer were selectively facilitated. Sectors which exhibited short latency responses were facilitated at short c/t intervals and sectors which exhibited longer latencies at longer c/t intervals. Stimulation of particular regions of the mucosa evoked facilitated responses in different sectors of the olfactory bulb, depending on the c/t interval. The selective enhancement of activity in a particular sector reduced the activity in other adyacent locations. These data suggest a widespread, non-homogeneous, pattern of activation of the olfactory bulb with a modular-like organization. Futhermore, the spatial distribution of activity in the bulb is influenced by spatial as well as temporal patterns of activity in the olfactory epithelium.

Supported by NIH, Pew Freedom Trust, and Dept. of Neurosurgery

626.3

ROLE OF STIMULATION FREQUENCY IN THE INDUCTION OF SELECTIVE LONG-TERM POTENTIATION IN THE PIRIFORM CORTEX. M. P. Galupo and J. S. Stripling. Department of Psychology, University of Arkansas, Fayetteville, AR 72701.

Repeated high-frequency stimulation of the granule cell layer of the olfactory bulb (OB) induces a long-term potentiation (LTP) in the piriform cortex (PC) that is selective to late components of the evoked potential (Stripling, Patneau, and Gramlich, Brain Res. 542: 107-122, 1991). In previous research we have typically induced selective LTP by stimulating the OB with trains of 10 pulses delivered at a frequency of 100 Hz. In the present experiment we examined the ability of 10-pulse trains varying in frequency from 5 to 400 Hz to induce selective LTP in the PC. Male Long-Evans rats with chronically implanted electrodes received one LTP treatment per day for 4 consecutive days. Each LTP treatment consisted of thirty 10-pulse trains applied to the granule cell layer of the OB at 5 sec intervals. Eight groups of animals received stimulation that differed only in the frequency of pulses within each train, which varied from 5 to 400 Hz. Animals receiving stimulation frequencies of 5 or 10 Hz failed to potentiate. In contrast, selective LTP was produced in animals receiving stimulation at frequencies of 25 to 400 Hz, with no significant differences within this frequency range. Examination of potentials evoked by the potentiating stimulation indicates that LTP is induced only by frequencies at which there is temporal overlap of the potentials evoked by each stimulus pulse. Previous research has demonstrated that afferent stimulation evokes an NMDA-mediated potential in hippocampal slices only at stimulation frequencies comparable to those that produced LTP in the present study (Collingridge, Herron, and Lester, J. Physiol. 399: 301-312, 1988). These observations are consistent with the involvement of NMDA receptors in selective LTP in the PC. Supported by NSF grant BNS 85-19700 and the Marie Wilson Howells Fund.

626.4

ALTERED RESPONSE TO PAIRED-PULSE STIMULATION FOLLOWING INDUCTION OF SELECTIVE LONG-TERM POTENTIATION IN THE PIRIFORM CORTEX. J. S. Stripling, D. K. Patneau, and M. P. Galupo. Department of Psychology, University of Arkansas, Fayetteville, AR 72701.

Stimulation of the olfactory bulb (OB) or lateral olfactory tract (LOT) evokes a potential in the piriform cortex (PC) whose initial component (A_1) represents monosynaptic activation of PC pyramidal cells by the LOT. In a naive animal, paired-pulse stimulation produces potentiation of A_1 due to Ca^{++} accumulation in presynaptic terminals. Repeated high-frequency stimulation of the granule cell layer of the OB induces a long-term potentiation (LTP) selective to late components of the evoked potential (Stripling, Patneau, and Gramlich, Brain Res. 542: 107-122, 1991). Although selective LTP leaves the expression of A_1 unaltered following single-pulse stimulation, paired-pulse stimulation causes a depression rather than a potentiation of A_1 (Stripling, Patneau, and Gramlich, Brain Res. 441: 281-291, 1988). This effect was investigated in male Long-Evans rats with chronically implanted electrodes by testing with paired-pulse stimulation before and after the induction of selective LTP. High frequency stimulation of the OB produced selective LTP and paired-pulse depression of A_1, while high-frequency stimulation of the LOT produced neither effect. Paired-pulse stimulation that caused depression of A_1 also depressed the pre-synaptic volley in the LOT. Even non-potentiated animals showed paired-pulse depression rather than potentiation of A_1 if very short inter-pulse intervals were used. Taken together, these results suggest that paired-pulse depression of A_1 following selective LTP is due to an enhancement of evoked inhibitory responses in the OB, resulting in activation of fewer mitral cells by the second pulse. This interpretation is consistent with previous evidence that selective LTP produces an enhancement of functional inhibition within the OB and PC. Supported by NSF grant BNS 85-19700 and the Marie Wilson Howells Fund.

626.5

CORTICOFUGAL INFLUENCE ON TASTE RESPONSES IN THE NUCLEUS OF THE SOLITARY TRACT OF THE RAT. S. Monroe and P.M. Di Lorenzo Dept. of Psychology,SUNY at Binghamton,Binghamton, NY 13902.

To investigate the influence of the forebrain on the neural code for gustation in the brainstem, the effects of reversible lesions of the gustatory neocortex (GN) on taste responses in the nucleus of the solitary tract (NTS) were studied in rats. Initially, taste-responsive NTS neurons were classified as "relay" or "non-relay" units based on the evoked response to electrical stimulation of the taste-responsive portion of the parabrachial pons. Electrophysiological responses to taste stimuli bathed over the tongue were then recorded before, after and following recovery from an infusion of procaine (10% in saline, 1 µl over 2 min) into the ipsilateral GN. Presentation of taste stimuli was then repeated in the same unit before, after and following recovery from a contralateral GN procaine infusion. Test stimuli consisted of sapid solutions of NaCl (.1 M), HCl (.01 M), quinine-HCl (.01 M), sucrose (.5 M) and Na-saccharin, (.004 M). Of 43 taste-responsive NTS units that were recorded in this paradigm, 12 units were identified as relay units and 13 were identified as non-relay units. Taste responses in 32 (74%) NTS units were different following procaine infusions into at least one side of the GN. These changes most often took the form of stimulus-specific enhancement or attenuation of the response to one or more taste stimuli within a given unit. The across unit pattern of response for NaCl in relay units, and for sucrose in non-relay units was altered following ipsilateral, but not contalateral, GN procaine infusions. These results point to the possibility that information about taste that enters the NTS may be affected by the GN differently according to where it will be relayed. This distinction may have important functional correlates.

This work was supported by a grant from the Whitehall Foundation to P.D.

626.6

TASTE NEURONS OF THE HAMSTER SOLITARY NUCLEUS: VARIATION IN IMPULSE RATE AND AMPLITUDE. T.P. Hettinger*, L.D. Savoy* and M.E. Frank. Dept. of BioStructure & Function, Univ. of CT Health Center, Farmington, CT 06030.

In extracellular recordings of taste-responsive neurons of the solitary nucleus (SN) of the golden hamster (Mesocricetus auratus), neurons differ in their stimulus profiles, latencies and interspike interval patterns. We have found that some SN neurons also show variation in impulse rate and amplitude. We recorded single-unit activity of SN neurons responding to anterior tongue stimulation, using micropipettes with 1-2 µm tips filled with 4% HRP and 0.5 M KCl in tris buffer, pH 7.6. Recording sites identified by HRP injections were located within the central and lateral subdivisions of the rostral SN, regions containing elongate, stellate and tufted cells and the preponderance of chorda tympani (CT) afferent endings. Neurons responding maximally to 0.1 M sucrose typically displayed bursting patterns of impulses, with as many as 20 impulses per burst. Bursting responses to sucrose have been found also in CT neurons. At high instantaneous rates (greater than 100 Hz), sucrose-responsive SN neurons often showed serial attenuation of amplitude within each burst, in some cases near to the point of extinction. Impulse attenuation has not been observed in CT fiber recordings. SN neurons responding maximally to 0.03 M NaCl or 0.1 M KCl did not generally show bursting or spike attenuation. The unique pattern of impulse rate and amplitude variation seen in sucrose-sensitive SN taste neurons probably reflects the incoming afferent signals and the integrative properties of the impulse generating sites, and may be important for the coding of sweet taste. NIH grant DC00853.

626.7

INTERSPIKE INTERVAL PATTERNS OF TASTE-RESPONSIVE NEURONS OF THE NUCLEUS OF THE SOLITARY TRACT: SIMULATIONS AND SPECIFIC AFFERENT INFLUENCES. S.C. Nuding and M.E. Frank. Center for Neurological Sciences and Dept. of BioStructure and Function, UCONN Health Center, Farmington, CT 06030.

Taste-responsive neurons in the nucleus of the solitary tract (SN) of the golden hamster (*Mesocricetus auratus*) generate two distinct interspike interval (ISI) patterns: simple unimodal ISI patterns and complex multimodal ISI patterns. We attempted to simulate simple and complex ISI patterns with computer-generated spike trains, using a random number generating function to place spikes in 1-ms bins. First approximations of simple ISI patterns were obtained with samples at one (eg. 2.5 hz) response rate, but complex ISI patterns required samples of at least two independent rates (eg. 2.5 hz, 50 hz). More exact simulation of neural responses may require consideration of refractory periods. We also attempted to restrict the afferent input to SN neurons with the sodium-channel blocker, amiloride. Amiloride can completely inhibit the NaCl responses of one class of afferents in hamsters (Hettinger and Frank, 1990). Response rates of some SN neurons to lingual application of 0.03 M NaCl are also much reduced by 0.01 mM amiloride and the two peaks in complex ISI patterns may be affected differently. As amiloride affects the perceptual quality of salts, ISI patterns could contribute to the code for taste quality. Supported by NIH grant DC00853.

626.8

EFFECTS OF DEPHASING OLFACTORY INPUT FROM RESPIRATORY CYCLE ON FIRING PROPERTIES OF OLFACTORY BULB NEURONS. E. Sobel and D.W. Tank. Biophysics Research Dept., AT&T Bell Laboratories, Murray Hill, NJ 07974

Single unit studies have demonstrated that mitral cells in the mammalian olfactory bulb can change the temporal structure of action potential firing in response to olfactory input without changing mean firing rate. Under natural breathing, the action potentials "bunch", becoming more frequent during the inspiratory phase and less frequent during the expiratory phase. There is uncertainty over the relative contributions to this phenomenon from, 1) the phasic nature of the olfactory stimulation and, 2) centrifugal control, synchronized to the respiratory rhythm.

To examine this issue, we have developed an experimental preparation that provides cyclical olfactory stimulation synchronized in frequency to the natural respiratory rhythm, but shifted in phase. We have examined the respiratory patterning of olfactory bulb unit activity while altering the relative phase of odor stimulation and the natural breathing cycle in anaesthetized rats.

Preliminary results suggest that the relative phase of on-going tracheal breathing does not affect the single unit activity evoked by phasic stimulation of the olfactory epithelium. Consequently, action potential "bunching" appears to be produced by the phasic nature of the stimulation and the inherent dynamics of the circuitry of the olfactory bulb.

626.9

CHANGES IN INFORMATION FLOW IN DISSOCIATED MOUSE OLFACTORY BULB CELL CULTURES J. Weil and S.P.Fracek Jr. Department of Biological Sciences and Center for Network Neuroscience, University of North Texas, Denton, TX 76203.

Bursting dominates the spontaneous electrophysiological activity of neural networks formed from cultured, dissociated mouse olfactory bulb cells. Measurements of various burst characteristics (e.g. duration, interval, period) are assumed to be a finite first order Markov chain (considering the relation of a burst X_n to the following burst X_{n+1}). A transition matrix is collected and the average stored information (ASI) is calculated using methods outlined by Shaw (The Dripping Faucet as a Model Chaotic System, Santa Cruz, CA: Aeriel Press, 1986). ASI is a map from a data set to a real number representing the complexity of the network activity. When used in conjunction with power spectra and phase space reconstructions, this measure helps to present a more complete quantification of the states occupied by the neural network. Spontaneous bursting activity is typically ergodic and thus creates information. This information can be stored for at least 5-10 measurements into the future. Some aspects of bursting activity can be viewed as the function of a dynamical system. GABAergic circuits may function as a control parameter, which can be modulated by bicuculline. As the concentration of bicuculline is increased, the ASI decreases, implying that the bursting activity is storing less and less information. This stored information is propagated less than three measurements into the future at high concentrations of bicuculline. These bicuculline effects are reversible. ASI compares favorably to the Lyapunov characteristic exponent in the case of the logistic equation. Generalization to higher dimensions (multiple burst characteristics) and consideration of higher order Markov processes are planned. Potentially ASI may be used to identify functional subpopulations of cells, which may correspond to the various cell types found in the bulb.

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626.10

LIMITS AND INFORMATION PROCESSING REQUIREMENTS IN OLFACTORY DISCRIMINATION. W. T. Nickell. Dept. of Anat. and Cell Biol., U. of Cincinnati Coll. of Med., Cincinnati, OH 45267.

In analogy with color vision and taste, it is a plausible hypothesis that the sensory quality of an odor is composed of a number of "primary odors." In the olfactory system the "primary odors" might be defined by the response properties of each of an unknown number of different classes of receptor neuron. If the response of each of the receptor classes to a particular odor can be described by a single numerical parameter, then the list of these numbers identifies the odor. It is therefore of interest to explore the limits and information processing requirements inherent in discrimination among odors specified in this way. We have approached this question by analysis of a simple model system. In this model an "odor" is simulated by a sequence of randomly generated numbers representing the responses of the receptor classes to the odor. A large number of these "odors" is then generated and stored as a table. The behavioral task of odor recognition is simulated by determining whether a matching procedure (linear correlation) can select the correct match between an arbitrarily selected test odor and the corresponding entry in the table. Numerical simulation and analysis demonstrate that, when the number of stored odors is plausibly large, reliable detection of a match requires many classes of receptor neurons. *Supported by NIDCD DC00347 and DAMD 86-C-6005.*

626.11

RAT PUPS CAN ACQUIRE NEUROBEHAVIORAL RESPONSES TO MORE THAN ONE ODOR. O. Carmi and M. Leon. Department of Psychobiology, University of California, Irvine, CA 92717.

Exposure of rat pups to an odor during postnatal days (PND) 1-18 with concurrent tactile stimulation results in an olfactory preference. This odor preference appears to correspond with physiological, anatomical and neurochemical changes in the olfactory bulb. Since previous studies of this phenomenon used only one odor, we considered the possibility that the neurobehavioral consequences of preference training was limited to a single olfactory cue. Therefore, we determined whether rat pups would develop neural and behavioral changes after training with two odors. During PND 1-18, all animals were exposed for 10 min/day to an odor while receiving perineal stimulation to peppermint odor only, orange odor only, or peppermint and orange odors on alternate days. On PND 19, the pups were tested for a behavioral preference between either: (1) orange and air, (2) peppermint and air, or (3) peppermint and orange. Neural activity in response to either odor was examined in other pups by assessing 14C-2-deoxyglucose (2-DG) uptake in the olfactory bulb.

Pups trained with only one odor had an olfactory preference only to that odor relative to either air or novel odor. However, pups trained with both odors developed preferences to both odors relative to air and showed an equal preference for the two odors when allowed to chose between them. The preferred odors also elicited changes in the uptake of 2-DG in the olfactory bulb glomerular foci. The data suggest that early experience with more than one odor can successfully induce behavioral preferences and neural changes in the olfactory bulb.

This work was supported by grant HD 24236 to M.L.

626.12

MODULATION OF OLFACTORY HABITUATION IN INFANT RATS BY A DEPRIVATION SENSITIVE NORADRENERGIC MECHANISM. C.M. Anderson, C.D. Kilts, G.L. Miller and W.G. Hall. Depts of Psychology, Pharmacology and Psychiatry, Duke University, Durham, NC 27706.

Deprivation of nutrients and maternal care affects activity and reactivity of infant rats (pups) and may decrease habituation to novel odors. Related to this deprivation effect is the finding of increases in noradrenaline (NA) turnover in the olfactory bulbs (OB's) of adult rats which is implicated in decreasing habituation to meaningful odors (Dluzen & Ramirez, 1989). To quantify deprivation-mediated influences on odor habituation, we compared the ability of 12-day old rat pups to habituate to 15 presentations of a novel odor after 8-hr nutrient deprivation (**FOSTER**), 8-hr deprivation of nutrients and maternal care (**TOTAL**), or no deprivation (**CONTROL**). In addition, NA turnover was estimated by assaying OB's of pups from the same conditions for [MHPG], the primary metabolite in rodent brain of NA. **TOTAL** pups exhibited more orienting in the first 3 blocks (3 trials/block) than **FOSTER** pups and more orienting than **CONTROL** pups in the 2nd and 3rd blocks. Overall, **TOTAL** pups oriented more than **FOSTER** pups in 4 out of 5 blocks and more than **CONTROL** pups in 3 out of 5 blocks. Matching the deprivation-induced changes seen in olfactory orienting were changes in [MHPG] in the OB's of individual pups from the same conditions; i.e., [MHPG] was only elevated in the OB's of **TOTAL** pups. **FOSTER** pups were not different from **CONTROL**s. In summary, the **TOTAL** pups were slower to habituate to a novel odor than **FOSTER** or **CONTROL** pups. MHPG is increased in **TOTAL** pups, but not in the other groups, suggesting that increased NA turnover may play a role in modulation of odor habituation at the level of the OB. *Supported by NICHD (HD17458).*

626.13

MICROSTRUCTURAL ANALYSIS OF INCENTIVE CONTRAST. P.S. Grigson, A.C. Spector[1], and R. Norgren. Dept. of Behavioral Science, College of Medicine, Pennsylvania State University, Hershey, PA 17033 and Dept. of Psychology[1], Univ. of Florida, Gainesville, FA 32611

Free-feeding and deprived rats were given 5 min access either to 0.1M sucrose for 14 days or to 1.0M sucrose for 10 days and then switched to 0.1M sucrose for 4 additional days. Subsequently, the deprivation conditions were reversed and the experiment was repeated. Free-feeding rats consumed less than deprived rats, but both groups licked more for 1.0M than for 0.1M sucrose. In addition, following reward downshift, both groups demonstrated an equivalent contrast effect, i.e. they licked significantly less 0.1M sucrose than did the unshifted controls. The microstructural analysis, however, revealed that the deprived and free-feeding rats achieved these similar shifts in overall lick frequency by altering different parameters of the behavior. For deprived subjects, both the concentration effect and the contrast effect were manifested by changes in the number of licks per burst, the burst duration, and the interburst interval. For free-feeding animals, both effects were accounted for solely by a change in interburst interval. The neural basis for these effects of reward shift are unknown, but a preliminary investigation suggests that the pontine parabrachial nucleus may play a role. Supported by PHS grants DC-00047, DC-00161, DC-00240, and MH-00653.

627.1

OPTICAL IMAGING OF CEREBELLAR AFFERENTS IN THE ISOLATED TURTLE CEREBELLUM. S.A. ELIAS, T.J. EBNER, C. NICHOLSON. DEPTS. NEUROSURGERY & PHYSIOLOGY, UNIV. MINNESOTA, MINNEAPOLIS, MN 55455 AND DEPT. PHYSIOLOGY & BIOPHYSICS, NYU MEDICAL CTR., NEW YORK, NY 10016.

The flat, non-foliated cerebellum of the turtle is superior to its highly convoluted mammalian counterpart for mapping afferent pathway distribution. Using optical recordings with a voltage-sensitive dye we observed the spatial patterns evoked by peduncular stimulation. Turtles were quickly decapitated and the entire cerebellum and peduncles removed. The tissue was stained with RH 795 for 30 minutes then placed, ventral side up, in a recording chamber below an epifluorescent microscope and imaged with a Photometrics CCD (14 bit A/D, 576X384 pixels). Stimulation of a peduncle with a tungsten microelectrode evoked a depolarizing optical signal that formed a narrow bundle, < 400 μm in width, which extended sagittally 4-5 mm from the electrode. Simultaneously recorded electrical field potentials were consistent with the image. The optical signal was recorded at various depths by changing the focus of the microscope. Signals were small (~ 0.02% change in fluorescence with a 2.5X, 0.08 n.a. objective) but reproducible and consistent. Supported by NIH Grants NS-27210 (TJE) and NS-13742 (CN).

627.2

DEBLURRING OF SERIALLY SECTIONED IMAGES OF THE OPTICALLY RECORDED PARALLEL FIBER BEAM IN THE RAT CEREBELLAR CORTEX *IN VIVO*. H. YAE*, S.A. ELIAS, T.J. EBNER. Depts. of Neurosurgery and Physiology, Univ. of Minnesota, Minneapolis, MN 55455.

Serially sectioned images of neuronal activity detected with voltage sensitive dyes contain significant amounts of out-of-focus information. We have used a deblurring algorithm to improve the spatial patterns of neuronal activity in the X-Y plane. Rats were anesthetized with ketamine/xylazine, the cerebellar cortex exposed, placed beneath an epifluorescent microscope (6.3 X 0.20 n.a.) and stained with the dye RH 795. A parallel fiber beam was evoked with a tungsten microelectrode. A series of images were taken with a Photometrics CCD camera, at 25μm increments of focus from the cortical surface to 350μm. Prior to deblurring, the depolarizing optical signal for the beam was observed throughout the entire depth series. However, depth profiles of evoked field potentials confined the Purkinje cell EPSP to a narrower range of depths, 50-75μm in extent. Application of the deblurring algorithm to this series of images restricted the appearance of the optical signal to the levels where the EPSP was recorded. Outside this range, the deblurred optical signals from the beam were absent. The net affect of the deblurring is to reduce the effective depth of field of the objective lens and reduce artifacts due to light scatter within the tissue. This method of restoration is a necessary prerequisite for three-dimensional analysis and rendering. Supported by NIH Grant #RO1-NS-27210.

627.3

DEVELOPMENT, PHARMACOLOGY, DISTRIBUTION, AND CELLULAR LOCALIZATION OF $GABA_B$ BINDING IN RAT CEREBELLUM. SM Turgeon, RL Albin, and S Gilman. Neuroscience Program and Department of Neurology, University of Michigan, Ann Arbor, Michigan 48109.

Receptor autoradiography using [3H] GABA under selective conditions was used to characterize the development, pharmacology, distribution, and cellular localization of $GABA_B$ binding sites in the rat cerebellum. Binding is greater in the molecular layer (ML) than in the granular cell layer at all ages and reached adult levels by postnatal day (PND) 14. A pattern of parasagittal zonation was observed in the ML of lobules IV-IX in adult rats. Transient expression of high levels of $GABA_B$ binding was observed in the deep cerebellar nuclei, peaking at PND 7 and decreasing to adult levels by PND 28. Drugs active at the $GABA_B$ binding site displaced [3H]GABA with the following order of potency: 3-APA >S-AVA = saclofen = 2-hydroxysaclofen > phaclofen. GTP-γ-S and GDP-β-S inhibited [3H]GABA binding in a dose dependent manner. The cellular localization of $GABA_B$ binding was investigated using lesion techniques. Neither MAM nor 3-AP lesions resulted in a decrease in [3H]GABA binding. Homozygote stumbler mutants, deficient in Purkinje cell dendrites, had a significant decrease in [3H]GABA binding in the ML. These results suggest that the majority of $GABA_B$ binding sites in the ML are located on Purkinje cell dendrites. Supported by NS 19613, NS 01300, MH 14279.

627.4

EXCITATORY AND INHIBITORY AMINO ACID BINDING SITES IN HUMAN DENTATE NUCLEUS. R.L. Albin, R.H. Price*, S.Y. Sakurai, J.B. Penney, and A.B. Young. Neuroscience Program and Department of Neurology, University of Michigan, Ann Arbor, MI 48104-1687.

We used receptor autoradiography to study the distribution of excitatory and inhibitory amino acid binding sites in human dentate nuclei. There was no binding to N-methyl-D-aspartate (NMDA) or $GABA_B$ binding sites, and there was a low level of kainate binding sites. α-Amino-3-hydroxy-5-methylisoxazole-4-propionic acid, metabotropic-quisqualate, benzodiazepine, and $GABA_A$ binding sites were present in moderate abundance. Our NMDA results are different than those found previously in rodents. $GABA_A$ receptors are probably the primary mediators of inhibitory neurotransmission within the deep cerebellar nuclei while α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid and metabotropic-quisqualate receptors are probably the primary mediators of excitatory neurotransmission within the human deep cerebellar nuclei. *Supported by USPHS grants NS01300, NS15655, NS19613, and AG 08671.*

627.5

CHANGES IN GAD IMMUNOREACTIVITY IN THE DEEP CEREBELLAR NUCLEI OF GENETICALLY DYSTONIC (*dt*) RATS AND RATS WITH 3-ACETYLPYRIDINE LESIONS. J. Lutes*, J.F. Lorden*, and B. Davis**. Dept. Psych.* and Dept. Cell Biol.**, UAB, Birmingham, AL 35294.

Both *dt* rats and rats with 3-acetylpyridine (3AP) lesions of the inferior olive display an increase in deep cerebellar nuclei (DCN) glutamate decarboxylase (GAD) activity. GAD, the synthetic enzyme for GABA, is located primarily in Purkinje cell terminals. In 3AP rats, increased DCN GAD activity coincides with increased Purkinje cell activity, following destruction of the climbing fibers. The *dt* rats, however, show lower than normal Purkinje cell activity. Immunocytochemistry was used to determine whether *dt* and 3AP rats showed different patterns of GAD distribution in the DCN. The size and density of GAD+ puncta were examined in three groups of 20-day-old rats: *dt*, normal saline-treated, and normal rats given 3AP (65 mg/kg) at 18 days of age. Separate groups of 3AP and saline controls were also examined at 14 days postinjection. An increase in the size of DCN GAD+ puncta in comparison with normal rats was seen only in the 3AP group at 2 days postinjection when GABA levels, as measured by HPLC, were also increased in the 3AP group only. Increases in puncta size were no longer present at 14 days postlesion, when GAD activity is still elevated but Purkinje cell activity has declined. Puncta density was reduced in the *dt* rats only. Other studies show that GAD activity remains elevated in 3AP rats after Purkinje cell activity declines. The *dt* rats resemble 3AP rats following extended survival. In both cases, increased GAD activity may represent a persistent change initiated by elevated Purkinje cell activity and may be related to a shift in the form and function of GAD that predominates in Purkinje cells at different times. (Supported by a grant from the Dystonia Med. Res. Fdn. and grant BNS 90-10187 from NSF.)

627.6

RELATIVELY SMALL NUMBERS OF PONTOCEREBELLAR AXONS PROVIDE COLLATERALS TO THE CEREBELLAR NUCLEI. G. A. Mihailoff, Dept. of Anatomy, University of Mississippi Medical Center, Jackson, MS 39216.

An issue that continues to be relevant to motor control studies is the question of whether information being transferred from sensorimotor cerebral cortex to the cerebellar cortex via the basilar pontine nuclei (BPN) is also distributed to the cerebellar nuclei (CN) through pontocerebellar axon collaterals. There is little data in the literature to support such a connection, yet schematic diagrams depicting cerebrocerebellar communication often include such circuitry. As an extension of ongoing research in our laboratory focused on the structure and function of the BPN, we have undertaken a study of this question using the orthograde axonal transport of the lectin *Phaseolus vulgaris* leucoagglutinin (PHAL). Long-Evans rats received electrophoretic or pressure injections of 2.5% PHAL into the BPN or nucleus reticularis tegmenti pontis (NRTP). After a survival period ranging from 5-19 days, rats were sacrificed and sections of the cerebellum reacted for the presence of PHAL using a biotin-avidin immunocytochemical procedure. Our observations indicate that only a small number of pontocerebellar axons give rise to collaterals that terminate in the CN, and of those that do so, the majority arise from NRTP and not the BPN. Accumulations of labeled fibers and terminals are found in the ventral and lateral portions of the lateral cerebellar nucleus, the posterior interpositus nucleus, and portions of the medial cerebellar nucleus. These findings suggest that sensorimotor cortical information does not have a relatively direct route to the CN but rather is transmitted to the CN after passage through the cerebellar cortex. Although other precerebellar nuclei such as the lateral reticular nucleus receive sensorimotor cortical projections, such projections are far less substantial than that received by the BPN and it is unlikely that these nuclei represent a major link between the cerebral cortex and CN. *Supported by USPHS grant NS12644.*

627.7

CORTICONUCLEAR AND -VESTIBULAR PROJECTION ZONES CORRESPOND TO ZEBRIN-POSITIVE AND -NEGATIVE ZONES IN ANTERIOR VERMIS OF RAT CEREBELLUM. J.Voogd (1),L.M.Eisenman (2), T.J.H. Ruigrok (1)*. Depts. of Anatomy Erasmus University Rotterdam 3000 DR The Netherlands (1) and Jefferson Med. Coll. Jefferson University Philadelphia PA 19107 (2).

Purkinje (P) cells with projections to vestibular nuclei are located in lateral A (A2) and B zones of anterior vermis (Voogd et al.,Arch.Ital.Biol.129:3'91). A2 borders on B in lobules I-III, but in IV-VI they are separated by the X zone which projects to the junction of the fastigial (F) and posterior interposed (IP) nuclei (Voogd, Exp.Brain Res.Ser 17:3'89). This zonal arrangement was compared to the compartmentalization for zebrin (Hawkes and Leclerc,J.Comp.Neurol.256:29'86) in the cerebellar cortex of the rat. Pcells were retrogradely labeled with WGA-HRP from injections in Deiters' and the F and IP nuclei. Cobalt-stabilized HRP reaction product and immunoreactivity for zebrin I were visualized in the same sections. Vestibular Pcells in the anterior vermis are exclusively zebrin-negative. HRP-labelled cells of the B zone correspond to the zebrin-negative N2 zone of Hawkes and Leclerc, A2 cells occupy the lateral part of N1. A2 is separated from the rest of N1 by a strip zebrin-positive Pcells in IV-VI. Pcells of the zebrin-positive P2 zone correspond to the X zone and can be labelled from F/IP. In lobule VIII the labelling is shifted laterally with respect to Hawkes and Leclerc's nomenclature with vestibular Pcells in N2 and N3 and F/IPcells in P3.

627.8

BRANCHING IN THE ROSTROCAUDAL AXIS WITHIN THE PROJECTION FROM THE INFERIOR OLIVE TO THE C_1 ZONE OF THE CAT CEREBELLUM. R. Apps* and J. R. Trott*. (SPON: Brain Research Association) Dept. of Physiology, School of Medical Sciences, University Walk, University of Bristol, Bristol, UK, BS8 1TD.

We have used a combined physiological and retrograde fluorescent technique to investigate the extent to which olive cells branch in the rostrocaudal axis to innervate the c_1 zone in the anterior (lobule V) and posterior (paramedian lobule, PML) lobes of the cat paravermal cerebellum.

Under full pentobarbitone anaesthesia (n=4 cats) the c_1 zone was identified physiologically at the two rostrocaudal levels. At each site injections of either rhodamine- or coumarin- tagged latex microspheres (Lumafluor) were made (total volume at each site 250-600 nl). After 4-5 days survival the cerebellum and brainstem were removed, cut at 50 and 30 µm respectively, and the distribution of retrogradely labelled cells within the contralateral inferior olive was plotted. Cells double-labelled (DL) ie. that have axons which branch to terminate within the c_1 zone in both lobule V and PML, were consistently located in a caudomedial to rostrolateral column within the Dorsal Accessory Olive (AP levels 12.00 to 10.25).

On average, DL cells represented 14% and 24% respectively of the total number of cells labelled from lobule V or PML injections. This contrasts to a previous study of branching in the mediolateral plane between the x and lateral c_1 zones (Apps, Trott and Dietrichs 1991, Exp. Brain Res., in press), in which olive cells that innervated both zones represented only 5-7% of either single-labelled population.

These results suggest that branching in the olivocerebellar projection is, on occasion, more extensive in the rostrocaudal than in the mediolateral axis.

Supported by the Wellcome Trust.

627.9

THREE-DIMENSIONAL VISUALIZATION OF THE INTERNAL MEMBRANE SYSTEM OF AVIAN PURKINJE CELL DENDRITES M. E. Martone, Y. Zhang*, V. M. Simpliciano*, B. O. Carragher* and M. H. Ellisman. San Diego Microscopy and Imaging Resource, Department of Neurosciences, University of California, San Diego, La Jolla, Calif. 92093-0608.

In a previous study from this laboratory, two proteins involved in intracellular calcium regulation, the IP3 receptor and the ryanodine receptor, were found to have distinct but partially overlapping distributions within cerebellar Purkinje cells. While the IP3 receptor was localized to the smooth endoplasmic reticulum (SER) in the cell body, dendritic shaft and dendritic spines, the ryanodine receptor was restricted to the SER in the cell body and dendritic shaft. Here, we employ 3-D computer reconstruction and high voltage electron microscopic (HVEM) techniques to determine whether the differential distribution of these proteins is due to the presence of physically separate SER networks within the dendrites of Purkinje cells.

Three-dimensional reconstructions were obtained from serial sections of chick cerebellum. Contours representing the SER and plasma membrane were traced and digitized for each individual section following which a tiling algorithm was used to fit a surface to these structures. The reconstructed region could then be inspected using a viewing program which allows for interactive modification of transformation parameters and image attributes. A second approach involves a volumetric analysis of selectively stained membrane systems viewed in thick sections with HVEM. A study is now underway to obtain volumetric reconstructions from both serial sections and tomographic reconstructions of single axis tilt series.

Preliminary results show that the SER forms a complex network of cisterns and tubules throughout the Purkinje cell dendrite. As noted previously (Harris et al., J. Neurosci., 8:4455, 1988), the SER of the spine shaft was found to be in continuity with the SER of the main dendrite. In most cases, the SER of the spine appeared to emanate from the hypolemmal cisternal system which, in turn, was continuous with the SER in the interior of the dendrite. These observations suggest that the IP3 receptor and the ryanodine receptor are present on a continuous SER network.

627.10

CEREBELLAR PROJECTIONS TO SPINAL MOTONEURONS. J.J. Smith*, A.R. Gibson, K.M. Horn and P.L.E. van Kan. Barrow Neurological Institute, Phoenix, AZ 85013

Intermediate cerebellum projects to the spinal cord via connections to magnocellular red nucleus. Rubrospinal terminals lie largely in lamina VII, but some lie among C8-T1 motoneurons serving distal limb musculature. Do other motoneuronal pools have specific relations with cerebellar output?

We first investigated spinal projections of the lateral vestibular nucleus, which receives afferents from the B zone of cerebellar cortex and medial nucleus. WGA-HRP injections in lvn produced heavy label among motoneurons supplying the neck and intraspinal muscles. Some lateral pools such as those innervating trapezius were conspicuously devoid of label. Therefore, the B zone and parts of the medial nucleus selectively target neck and intraspinal motoneurons via lvn.

Determining cerebellar relations with motoneurons serving proximal limb musculature has been more difficult. A large injection into the medullary reticular formation that produced heavy retrograde label throughout the cerebellar medial nucleus did not label terminals among proximal limb motoneuronal pools. A small injection placed in the biceps motoneuron pool at C6 failed to identify any brainstem area as a major source of input. However, the reticular injection resulted in heavy anterograde label in C3-C4 propriospinal neurons, which were also retrogradely labeled by the C6 injection. We are now investigating the possibility that medial nucleus targets proximal limb musculature via connections through brainstem reticular and C3-C4 propriospinal neurons.

627.11

SPATIAL ORGANIZATION OF SPINOCEREBELLAR INPUT TO THE UNFOLDED CEREBELLAR ANTERIOR LOBE. D.L. Tolbert, K. Yates-Siilata and B.R. Clark. Departments of Anatomy and Neurobiology and Surgery (Neurosurgery), St. Louis Univ. Sch. of Med. and Program in Physical Therapy, Washington Univ. Sch. of Med., St. Louis, MO

Lower thoracic-upper lumbar (LTUL) projections to the cerebellar anterior lobe were studied in adult rats using WGA-HRP orthograde labeling techniques and image analysis software (Bioquant) that graphically unfolds the cortex while maintaining spatial relationships between identified spinocerebellar (SpCb) mossy fiber terminals and the overlying surface. Small injections restricted to approximately one LTUL segment labeled SpCb terminals located mainly near the junctions of lobules I-II, II-III, and III-IV. Computer reconstruction showed that these terminals formed medial-lateral oriented bands that extended from midline though intermediate regions but not into lateral areas of these lobules. Fewer terminals were distributed in other areas of these lobules and in lobule V. WGA-HRP injections into two adjoining LTUL segments labeled greater numbers of terminals in the medial-lateral oriented bands at the junction of lobules I through IV and more terminals in superficial parts of the lobules. Computer reconstructions suggest two levels of organization of adjoining segmental input to the anterior lobe. First, in each lobule SpCb terminals form two to three medial-lateral oriented bands separated by well circumscribed similarly oriented terminal free areas. Along the medial-lateral bands the number of terminals was not uniform, rather alternating high and lower densities of terminals existed. These high and lower terminal densities were respectively aligned anterior-posteriorly forming a secondary pattern of organization of sagittally oriented stripes. Supported by NIH grant NS20227.

627.12

PRELIMINARY CHARACTERIZATION OF A MODEL OF HEREDITARY PURKINJE CELL-INFERIOR OLIVARY DEGENERATION IN RATS. K. Yates-Siilata, M.G. La Regina, L. Woods, T. Pittman, and D.L. Tolbert. Depts. Anatomy and Neurobiology, Comparative Medicine, and Surgery (Neurosurgery). St. Louis Univ. Sch. of Med., St. Louis, MO

The cerebellum and brainstem of adolescent and young adult Sprague-Dawley rats from a colony which spontaneously displays abnormal motor signs characterized by ataxia, wide-based hindlimb posture, and dorsal arching of the tail (shaker rats) were studied by light microscopy. The histological organization of the cerebellar anterior lobe cortex was dramatically altered in shaker rats, due to the complete absence of Purkinje cells (PCs) and shrinkage of the molecular layer. These changes were not as dramatic in the posterior lobe. Along the PC monolayer some cells have degenerated but immediately adjacent PCs persisted. The overall number of surviving PCs increased from anterior (VI) to posterior (IX). The cerebella from shaker rats were analyzed morphometrically and the findings compared with data from similar measurements in age and weight matched controls. The total area of the midline cerebellum from shaker rats was 39% smaller than in controls. When analyzed separately the shaker anterior lobes were 50% smaller than controls. The largest percentage decrease in area occurred in the molecular layer and the white matter.

Brainstem pontine, lateral reticular and inferior olivary precerebellar nuclei were examined in the shaker rats. The only apparent neurohistological alterations were localized to the inferior olivary nuclei where almost all neurons had degenerated. This animal model of hereditary PC-inferior olivary degeneration warrants futher study because of similarities with human cerebellar disease states. Supported by NIH grant NS20227.

627.13

CALCITONIN GENE-RELATED PEPTIDE IN THE CAT'S CEREBELLUM. GEORGIA A. BISHOP Dept. of Cell Biology, Neurobiology and Anatomy and Neuroscience Program, The Ohio State University, Columbus, OH 43210

In the present study the distribution, origin and physiological effects of calcitonin gene related peptide (CGRP) were analyzed in the cat's cerebellum. The PAP technique revealed that CGRP-immunoreactivity (IR) is present in profiles that have morphological characteristics of mossy fibers. These CGRP-IR mossy fibers have a heterogeneous distribution. In the vermis, the densest distribution is in lobules VII, VIII and the dorsal folia of IX. In anterior vermal lobules only scattered fibers, located primarily at the apex of the folia, are present. Laterally, CGRP-IR mossy fibers are located in the paramedian lobule, paraflocculus and crus II. No CGRP-IR mossy fibers are present in lobule X or the flocculus. Our distribution data are in partial agreement with the findings of Sugimoto et al (Brain Res. 439:147, 1988) who described CGRP-IR mossy fibers and granule cells. Whereas CGRP positive mossy fibers were identified in the present study, no CGRP-IR granule cells or parallel fibers were evident. A double label technique revealed that CGRP-IR mossy fibers arise from neurons located in the lateral reticular nucleus, external cuneate nucleus, inferior vestibular nucleus and basilar pons. Physiologically, CGRP has a weak suppressive effect on spontaneous activity in most Purkinje cells. In addition, this peptide decreases the level of the cell's excitatory response to simultaneous application of glutamate or aspartate. Occasionally, CGRP increased neuronal excitability. Taken together, these data indicate that there is a chemically and physiologically distinct population of mossy fibers that arises from specific precerebellar nuclei. Functionally, CGRP likely modulates Purkinje cell activity in restricted regions of the cerebellar cortex by altering the responsiveness of these cells to the excitatory amino acids glutamate and aspartate. (Supported by NS18028).

627.14

5-HT IN THE CAT'S CEREBELLAR CORTEX: ULTRASTRUCTURAL ANALYSIS AND IDENTIFICATION OF RECEPTORS. C.W. Kerr, Y.F. Chen, J.S. King and G.A. Bishop Dept. of Cell Biology, Neurobiology and Anatomy and Neuroscience Program, The Ohio State University, Columbus, OH 43210

Serotoninergic neurons in the caudal reticular formation project to the cat's cerebellar cortex and terminate as a beaded fiber plexus in the granule and Purkinje cell layers (JCN, 304:502, 1991). Ultrastructural analysis revealed serotonin-immunoreactive (5-HT-IR) profiles approximating the dendrites and somata of granule cells as well as the somata of a few Purkinje cells. In addition, 5-HT-IR fibers contributed to the formation of the mossy fiber glomerulus. To date however, no conclusive synaptic specializations have been observed. Functionally, 5-HT potently reduces the firing rate of spontaneously active Purkinje cells as well as activity induced by the application of glutamate or aspartate, the putative neurotransmitters of mossy and climbing fibers, respectively. 5-HT also potentiates the inhibitory effects of GABA. In addition to decreasing Purkinje cell firing rate, 5-HT increases the interval between firing bursts in approximately 45% of the tested cells. Iontophoretic application of the 5-HT_{1A} agonist, 8-OH-DPAT, mimicks the suppressive action of 5-HT in a dose-dependent manner. This response, as well as the 5-HT mediated suppression are blocked by application of spiperone a 5-HT_{1A} antagonist. Compounds selective for the 5-$HT_{1C,2}$ and $_3$ receptors are physiologically ineffective. Preliminary experiments have demonstrated that the 5-HT_{1B} selective agent, TFMPP, may act as a partial agonist when compared with the actions of 5-HT. The present data are in agreement with previous studies in the rat cerebellar cortex which reported that selective activation of the 5-HT_{1A} receptor replicates the 5-HT-mediated suppression of Purkinje cell activity (Neurosci. Abst., 15:406, 1990). However, our study did not find evidence for the functional presence of the 5-HT_2 receptor, a putative heteroreceptor on glutaminergic neurons in the rodent cerebellar cortex (Br.J.Pharm., 82:271, 1984). In summary, 5-HT may mediate its suppressive effects on Purkinje cell firing through non-junctional transmission and subsequent activation of 5-HT_{1A} receptors in the cat's cerebellar cortex. (Supported by NS 18028).

627.15

DO TRANSIENT CLIMBING FIBERS PROVIDE A TEMPLATE FOR THE SAGITTAL ORGANIZATION OF MOSSY FIBERS? J.S. King and G.A. Bishop Dept. of Cell Biology, Neurobiology and Anatomy and Neuroscience Program, The Ohio State University, Columbus, OH 43210.

The PAP method was used to detect cholecystokinin (CCK) in the developing cerebellum of the opossum. In the adult, CCK immunoreactivity is present almost exclusively in mossy fibers (King & Bishop,'90,JCN 298:373). During development, CCK-positive fibers are present in the cerebellar peduncle by postnatal day (PD) 1, where they wait and then penetrate the cerebellar anlage on PD 7. The early arriving fibers remain in the medullary core of the cerebellum until PD 26. At that time, in the anterior lobe vermis, some CCK-positive puncta organize in four sagittal bands which overlie populations of Purkinje (PK) cells; at this stage of development PK cells are in a layer 4-5 cells deep. Most of these immunoreactive elements are transient as they do not persist beyond PD 68. A population of inferior olivary neurons also transiently expresses CCK during this time interval. Prior to the disappearance of these transient elements and subsequent to granule cell migration sagittal bands of CCK puncta develop in the internal granule cell layer immediately below the transient sagittal bands in the PK cell layer. In contrast, in other areas of the vermis and hemispheres CCK positive fibers do not penetrate the PK cell layer. Rather, they remain in the medullary core of the cerebellum until the internal granule cell layer (IGL) can be differentiated histologically (PD 33); coincident with this developmental event CCK axons grow into the IGL. Arsenio-Nunes et al. ('88,JCN 273:120)) have suggested a primary role for the Purkinje cell in organizing spinocerebellar afferents, as spinal axons (mossy fibers) are arrayed in sagittal bands in the vermis even in the absence of their target cell (the granule cell). Our findings provide additional morphological evidence to support an organizational role for populations of Purkinje cells in the anterior lobe and their associated climbing fibers in the subsequent development of sagittal bands of mossy fibers; they are likely derived from the external cuneate or lateral reticular nuclei (King and Bishop, 90). However, in other areas of the vermis and hemispheres additional cues important for target recognition and afferent organization must be present. (Supported by NS 08798).

627.16

ACTIVITY-DEPENDENT EXPRESSION OF CRF IN THE OLIVO-CEREBELLAR AXIS. S.L. Cummings, G.P. Chrousos, and W. S. Young, III. U. C. Davis, CA, DEB/NICHD and LCB/NIMH, Bethesda, MD

Corticotropin releasing factor (CRF) is widely distributed within brainstem-cerebellar climbing and mossy fiber afferents, and has been demonstrated to modulate the physiological effects of aspartate, glutamate, and GABA in the cat cerebellum. CRF potentiates the excitatory effects of aspartate and glutamate on Purkinje cell firing, and does so in a dose-dependent manner. In addition, CRF overcomes the supressive effects of GABA on aspartate and glutamate induced-activity in the cerebellar cortex (Cummings, Bishop and King, Anat. Rec. (1990) 226:294A; Bishop, Neurosci. (1990) 39:251). Results of studies utilizing in situ hybridization histochemistry and radioimmunoassay now indicate that CRF expression within the olivocerebellar axis is altered by electrophysiologically- and pharmacologically- induced activity, in paradigms known to synchronously activate the GABA-containing Purkinje cells and deep cerebellar nuclei. CRF mRNA and CRF are increased in the inferior olivary complex (IOC), and CRF is depleted from the cerebellar cortex of the cat in response to 6 hr of electrophysiological stimulation of the IOC (0.1mA, 0.1ms at 1 Hz), stimulation of the dorsal column afferent projection to the IOC with equivalent parameters, or 6 hr after administration of the tremor-inducing ß-carboline harmaline (10mg/kg, i.p.). Following harmaline treatment, increased CRF hybridization signal is evident in the dorsal and medial accessory olives and in areas of the IOC thought previously to be unresponsive to harmaline. No difference existed between CRF levels in the vermis as compared to the hemispheres in normal or experimental animals. Results of these studies support suggestions that CRF-coded circuits function in motor responses generated via the olivocerebellar axis.

627.17

CHOLINERGIC AND CRF-CONTAINING NEURONS IN VESTIBULAR NUCLEI AND RELATED CELL GROUPS PROVIDE CEREBELLAR MOSSY FIBER PROJECTIONS.

M. Ikeda, T. Houtani*, T. Ueyama and T. Sugimoto.
Dept. of Anatomy, Kansai Medical Univ., Osaka 570, Japan.

By avidin-biotin peroxidase immunohistochemistry, choline acetyltransferase (ChAT) and corticotropin-releasing factor (CRF) were examined in cat vestibular nuclei and related cell groups. Surgery was conducted on adult cats under general anesthesia with intraperitoneal pentobarbital (40 mg/kg). Alternate sections from colchicine-treated cats stained for ChAT or CRF revealed a similar but different localization of ChAT neurons and CRF neurons in the medial and descending vestibular nuclei, prepositus hypoglossi, intercalatus and solitary nuclei, nucleus of Roller and cell groups f, x and z. ChAT neurons predominated in the caudal half of the medial and descending vestibular nuclei while CRF neurons concentrated in their rostral half; ChAT and CRF did not show colocalization in these neurons. In the cerebellum ChAT- and CRF-positive mossy fibers and rosettes were observed in many cortical areas which receive vestibular projections. Following unilateral destruction of vestibular nuclei, these mossy fiber elements were largely decreased ipsilaterally while CRF-positive climbing fibers remained unchanged. Following WGA-HRP injections in the vermal lobules I, II, IX and X, most of the ChAT and CRF neurons in the vestibular area were retrogradely labeled by the tracer. The results suggest that the vestibular area contains two independent populations of vestibulocerebellar projection neurons chemically coded by ChAT and CRF.

628.1

GLUTAMIC ACID DECARBOXYLASE (GAD) ACTIVITY AND mRNA LEVELS IN CEREBELLAR PURKINJE CELLS AFTER CHANGES IN CELL FIRING. J. Litwak*, Z. Yin*, M. Beales* and G. A. Oltmans. Dept. of Pharm. and Mol. Biol., Chicago Med. Sch., N. Chicago, IL 60064

The neurotoxin 3-acetylpyridine (3-AP) destroys the climbing fiber input to the cerebellar cortex, abolishing Purkinje cell (PC) complex spike (CS) activity and increasing simple spike (SS) activity (+100%). The SS activity returns to pre-lesion levels over a 30 day period, while GAD activity in PC terminals gradually increases, remaining elevated up to 45 days post-lesion. GAD is the synthetic enzyme for GABA, the PC neurotransmitter, and the increase in GAD activity presumably reflects increased GABA utilization as a consequence of increased SS activity. In contrast, the drug harmaline activates climbing fibers, increasing the low frequency CS activity (+10-fold) and strongly inhibiting SS activity. This produces a decline in overall PC activity, and should reduce GABA demand and, subsequently, GAD activity. In the current study, the effects of prolonged climbing fiber stimulation on PC GAD activity were measured. In addition, in situ hybridization was used to establish the time course for GAD mRNA induction after lesion-induced increases in PC firing.

Rats were administered a single injection of 3-AP (70 mg/kg) or six injections of harmaline (20 mg/kg/4h) or saline. Animals administered 3-AP were euthanized 1, 3, 7 and 30 days post-lesion, and those given harmaline after 24 h. GAD mRNA and GAD activity were measured in PC bodies, or terminal areas in the deep cerebellar nuclei (DCN), respectively. In climbing fiber-lesioned animals GAD mRNA levels in PC bodies were significantly increased at 1 (+38%), 3 (+42%), 7 (+42%), and 30 (+22%) days post-lesion. In harmaline-treated subjects GAD activity was significantly decreased in the medial (-29%), and interpositus (-20%) subdivisions of the DCN. These results suggest that decreases in overall cell firing (harmaline treatment) reduce GAD activity in PC terminals, while increases in firing (3-AP treatment) increase both GAD mRNA levels and GAD activity in these cells. The effects of harmaline on PC GAD mRNA levels are currently being studied. (Supported in part by the Dystonia Medical Research Foundation)

628.2

QUIPAZINE HAS DIFFERENTIAL ELECTROPHYSIOLOGICAL EFFECTS IN THE DEEP CEREBELLAR NUCLEI OF DEVELOPING NORMAL AND GENETICALLY DYSTONIC RATS. V.L. Michela and J.F. Lorden. Dept. Psych., Univ. of Alabama at Birmingham, Birmingham, AL 35294.

Systemic administration of the serotonin (5-HT) agonist quipazine produces an 18 Hz forepaw tremor in newborn rats. The effect declines to negligible levels by postnatal day 16 in normal rats but persists indefinitely in the genetically dystonic rat (dt), a mutant that shows a movement disorder appearing around postnatal days 9-10. Since quipazine acts on the olivo-cerebellar pathway, extracellular recordings were made of the Purkinje cells in the cerebellar vermis of rats aged 16-25 days, both before and after treatment with quipazine. Significant increases in complex spikes and decreases in simple spikes were observed only in the mutant rats. Although the enhanced sensitivity of Purkinje cells in dt rats paralleled the behavioral sensitivity of the mutants, the magnitude of the change appeared insufficient to explain the observed tremor. When the experiment was repeated in the deep cerebellar nuclei (DCN), quipazine induced significant increases in firing rate and rhythmicity of cells in normal rats, suggesting that quipazine can act directly on serotonin receptors in the DCN. In dt rats, the spontaneous firing rate and rhythmicity of DCN cells were significantly greater than in normal rats. Quipazine increased firing rate in dt rats, however, unlike cells in normal rats, cells in dt rats either showed no change in rhythmicity or showed increases that greatly exceeded those seen in controls. The tremor may result from activity in a subset of DCN neurons in the mutant rats. (Supported by a grant from the Dystonia Medical Research Foundation and grant BNS 90-10187 from NSF.)

628.3

ACUTE UNILATERAL SENSORIMOTOR CORTEX INJURY IN RATS BLOCKS d-AMPHETAMINE INDUCED NOREPINEPHRINE RELEASE IN CEREBELLUM. L.B. Goldstein* and V. MacMillan. V.A & Duke Medical Centers, Durham, NC 27705

Pharmacologic studies have implicated norepinephrine (NE) in amphetamine-facilitated motor recovery following sensorimotor cortex (SMC) injury in rats. We studied the acute effects of unilateral SMC ablation on the release of NE in cerebellum with in vivo microdialysis. Diasylate samples were collected in 10 min. fractions and NE assayed by HPLC-ECD. All studies were carried out in anesthetized rats.

Baseline levels of NE were undetectable in the cerebellar diasylate. The administration of a single dose of d-amphetamine (2.6 mg/kg base weight, i.p.) resulted in a significant increase in dialyzable NE reaching its peak 30 min. later (~15 pg/µl, not corrected for recovery). However, the administration of the same dose of d-amphetamine to naive rats 60 min. following a suction-ablation lesion of the right SMC did not result in NE release into the cerebellar diasylate.

This data provides evidence for an acute remote effect of SMC injury on amphetamine-induced NE release in the cerebellum (diaschisis). Experiments are in progress to establish the duration of this effect.

Supported by the N.I.H. (NS 01162) and the V.A.

628.4

TRIPHASIC GABA RESPONSES RECORDED FROM PURKINJE CELL DENDRITES IN THE RABBIT CEREBELLAR SLICE. B. G. Schreurs, J. V. Sanchez-Andres, and D. L. Alkon. Neural Systems Section, NINDS, NIH, Bethesda, MD 20892.

Gamma-aminobutyric acid (GABA) has been shown to elicit triphasic neuronal responses in a number of mammalian CNS locations including the somatosensory cortex and the primary visual cortex. We report a triphasic GABA response recorded intradendritically from Purkinje cells (n=50) in vermal slices of the rabbit cerebellar cortex.

Focal pressure applications of GABA (2 mM, 50-500 ms) to the Purkinje cell layer of a rabbit cerebellar slice produced a three-phase response consisting of (1) rapid hyperpolarization ($GABA_{hf}$); (2) slower depolarization ($GABA_d$); and (3) later long-lasting hyperpolarization ($GABA_{hl}$). The three phases were associated with varying increases in membrane conductance. Elicitation of the triphasic response in the presence of TTX confirmed that it was intrinsic to the recorded cell.

Bath application of $GABA_A$ antagonists (bicuculline or picrotoxin) eliminated $GABA_d$ and $GABA_{hl}$ while leaving $GABA_{hf}$ intact, suggesting that $GABA_{hf}$ was mediated by bicuculline-insensitive or possibly $GABA_B$ receptors whereas the $GABA_d$ and $GABA_{hl}$ response were mediated by $GABA_A$ receptors. Previous immunohistochemical evidence for the predominance of $GABA_A$ receptors in the Purkinje-cell dendrites and $GABA_B$ receptors at the cell body suggests that the present triphasic response arose from distinct compartments of the Purkinje cell. This hypothesis was supported by our observation that somatic spikes failed to occur despite the fact that membrane potentials during $GABA_d$ exceeded the threshold for passive propagation of somatic spikes to the recording site.

628.5

IMAGING OF REGIONAL VARIATIONS OF ELECTRICALLY EVOKED SODIUM INFLUX IN CEREBELLAR PURKINJE CELLS. N. Lasser-Ross and W.N. Ross, Dept. of Physiology, New York Medical College, Valhalla, NY 10595.

We used the fluorescent sodium indicator SBFI to monitor changes in $[Na]_i$ associated with intrasomatically and synaptically stimulated events. Neurons in sagittal slices from the guinea pig cerebellum were injected with the impermeant form of the dye with an electrode also used for stimulating and recording. Changes in fluorescence, excited at 380 nm, were recorded with a high speed CCD camera.

Intrasomatically evoked bursts of fast action potentials caused an increase in $[Na]_i$ limited to the soma and axon. The optical signal was proportional to the number of fast action potentials. The change in $[Na]_i$ (measured by $\Delta F/F$) was much larger, and the recovery time much faster ($\tau_{\frac{1}{2}} < 1$ sec), in the axon than in the soma. The larger surface to volume ratio in the axon is probably one factor determining these differences. There was no detectable change in $[Na]_i$ in the dendrites corresponding to either Na or Ca dependent action potentials. The lack of signal also shows that SBFI was not responding to changes in $[K]_i$.

Climbing Fiber activation caused a small change in $[Na]_i$ in the lower part of the dendritic tree. The signals showed paired pulse depression consistent with whole cell voltage clamp recordings (Konnerth et al., PNAS 87, p.2662-2665 (1990)). Therefore, these synaptic signals probably reflect Na entry through ligand gated channels.

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628.6

INTRINSIC DETERMINANTS OF DENDRITIC CALCIUM INFLUX IN TURTLE PURKINJE CELLS IN VITRO. J. Midtgaard, N. Lasser-Ross & W.N. Ross. Institute of Neurophysiology, Univ. of Copenhagen, DK-2200 Copenhagen N, Denmark and Dept. of Physiology, New York Medical College, Valhalla, NY 10595.

In order to determine the significance of active membrane properties in synaptic integration simultaneous electrical and optical recordings were made from Fura-2 injected Purkinje cells in slices from the turtle (Pseudemys scripta elegans) cerebellum. Fluorescence changes were imaged with a high speed CCD camera. Voltage dependent Ca influx in the soma was mostly correlated with fast, TTX-sensitive spikes. Ca transients in the spiny dendrites were correlated with calcium spikes and had much faster rise and fall times. In some cases the locations of Ca influx varied from spike to spike implying localized and varied spiking in the dendrites. In these aspects turtle Purkinje cells resemble guinea-pig Purkinje cells. When an external electric field was applied across the cerebellum Ca spikes were evoked with Ca influx confined to the tips of the spiny dendrites demonstrating that very localized firing can take place in the spiny dendrites. Both PF and CF stimulation evoked voltage dependent Ca influx. The distribution and amplitude of synaptically evoked Ca influx was dramatically affected by a 4-AP sensitive A-like potassium conductance (Chan, Hounsgaard & Midtgaard, J.Physiol. 409, 143-156, 1989).

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628.7

ELECTROPHYSIOLOGICAL PROPERTIES OF IDENTIFIED GRANULE CELLS. C. Huang, C.-F. Hsiao and R. Huang*. School of Basic Life Sciences, Univ. Missouri-Kansas City, Kansas City, MO 64110

The cerebellar granule cells provide important sources of excitation for all cell types in the cerebellar cortex. Yet the electrophysiological properties of granule cells remained poorly known. Indeed, it was unclear whether the somata of mature granule cells possess the necessary combination of ion channels to generate conventional action potentials. We have recorded single granule cells intracellularly followed by horseradish peroxidase (HRP) injection in cats under chloralose anesthesia (70mg/Kg). The site of recording and injection was confirmed to be at the soma of the injected granule cell. The resting membrane potential was -43.5±6.7mV (n=31). The action potentials were 7.7±2.6mV extracellularly and 53.4±15.8mV intracellularly (n=22). Among the 31 granule cells, 26 responded to sound (60dB above threshold) with average latencies at 24.9±8.2ms while 17 responded to light with longer average latencies at 53.0±11.0ms. Five granule cells, however, did not respond to either sound or light. The mean inter-spike interval of granule cells was 152.7±68.8ms (n=21). (Supported by PHS grant AA07643).

CONTROL OF POSTURE AND MOVEMENT V

629.1

A NEURAL NETWORK FOR LEARNING MUSCLE COORDINATION J.B.J. Smeets*, and J.J. Denier van der Gon*, Utrechts Biofysica Instituut, University of Utrecht, Princetonplein 5, Utrecht, The Netherlands. (SPON: European Neuroscience Association).

In the human arm, there are more muscle pairs than degrees of freedom. To make a specific movement, the nervous system has ample choice from combinations of muscle activations. We present a neural network model which learns, unsupervised, from afferent signals to coordinate muscles in a realistic way.

The model consists of a planar arm with two joints, controlled by four mono-articular and two bi-articular muscles. As a substitute for a homogeneously innervated motoneuron pool we used one motoneuron. A pooled Ia-afferent signals muscle stretch. An array of central neurons innervates the motoneurons. Movements are generated by the activation of abutting central neurons. All central neurons and all afferents are connected to all motoneurons. The weights of these connections are adapted during centrally induced movements of the arm, using only information present at the location of that neuron. Simulations start with poor coordination: each central neuron has only one non-zero connection with a motoneuron.

The resulting coordination of the muscles conforms to experimental data on muscle activation. Furthermore, the model predicts homonymous and heteronymous monosynaptic reflexes in a realistic way, for instance a heteronymous reflex in the mono-articular elbow flexor when shoulder-flexors are stretched.

629.2

THE RELATIONSHIP BETWEEN LATERAL DOMINANCE AND TURNING PREFERENCE ON THE STEPPING TEST. Fred H. Previc and Joanna Saucedo*. Armstrong Laboratory, Brooks AFB, Tx 78235-5000.

Previous research has shown a relationship between handedness and the direction of turning during Fukuda's stepping test. This study attempted to correlate directional preference on the stepping test with several measures of lateral dominance, including handedness. A total of 111 students from a local high school participated as subjects. The vast majority of subjects (89%) were right-handed, while a smaller percentage (59%) were right-footed and right-eyed. Subjects performed the stepping test with their vision occluded and their arms folded in front of them, and their angular rotation during a 1-min interval was measured. Although only a slight majority of subjects turned rightward, the direction and magnitude of turning correlated significantly with footedness and eyedness (but not handedness). For example, 60% of right-footed and right-eyed subjects turned rightward, whereas less than 40% of subjects with left and mixed preferences did so. The results indicate that axial turning preferences and various lateral dominance tendencies may at least partially share a common mechanism, possibly involving the vestibular system.

629.3

EFFECTS OF DORSAL ROOT CUT ON FORCES EVOKED BY SPINAL STIMULATION IN SPINALIZED FROGS. E.P. Loeb, S.F. Giszter, E. Bizzi, and F.A. Mussa-Ivaldi* Dept. of Brain and Cognitive Sciences, MIT, Cambridge, MA 02139.

Recent experiments in spinal frogs have shown that focal microstimulation of a single site in the lumbar grey, in conjunction with positioning the leg in different workspace locations, results in the generation of a force field with a single equilibrium point. The equilibrium point is that spatial location at which the leg would be at steady state were it free to move. The force fields are produced by spinal circuits and modulated by afferent feedback. The purpose of this work was to investigate the contribution of afferent feedback to the force fields. We measured the force fields before and after cutting the dorsal roots without moving the stimulating electrode. After cutting the dorsal roots we found that the current threshold required to induce a force field was temporarily elevated, and when induced the dynamics of the rise and fall of the force traces was altered. This suggests a role for afferent activity in the maintenance of spinal circuit excitability, and shaping of the force fields. We will present a quantitative analysis of the effects of the dorsal root cuts on the shape of the force traces.

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629.4

DISCRIMINATING AMONG RHYTHMICAL BEHAVIORS IN THE CHICK. R. M. Johnston, S.M. Woolley, M.B. Smith and A. Bekoff. Dept. of E.P.O. Biology, University of Colorado, Boulder, CO 80309-0334.

Our intent in this study was to quantify the degree of relatedness among different rhythmical hindlimb behaviors using discriminant function analysis. We chose to compare the kinematic profiles of five behaviors: walking, swimming, airstepping, foot shaking and head scratching. The following results do not yet include head scratching.

Small dots of black ink placed on the skin overlying the lateral surface of the right hindlimb were used to define the hindlimb and the hip, knee and ankle joints. Video recordings were made as chicks produced one of the five behaviors being examined. The hindlimb movements were digitized and we quantified several features of intra- and interjoint coordination patterns. Discriminate function analyses were performed on groups of non-complementary variables using a cross validation approach for behavioral classifications.

Our results show that functions based on the actual time (ms) of kinematic features discriminate unilateral foot shaking from the three bilateral behaviors. We conclude that this separation corresponds to a cycle duration constraint between unilateral (max $\leq$ 150 ms) and bilateral behaviors (min $\geq$ 150 ms). When variables were expressed as a proportion of their cycle durations, converting real time to relative time, functions discriminate walking, which involves weight support, from the other three behaviors which lack weight support. When walking is removed from these analyses relative latencies and not relative durations discriminate among the remaining three closely related behaviors. These results suggest that the behavioral implications of weight support are dramatic and that discrimination among the more closely related behaviors may reflect different interjoint coordination patterns in each behavior. By establishing the relatedness among diverse behaviors we provide the foundation for interpreting the differences in the motor patterns underlying these behaviors. Supported by NIH grant NS20310.

629.5

WAVEFORMS AS A MEASURE OF CHANGES IN EMG ACTIVITY. J.A. Hodgson*, R.R. Roy, C.P. de Guzman*, R. de Leon*, R.J. Prober*, A.J. Garfinkel and V.R. Edgerton, Dept. of Kinesiology and Brain Research Institute, UCLA, Los Angeles, CA 90024-1527.

Fluctuations in the amplitude of the EMG envelope throughout a step cycle have been reported for some muscles and it appears that the rectified and smoothed EMGs reflect the frequency of discharge of motor units in a muscle (Hoffer et al., J. Neurophysiol. 57:530, 1987). Thus EMG waveforms can be interpreted to be indicative of the patterns of activation experienced by motoneuron populations. We have computed averaged EMG waveforms for several muscles in normal and spinal cats and normal rats walking on a treadmill over several speeds. It was found that the speed of locomotion, level of loading and selected neuropharmacological agonists and antagonists had unique and repeatable effects on the waveforms of some muscles. These changes in waveform were restricted to only one part of a burst in some muscles (soleus, medial gastrocnemius, tibialis anterior). In muscles exhibiting two bursts per step cycle (vastus lateralis, rectus femoris), these interventions often affected only one burst. These waveform modifications may reflect changes in timing (soleus) or amplitude (tibialis anterior) or both (medial gastrocnemius, vastus lateralis, rectus femoris). These results indicate that the timing and amplitude of EMGs may be controlled separately and that the mechanisms contributing to the timing or amplitude of a burst pattern may change throughout the duration of the burst. The extent to which the unique waveforms which characterize each motor pool output during locomotion reflect central pattern generation versus supraspinal and/or sensory feedback can be defined. For example, the EMG waveforms executed during carefully controlled locomotor tasks can be compared with the efferent output of the surgically isolated spinal cord during fictive locomotion. *Supported by NIH Grant NS16333.*

629.6

EFFECTS OF LOW THORACIC SPINALIZATION ON EMG OF ADULT CATS. D.J. Pierotti, R.R. Roy, J.A. Hodgson*, R. Prober*, C.P. de Guzman*, R. de Leon* and V.R. Edgerton. Brain Research Institute and Kinesiology Department, UCLA, L.A., CA 90024-1527.

The hindlimbs of adult spinal cats (T12-T13) can be induced to step on a treadmill and daily training can improve this locomotor capability (Lovely et al., Exp. Neurol. 92:421, 1986; Barbeau and Rossignol, Brain Res. 412:84, 1987). Although the general pattern of EMG bursts of selected extensors and flexors appear to be similar in spinal and intact cats, some effects of spinalization are evident. In the present study, the EMG patterns were studied in the same cat during quadrupedal and bipedal stepping and during bipedal stepping at 1 and 2 months postspinalization. EMG recording electrodes were implanted in the soleus (Sol), medial gastrocnemius (MG), tibialis anterior (TA), vastus lateralis (VL), semitendinosus (St), gluteus medius (GM) and iliopsoas (IP) as described by Pierotti et al. (Brain Res. 481:57, 1989). Treadmill training was initiated within 1 week postspinalization in contrast to our previous studies where training was initiated after 1 month. Some differences in the EMG waveforms during quadrupedal and bipedal walking were evident. The general features of the EMG waveforms recorded postspinalization were remarkably similar to those recorded bipedally before spinalization. In contrast to previous studies, clonus was absent in the cats in which training was initiated 1 week after spinalization. The major effect on burst patterns was an increased amplitude of the EMG in primary flexors (TA, IP, St), but not primary extensors (Sol, MG, VL, GM). After spinalization, a double burst occurred in the St, with the initial burst doubling in amplitude compared to prespinalization. In addition, the GM, IP and MG had less distinct bursts 1 month after, than before spinalization. Normal waveforms were reestablished by 2 months postspinalization. These data indicate that, with the exception of the St, the EMG patterns of activation of each of the hip, knee and ankle muscles studied in normal cats can be reestablished following spinalization by appropriate locomotor training techniques.
Supported by NIH Grant NS16333.

629.7

PHARMACOLOGICAL EFFECTS ON EMG ACTIVITY OF HINDLIMB FLEXORS AND EXTENSORS IN ADULT SPINAL CATS. C.P. de Guzman*, J.A. Hodgson*, R.R. Roy, R. de Leon*, R.J. Prober*, S.C. Bodine-Fowler and V.R. Edgerton, Dept. of Kinesiology and Brain Research Institute, UCLA, Los Angeles, CA 90024-1527.

Two adult cats underwent different training regimens following spinalization (T12-T13). One was trained to walk on a treadmill at varying speeds (0.2-1.0 m/s) and the other was trained to stand for 30 min/day, 5 days/week. Once the locomotor performance had reached a plateau, the effects of i.p. administration of several pharmacological agonists and antagonists of neurotransmitters and modulators were tested during treadmill locomotion. The EMG activity of hindlimb flexors and extensors, i.e. semitendinosus (St), vastus lateralis, gluteus medius, iliopsoas, soleus (Sol), medial gastrocnemius (MG) and tibialis anterior during stable treadmill stepping over a range of speeds before and after administration of each drug was studied. The cat trained to stand was unable to step when first tested. One of the most dramatic pharmacological effects was the initiation of full-weight supporting stepping after the administration of 0.02 mg/kg of strychnine (specific binding to glycine receptors). On two subsequent occasions, the administration of strychnine (0.02-0.06 mg/kg) also improved locomotion dramatically. Similar doses of strychnine had minimal effects on the locomotor behavior of the cat that was already trained to walk. A dosage of 150 or 300 *ug*/kg of clonidine had no noticeable effect on locomotion prespinalization. In the cat trained to walk, clonidine (60 *ug*/kg) increased the EMG burst amplitude and duration of the St during stepping and restored the waveform to its appearance before transection. Clonidine also increased the amplitude of the Sol, but not the MG. These data show that the effects of selected neuromodulators and neurotransmitters can be very specific to selected motor pools and even to selected segments of the burst pattern of a single muscle during stepping. *Supported by NIH Grant NS16333.*

629.8

PERCEPTUAL AND NYSTAGMIC RESPONSES TO BODY TURNING: SOMATOSENSORY AND MOTOR INFLUENCES. P. DiZio, J. R. Lackner, J. Segal*. Ashton Graybiel Spatial Orientation Laboratory, Brandeis University, Waltham, MA 02254.

Somatosensory and motor inputs from active stepping in a circle elicit compensatory nystagmus with a gradual rise in slow phase velocity (SPV) in normal individuals. We simultaneously assessed in seated subjects how nystagmus and perceived self-motion (PSM) are influenced by pedalling or dragging movements of the feet.

Subjects (N=10) sat in a rotating chair in darkness and were turned for 60 s at 90 deg/s. They either sat passively, dragged their feet, or pedalled in time with the chair's motion the whole time, or began dragging or pedalling after 45 s when vestibular nystagmus and PSM had decayed. Eye position and PSM were continuously measured during and after rotation.

Relative to the passive conditions, SPV and PSM decayed more slowly (sometimes not at all for PSM) in the dragging and pedalling conditions. When dragging or pedalling commenced after vestibular nystagmus and PSM had decayed, PSM returned immediately but SPV showed no significant increment. Neither the initial SPV of post-rotary nystagmus nor its decay rate depended on the prior conditions, but PSM either decayed quicker or failed to reverse following active pedalling as compared to passive turning.

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629.9

THE BACKDRIVING ALGORITHM FOR THE CONTROL OF REDUNDANT MOTOR SYSTEMS: ANALYSIS AND PREDICTIONS. J. McIntyre, F.A. Mussa-Ivaldi* and E. Bizzi,
Department of Brain and Cognitive Sciences,
Massachusetts Institute of Technology, Cambridge, MA 02139

Biological motor system are redundant with respect to many of the tasks required of them. This redundancy exists in terms of both the positions assumed by a limb and the forces which are produced by the motor system on its environment. For instance, there are typically more degrees of freedom in the joints of an arm than are required to achieve a given hand position and similarly there are more muscles acting around the joints than are necessary to produce a desired torque or force. The CNS must resolve, and perhaps exploit, this redundancy to produce the muscle activations necessary to achieve a given task.

In this study we analyzed and tested the *backdriving* algorithm, which has been proposed as a model for the control of vertebrate motor systems [Mussa-Ivaldi et al. 1989]. According to this algorithm, active coordination is obtained by reproducing the patterns of passive forces and motions which are induced by the environment at the point of contact with the motor system.

First, it was shown that the backdriving algorithm can deal with a variety of problems faced by the CNS, including redundancy, non-linear geometries, kinematic singularities and muscle saturation. Second, the backdriving algorithm was used to predict the behavior of the human motor system. Using anatomical data acquired from the literature [An et al. 1981], we simulated the backdriving algorithm on a computer to predict the muscle activations utilized by the CNS to produce a desired output in an isometric force task. These predictions were compared with published experimental data for the same tasks [Buchanan et al. 1986]. The backdriving algorithm successfully predicts a number of the features of the observed muscle behavior, including the direction of maximal activation for a given muscle and the agonist/antagonist pattern of activation for a group of muscles.

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629.10

ELBOW FLEXION MOVEMENTS IN C5/C6 QUADRIPLEGICS WITH NO (WEAK) TRICEPS AND WITH ARTIFICIAL TRICEPS SUPPORT. M. M. Wierzbicka and A.W. Wiegner West Roxbury VA Medical Center & Harvard Medical School, Boston MA 02132.

We have previously shown using a mathematical model and with electrical stimulation (Wierzbicka at al. Exp Brain Res 63: 331, 1986), that in fast one-joint movements the antagonist muscle provides an effective means to reduce movement time. To further study the role of the antagonist we have now analyzed movements performed by patients with C5/C6 spinal cord injury who had relatively normal biceps and little or no voluntary control of triceps. We also explored the effect of an "artificial triceps", provided to the elbow joint by an external torque motor, on movement characteristics. Four quadriplegic patients and 6 control subjects performed elbow flexion movements "as fast and accurately as possible" to targets of 10°, 20°, 30°. Despite the lack of antagonist, patients used the same "pulse height" control strategy as normal subjects to scale their responses with movement amplitude. However, patients' movement time was on average twice that of normal subjects, and durations of both accelerative and decelerative phases of movement were increased. Movement speed and accelerations were significantly reduced (2-5 times) in comparison to the corresponding values of normal subjects. Patients overshot all targets to a larger extent than normal subjects, particularly 10° targets, with nearly twice the error. When a constant 2.5 or 5 Nm extensor torque was provided by a motor, patients were able to move faster than without triceps assistance and movement accuracy was within the normal range. These results provide direct evidence that the antagonist has an important effect on time and accuracy of fast goal-directed movements. (Supported by NIDRR and VA)

629.11

SUPERIOR COLLICULUS LESIONS IMPAIR OBSERVATIONALLY-RATED ORIENTING RESPONSES, BUT NOT LICK SUPPRESSION OR HEART RATE CHANGES, TO ACOUSTIC STIMULI IN RATS. B.J. Young and R.N.Leaton. Dept. of Psychology, Dartmouth College, Hanover, NH 03755.

Despite more than two decades of research, there continues to be debate concerning the nature of the orienting deficit produced by superior colliculus (SC) lesions. We examined the nature and extent of the impairment in orienting to auditory stimuli in rats with SC lesions. Three measures of orienting response were used: heart-rate changes, lick suppression, and a measure of behavioral orienting scored from video records. Bilateral electrolytic SC lesions were made in 7 rats, and another 7 rats served as sham-operated controls. All animals were water deprived for 23.5 hours per day and trained to lick at a drinking tube. Pulsed white noise stimuli (65-dB SPL, 0.5-s duration, 1-Hz) were presented through small peripherally located speakers. Each animal received four presentations of the stimulus, two on each side, on each of three test days. The SC-lesioned animals were significantly impaired on the video-scored behavioral orienting measure, but they did not differ from controls on the lick-suppression and heart-rate measures. That the lesions impaired orienting on only one of the three response measures suggests a response-based rather than a sensory-based deficit. The failure of the lick suppression measure to differentiate between the groups emphasizes the importance of selecting an appropriate response measure, and may account for some previous negative results with auditory stimuli. Our previous work showed that SC-lesioned rats were significantly impaired in their orienting responses to visual stimuli on both the video rating and lick-suppression measures.

629.12

Speed-accuracy tradeoff must take into account the biomechanics of the movement. C.C.Bassile, T.Kaminski, Teachers College, Columbia University, New York, NY 10027.

Previous speed-accuracy investigations have identified relationships between movement time (MT) or peak velocity (PV) and extrinsic variables (i.e. displacement, accuracy requirements, contextual demands). This investigation demonstrated that biomechanical variables also affect MT and PV. Four subjects performed two types of pointing movements, reaches (shoulder and elbow moving in opposite directions) vs. whips, (shoulder and elbow moving in same directions) under two conditions, fast vs. fast/accurate. These multijoint arm movements were compared across three amplitudes of displacement (10.16cm, 20.32cm, 30.48cm) in the horizontal plane. As expected, we found: (1) MT and PV were linearly related to displacement, and (2) accuracy demands increased MT and decreased PV. However, MT was briefer and PV was higher for whips under both fast and fast/accurate movements of comparable displacements. These MT/PV differences between whips and reaches are not accounted for by Fitts' Law (Fitts, P.M.,*JEP* 47: 381,1954) or subsequent modifications (Schmidt,R.A. et.al., *Psychol Rev* 86:415, 1979; Meyer D.E. et.al., Attention and Performance XIII,173,1990). Therefore, the speed at which a movement proceeds is based not only on the accuracy and distance requirements of the task but also on biomechanical factors, such as, the summation of interactional forces.

629.13

DIRECTED MOVEMENT IN THE FROG: EXPLORATIONS USING BACK PROPAGATION NETWORKS J. N. Carr*, D. Louca*, and P. Grobstein, Dept. of Biology, Bryn Mawr College, Bryn Mawr, PA 19010.

Prey orienting behavior in the frog depends on successive map-like and parcellated representations of target location in space. Both forms of spatial representation underlie a variety of behaviors in a diversity of organisms, suggesting that they exist for reasons unrelated to the idiosyncrasies of particular organisms or tasks. To explore this, we are studying the behavior of back propagation networks presented with frog-like sensorimotor problems, to see whether such networks create solutions with either map-like or parcellated spatial representations and, if so, under what circumstances.

Networks given simple sensorimotor problems (direction of target location to direction of movement; 1-D) frequently created solutions involving distributed rather than map-like spatial representations. Maps are thus not an inevitable consequence of the need to associate each of a set of input locations with the appropriate output directions. We were able to bias the networks towards the generation of map-like solutions by manipulation of either the range of initial synaptic weights, or the parameters governing subsequent synaptic modification. Hence, neither kind of representation is an inevitable consequence of simple sensorimotor tasks; the starting conditions and methods by which a solution is found influence its form.

Given analogues of slightly more complex problems (direction and distance of target location to direction and distance of movement; 2-D), networks again frequently created distributed spatial representations, in which two spatial dimensions are coded together, rather than parcellated representations, in which the two dimensions are coded independently. Hence, parcellated representations are also not inevitable consequences of simple sensorimotor tasks. Networks modified to include simulated inhibitory interneurons did tend to form parcellated representations. This suggests that a distinction between excitation and inhibition is among the starting conditions which can influence the form of a solution. The nature of the problem presented also appears to bias the networks towards solutions involving parcellated spatial representations, and this is currently being further explored.

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629.14

DIRECTED MOVEMENT IN THE FROG: ELECTROPHYSIOLOGICAL STUDIES OF A TECTO-TEGMENTAL PATHWAY. C. Smeraski and P. Grobstein, Dept. of Biology, Bryn Mawr College, Bryn Mawr, PA 19010.

Tectofugal signals representing the horizontal eccentricity of the targets of prey orienting movements apparently descend on axons originating from a large cell column (LCC) in the ventral midbrain tegmentum. The anatomically defined LCC is also identifiable physiologically, as a region which exhibits distinctive spontaneous activity. The present studies were undertaken to determine whether neurons in this region could be activated by local stimulation of the tectum, and to begin a characterization of the physiological organization of such a tecto-tegmental projection.

Tungsten microelectrodes were used to record in the LCC while stimulating at various locations in one tectal lobe. A second recording electrode, in the opposite tectal lobe, monitored activation of the topographically organized tecto-isthmo-tectal pathway, so as to verify that the stimulus strengths employed activated only local tectal regions. Such stimuli did produce unit activity in the LCC, at variable latencies as would be expected of post-synaptic responses. Theshhold responses in the LCC occurred when the stimulating electrode was in tectal laminae deeper than those at which threshhold responses in the opposite tectal lobe were observed, suggesting that tecto-isthmal and tecto-LCC projections arise from different tectal laminae.

Responses at a given location in the LCC could be produced by local activation of either tectal lobe, and from a wide array of locations within one tectal lobe. The findings are consistent with prior work suggesting that 1) activity in the LCC on one side of the brain represents the horizontal eccentricity of targets to one side of the body irrespective of the tectal lobe activated by such targets, 2) projections from tectum to LCC lack a clear topographic organization, and 3) it is activity over a wide area of the LCC rather than the particular region of LCC activated which codes the relevant information. Further studies of LCC responses to local tectal stimulation may provide new information about the nature of the LCC population code and how a transformation from topographic coding to activity coding is achieved.

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629.15

THE EFFECTS OF CLONIDINE ON METHYLPHENIDATE-INDUCED MOTOR BEHAVIORS IN RATS. Lester, C.C.* and Moss, D.E. Dept. of Psychology, Univ. of Texas at El Paso, El Paso, TX 79968.

The success of clonidine, an alpha-2 adrenergic agonist, in some Tourette's syndrome patients implicates noradrenergic involvement in this hyperkinetic motor disorder which has traditionally been viewed to be the result of dopaminergic dysfunction. In order to examine the role of noradrenergic control of dopamine-dependent behaviors, the effect of clonidine on methylphenidate-induced motor behaviors was examined. Female rats were assigned to one of three groups receiving methylphenidate HCl (MP, 30 mg/kg) i.p. alone, clonidine HCl (CL, 150 ug/kg) i.p. alone, or CL (150 ug/kg) i.p. followed 40 minutes later by MP (30 mg/kg) i.p. All three groups were rated for stereotypy and locomotor movements were counted. The group receiving CL followed by MP had significantly decreased stereotypy and, paradoxically, increased locomotor activity. These data suggest an important interaction between the noradrenergic and dopaminergic systems in extrapyramidal motor functioning. (Supported by MH47167.)

629.16

EFFECT OF FOCAL TRANSCRANIAL MAGNETIC STIMULATION ON SIMPLE REACTION TIME. A. Pascual-Leone, J. Valls-Solé*, E.M. Wassermann, J. Brasil Neto*, L.G. Cohen, and M. Hallett. NINDS-NIH, Bethesda, MD 20892.

We studied the effect of transcranial magnetic stimulation (TMS) on simple reaction time (RT) to auditory, visual, and somatosensory stimuli. The subjects were requested to flex the right arm rapidly in response to the go-signal. RT was measured from go-signal to onset of biceps EMG activity. TMS was delivered at different intensities, over different scalp positions, and at variable delays before or after the go-signal. We also studied motor cortex excitability during RT by determining the probability of inducing motor evoked potentials (MEP) with a subthreshold transcranial stimulus (S) delivered at variable times before movement onset. We compared trials using a visual go-signal (control) with trials using a visual stimulus coupled with a subthreshold TMS stimulus (test).

TMS shortened RT to the different go-signals by approximately 30 ms when delivered over the left motor cortex at intensities below motor threshold. This effect was present at TMS delays of -30 to +30 ms, and was maximal at approximately +10 ms delay. TMS over other left-sided scalp areas did not affect RT. S did not evoke MEPs until the time to EMG onset was ≤ 80 ms. Thereafter, probability and amplitude of MEPs increased with decreasing interval. We found no differences between control and test trials.

In the RT paradigm used, the subjects were able to plan the response completely during the foreperiod. The motor program had to be held in memory, and upon arrival of the go-signal, be executed. TMS shortens RT by speeding up initiation of the execution, without affecting execution itself.

629.17

AN ANIMAL MODEL OF OPTIC FLOW UTILIZATION IN THE CONTROL OF LOCOMOTION. H. Sun*, D. P. Carey and M. A. Goodale. Univ. Western Ontario, London, Ontario, Canada.

In spite of numerous claims of flow-field processing in visual neurons of the CNS (Steinmetz, et al., 1987, *J. Neurosci.*), few investigators have manipulated optic flow to examine its effects on behaviour. Although there have been some laboratory studies of the use of optic flow by humans and some demonstrations of its use in free-living birds, there have been no laboratory studies of the use of this important visual cue in non-human mammals. In the present study, eight male Mongolian gerbils were trained to run random distances (varying from 95 to 165 cm) to a target presented on a computer screen at the end of an elongated runway. The target consisted of a solid white circle on a black background. During training days and many of the trials on test days, the size of the circle remained constant during an individual trial but was varied across trials from 1.5 to 6 cm. On probe trials, however, the size of the circle suddenly expanded (from 3 cm to 6 cm) or contracted (from 3 cm to 1.5 cm) over a period of 450 ms after the animal crossed an infra-red photobeam 70 cm away from the target. Analysis of digitized video recordings of the runs suggested that when the target size was increased the gerbils tended to decelerate earlier and/or more rapidly than on control runs to a constant-size target (3 cm) at the same distances. Contracting the target during the run had the opposite effect. These results provide strong support for the use of the relative rate of retinal expansion as an important variable in locomotor control. Moreover, this paradigm may provide a useful means of studying the neural mechanisms that are critical for the extraction of optic flow by moving animals. Supported by NSERC grant #A6313 to MAG and ITRC Research Fellowships to HS and DPC.

629.18

CHANGES IN FORCE RESULT IN INCREASED AMPLITUDE OF SCALP-RECORDED MOVEMENT-RELATED POTENTIALS. **D.H. York, D. Fousek*, Dept. of Physiology, School of Medicine, University of Missouri, Columbia, MO. 65212.**

The question of whether alterations in force opposing movement could be detected in ballistic movements of the thumb was evaluated with scalp recordings in human subjects. Bipolar recordings were made overlying the precentral gyrus and referenced either anterior or posterior to a site lying 6 cm lateral to the vertex. Subjects performed either a flexion and hold movement or a flexion-extension movement against a spring load at constant displacement and movement time. Increases in initial resting spring tension from 200 - 500 gm did not result in significant changes in cortical potentials. However, increases in spring constant resulted in significant increases in amplitude of an N1-P1 component defined in anterior referenced recordings but not in posterior referenced recordings. Posterior recordings did show a profile of the acceleration changes occurring during movement. Increases in velocity of movement were associated with increases in N1-P1 amplitude and decreases in latency of a post movement, P2 component.

Supported by NIH Grant NS24960.

629.19

LATERALIZATION IN HAPTIC PROCESSING: SEX AND HAND DIFFERENCES IN EXPLORATORY STRATEGIES. J. Fagot, J. Vauclair and J. Requin. C.N.R.S., L.N.F, Unit of Cognitive Neurosciences, 31 ch. Joseph Aiguier, 13402 Marseille, France.

Right-handed adults (12 males and 12 females) had to touch unseen non-sense stimuli for a maximum of 10 sec., and then to recognize their outline drawings. Tactual stimuli were composed of 8 independent units not haptically discernible. The computer recorded hand contacts and duration with each unit. Analysis included the mean number of units simultaneously touched, the number and duration of complete explorations during the 10 sec., and the accuracy of the recognition phase. In average, males touched a greater number of units than females ($M=5.56$, $F=4.59$, $p<.02$) and they reexplored more frequently the stimulus ($M=3.75$, $F=2.88$, $p<.05$) with a shorter duration ($M=2.12$ sec., $F=3,07$ sec. $p<.05$). For the 24 subjects, the left hand touched simultaneously more units than the right ($L=5.21$, $R=4.96$, $p<.001$). Neither hand nor sex differences were found regarding recognition accuracy. It is suggested that the strategy is more sensitive to laterality effects than measurement of the accuracy.

629.20

DISCHARGE PROPERTIES OF ANTIDROMICALLY ACTIVATED AMBIGUOUS NEURONS DURING VOCALIZATION IN THE AWAKE MONKEY. Y. Yajima* and C.R. Larson. Dept. of Physiology, Hyogo College of Medicine, Nishinomiya, Hyogo, 663 Japan and Dept. of Communication Sciences and Disorders, Northwestern University, Evanston, IL 60208.

As a way of understanding neural mechanisms controlling vocalization, neurons in and near the nucleus ambiguus (NA) were extracelluraly recorded in an awake, vocalizing Macaca nemestrina monkey. Motoneurons within NA were identified by antidromic activation and collision testing from stimulation of the recurrent laryngeal nerve. Peri-event time, interspike interval and auto-correlation histograms were used to further classify the neurons. Other neurons within the NA where antidromic potentials were recorded were classed as respiratory related, swallowing related and unspecified neurons. A variety of discharge patterns such as burst with vocalization or suppression with vocalization were noted. Motoneurons were found at distinct locations in the NA compared with other types of cells. Motoneurons differed from the other cell types on the basis of their pre-vocalization onset times, duration of discharge during vocalization, and time of peak discharge frequency. Most respiratory related neurons discharged in phase with inspiration and also before vocalization. Swallowing related neurons did not discharge with vocalization but were active with swallowing following vocalization. Several other types of neurons related to vocalization close to the NA, which did not show an antidromic response, were also observed.

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629.21

TACTILE PLACING IN NORMAL CATS IS DIRECTIONALLY DISCRETE: A VIEW FROM DOWN UNDER. N.S. Bradley and S.H. Chambers. School of Physical and Occupational Therapy, McGill Univ., Montreal, PQ, Canada H3G 1Y5.

Spinal lesion studies assuming the view that tactile placing (TP) is a step in isolation have typically treated rostral, caudal, medial and lateral responses as separately controlled behaviors. Observation of forward trajectory of the paw following tactile stimulation of guard hairs has led some investigators to conclude that rostral TP can be controlled by the spinal cord. Our study provides new data that describes the directionally discrete trajectory of the paw in normal adult cats and suggests that responses limited to a single vector space are not equivalent.

Fore- and hindlimb TP was tested in normal adult cats while held with 1 limb relaxed and pendent over a circular opening in a plexiglass testing surface and videotaped from below. Testing fibers (6) were placed at 60° intervals around the paw in the horizontal plane; fiber selection for each trial was randomized.

Of nearly 2,000 trials videotaped to date, forelimb TP (20-40%) was more readily evoked than hindlimb TP (10-20%). Preliminary analyses indicate that nearly all placements were within 30° of the test fiber and half were within 15° regardless of fiber location. Subsequent computer analysis will include polar coordinate representation of paw trajectory leading to paw placement.

This work was supported by the McGill Faculties of Medicine and Graduate Studies, FCAR and NSERC.

629.22

PRIMATE LESION FILM COLLECTIONS. J.A. Vilensky, S. Gilman and A. Moore*. Dept. of Anatomy, Indiana Univ. Sch. Med., Ft. Wayne, IN 46805, and Dept. of Neurology, Univ. of Michigan Sch. Med., Ann Arbor, MI 48109.

Interest in the primate motor system was dominated during the early and middle parts of this century by such renowned scientists as Sherrington, Fulton, Hines, Tower, Woolsey, Mettler, Bucy, Denny-Brown and Kuypers. Some of these individuals used film to record the behavior of the lesioned animals. The historical value of these films is undoubted. However, further analyses of these films by contemporary scientists might provide "new" information on motor function, or could be used to evaluate current theories. Accordingly, we have been collecting all the available films of CNS-lesioned monkeys, as well as the accompanying materials (eg, surgical protocols, histological slides, photographs). By far the largest collection is that of Denny-Brown, which consists of approximately 3000 rolls of film of about 450 monkeys. In this presentation we primarily describe the Denny-Brown collection. Briefer descriptions are provided for some of the smaller collections (eg, Woolsey, Bucy, Kuypers with Lawrence). We ask anyone with an interest in using any one of these collections, as well as anyone knowing the whereabouts of additional collections, to contact us. Finally, videotapes of a few of the films from the Denny-Brown collection are available for viewing at this meeting.

629.23

NEURONAL PATHWAYS MEDIATING CRAWLING BEHAVIOR IN THE MEDICINAL LEECH, *Hirudo medicinalis*. A. Baader and W.B. Kristan Jr., Department of Biology, UCSD, La Jolla, CA 92093-0322.

Leeches produce two distinct locomotor behaviors: they can swim or crawl towards their targets. While swimming is a rather fixed oscillatory behavior which, once elicited, is hardly influenced by sensory information, crawling consists of many behavioral subcomponents that are strongly sensory-controlled. We are interested in a) finding neuronal pathways participating in crawling and b) the extend to what the nervous system recruits neuronal elements involved in other behaviors to produce crawling.

In freely behaving animals changes in crawling behavior were analyzed after cutting peripheral nerves and transecting the connectives. Deafferentation of several midbody segments (e.g. segments 8 through 18) does not hinder co-ordinated head and tail sucker activity, but it decreases the velocity of body contractions. Transection of both connectives (between segments 11 and 12) blocks the propagation of the elongation wave, but does not prevent contraction of posterior segments and reattachment of the tail sucker, while animals with only one connective cut can still crawl in a co-ordinated manner. Thus crawling seems to be mediated both by central information transfer through the connectives and partly by peripheral pathways.

In tethered crawling leeches, intra- and extracellular recordings were performed in midbody ganglia while the behavior was video-monitored simultaneously. The activity profiles of identified interneurons (e.g 204, 208, S) were determined during crawling. The S-cell, for example, which forms a fast conducting pathway through the whole nerve cord is activated during the contraction phase. The S-cell also excites motoneurons which cause the inhibition of other circular motoneurons producing the elongation of the body during crawling. Supported by a Deutsche Forschungsgemeinschaft grant to AB and a USPHS grant MH43396 to WBK.

CIRCUITRY AND PATTERN GENERATION III

630.1

RESPIRATORY OSCILLATIONS IN MEDULLARY SLICES: CRITICAL ROLE OF EXCITATORY AMINO ACIDS (EAAs). G.D. Funk, J.C. Smith & J.L. Feldman. Systems Neurobiology Lab., Dept. Kinesiology, UCLA, Los Angeles, CA, 90024-1527.

Our previous *in vitro* studies identified a limited region of the neonatal rat ventrolateral medulla, the pre-Bötzinger Complex (pre-BötC), that contains neurons generating respiratory rhythm. Medullary slices (400-600 µm thick) containing this region generate respiratory oscillations in cranial nerves XII and/or IX (Smith *et al.*, SN abs. 465.1, '90), allowing analysis of mechanisms of rhythm generation and rhythmic drive transmission in an isolated, active respiratory circuit. To determine the role of EAAs in rhythmogenesis and synaptic transmission in this circuit, effects of local microinjection of NMDA (MK801) and non-NMDA (CNQX) antagonists were studied. Unilateral injection of 20 µM CNQX solution (10 - 30 nl; 200 - 600 fmoles) into the pre-BötC produced a dose-dependent decrease in respiratory frequency and ultimately eliminated rhythm generation; MK-801 injections (up to 20 nl, 1 mM solution) did not perturb rhythm. CNQX injections 300 µm distant from the site of maximum response did not affect rhythm, confirming site-specificity. Unilateral injection of 20 µM CNQX (2 - 25 nl) into the hypoglossal motor nucleus produced a dose-dependent decrease in burst amplitude, and completely blocked the motor discharge of XII nerve ipsilaterally, without blocking rhythmic discharge on the contralateral nerve. MK801 (up to 50 nl, 1 mM) or AP4 (up to 50 nl, 1 mM) injected into the same site had no effect on XII motor discharge. These results indicate that endogenously released EAAs activating non-NMDA receptors are essential for rhythmogenesis in the pre-BötC, consistent with our hypothesis that depolarization of rhythm generating neurons mediated by EAAs is a necessary condition for rhythm generation. These EAAs may be released at synaptic connections between the rhythm generating neurons, and/or from synapses on these cells from a separate population of neurons. Synaptic transmission of rhythmic drive to hypoglossal motoneurons is also mediated primarily by non-NMDA EAA receptors, consistent with our previous results in *en bloc* brainstem preparations that these receptors mediate transmission in medullary synaptic pathways to cranial motoneurons (Greer *et al.*, J. Physiol. 437: 727-749, '91). Supported by NIH grants HL 40959 and HL 02204 and NSERC of Canada.

630.2

OSCILLATORY PROPERTIES OF NEURONS IN AN ISOLATED RESPIRATORY CIRCUIT ANALYZED BY WHOLE-CELL PATCH-CLAMP TECHNIQUES. J.C. Smith, K. Ballanyi*, D.W. Richter, & J.L. Feldman Systems Neurobiology Lab, UCLA, Los Angeles, CA 90024 & Physiol. Institut, Univ. Göttingen, 3400 Göttingen, F.R.G.

Slice preparations of the neonatal rat medulla have been developed that contain neurons generating respiratory rhythm and a local circuit for motor output generation (Smith *et al.*, Soc. Neurosci. Abs. 16: 1130, '90). These slices provide a novel tool for analysis of rhythm generation and oscillatory drive transmission in an isolated, functionally active motor network *in vitro*. Whole-cell patch-clamp recording techniques were applied to analyze oscillatory properties of neurons in the network. In the pre-Bötzinger Complex of the ventrolateral reticular formation, the region previously identified as the locus for rhythm generation (*ibid*; Smith *et al.*, Soc. Neurosci. Abs. 15: 505, '89), a population of neurons was identified with voltage-dependent pacemaker properties that are candidates for the rhythm generating neurons. These neurons generate 10 - 15 mV regenerative membrane potential oscillations and rhythmic bursts with a voltage-dependent oscillatory frequency when the membrane potential is depolarized into the -55 – -45 mV range under current clamp. When the network is rhythmically active, these neurons receive depolarizing inputs that synchronize the oscillation of the pacemaker neuron population, so that the rhythmic discharge of these cells results from the interaction of synaptic currents and the intrinsic pacemaker properties. Other respiratory neurons in the network do not exhibit intrinsic voltage-dependent oscillatory properties; their rhythmic discharge results from large amplitude synaptic drive currents (400 – 700 pA currents, reversal potentials near 0 mV under voltage clamp). These results are consistent with a model in which respiratory rhythm is generated by a population of coupled, synchronized conditionally bursting pacemaker neurons. The current-voltage properties of these neurons that promote oscillatory behavior also facilitate a rapidly propagating, synchronized depolarization within the pacemaker cell population. Supported by NIH HL 40959, HL 02204, DFG, & the Alexander von Humboldt Foundation.

630.3

PLATEAU-POTENTIALS CONTRIBUTE TO THE GENERATION OF RHYTHMIC DEPOLARIZATIONS IN LOCUST FLIGHT INTERNEURONS. J.M.Ramirez and K.G.Pearson, Dept. of Physiology, University of Alberta, Edmonton, Canada, T6G 2H7.

During flight in the locust the membrane potential of interneurons oscillates over a wide amplitude range (up to 25mV). We have found that in many interneurons (e.g. 308, 504, 514, 566 and 567) voltage-sensitive plateau-potentials contribute to the generation of these large oscillations. Hyperpolarizing currents injected into these interneurons caused the amplitude of rhythmic depolarizations to drop suddenly by up to 50% in both intact and deafferented flying locusts. Plateau-potentials could be triggered by short depolarizing pulses and prematurely terminated by short hyperpolarizing pulses. A characteristic feature of these potentials was that they were rarely expressed in the quiescent locust. In the absence of flight activity most neurons displayed only passive membrane properties in response to current injection. These data indicate that plateau-potentials are induced during flight activity. Induction of these active membrane properties during flight may depend on the natural release of octopamine or may be due to octopamine modulating active membrane properties induced by other neuromodulators. This is suggested by the findings that 1) octopaminergic neurons are activated at the onset of flight, and 2) exogenously applied octopamine induces plateau-potentials and endogenous bursting in quiescent locusts. The voltage-sensitivity, the amplitude and the duration of these octopamine induced plateau-potentials are similar to the potentials seen during flight, and the frequency of endogenous oscillations is close to that in deafferented flying locusts.
Supported by grants from the Medical Research Council of Canada and the Alberta Heritage Foundation for Medical Research

630.4

SEROTONIN DEPENDENT PERIPHERAL SPIKE INITIATION ZONES IN THE AXON OF A MOTOR NEURON. J.M.Weimann, P.Meyrand*, and E.Marder. Biology Dept., Brandeis Univ., Waltham, MA 02245 and Neurobiologie, CNRS, Arcachon, France.

The lateral gastric (LG) neuron of the stomatogastric ganglion (STG) in the crab, *Cancer borealis*, makes inhibitory synaptic connections within the neuropil of the STG, and also projects to the periphery where it innervates muscles that control the movements of the gastric mill. The LG motor neuron has a spike initiation zone close to its neuropilar integrative regions. Spikes initiated here evoke IPSPs in LG followers. During spontaneous gastric activity in semi-intact preparations (muscles attached) spikes can also be initiated at peripheral axonal sites ~2 cm from the LG soma. Peripherally initiated spikes propagate antidromically into the STG where they do not evoke IPSPs onto LG follower neurons and also propagate to the muscles to prolong the contraction of the gm5b muscle. When the muscles are removed, long depolarizations of the LG soma (>3 seconds) together with serotonin applied to the nerve, also evoke peripheral spike initiation. The activity of the peripheral zone can be controlled by current injection into the soma to either initiate or terminate the spikes, suggesting the presence of plateau-like properties in the LG axon. Thus, the LG neuron can send different signals to its peripheral and central targets.
Supported by NS17813.

630.5

A MODEL NEURON WITH A PERIPHERAL SPIKE INITIATION ZONE. L.F. Abbott*, Barry Feedman*, J.M. Weimann, T. Kepler and E. Marder. Center for Complex Systems, Brandeis University, Waltham, MA 02254.

We have constructed a multi-compartmental, conductance-based model to study the serotonin activated peripheral spike initiation zone found in the Lateral Gastric neuron of the stomatogastric ganglion in *Cancer borealis*. The model neuron consists of two large passive regions representing the soma and primary neurite connected to an axon and a secondary process both of which have active Hodgkin-Huxley sodium and delayed rectifier conductances. The peripheral spike initiation zone located distally on the axon is modelled with an additional current that can produce a plateau potential resulting in a burst of action potentials.

As in the experimental preparation, we find the following features in the model: 1) The orthodromic and antidromic action potentials appear highly attenuated when recorded from the soma and have different shapes. 2) The peripheral zone can be activated by action potentials propagating outward from the primary initiation zone. Activation may require anywhere from one to a large number of spikes. Once activated, the peripheral initiation zone can generate a number of spikes or it can fire continuously. 3) As well as travelling down the axon, orthodromic spikes propagate down secondary processes to inhibitory synapses. Antidromic spikes also propagate along the axon but they fail to propagate down secondary processes.

The third result shows that the postsynaptic effect of an action potential propagating along a neuron depends on the location of the spike initiation zone. Supported by MH46742

630.6

PILOCARPINE INDUCES RHYTHMIC ACTIVITY IN LEG MOTOR NEURONS IN ISOLATED THORACIC GANGLIA OF LOCUSTS. Sylvie Ryckebusch and Gilles Laurent. Computation and Neural Systems, California Institute of Technology, Pasadena, CA 91125.

We demonstrate that in isolated thoracic ganglia of the locust, *Schistocerca americana*, leg motor neurons will express rhythmic activity similar to that which occurs during walking in an intact animal. The centrally generated rhythm, induced by bath application of the muscarinic agonist pilocarpine, was recorded both intracellularly from leg motor neurons and extracellularly from leg motor nerves.

The observed rhythm appeared similar to walking in an intact animal, as motor neurons to antagonistic muscle groups in the metathoracic ganglion were active in antiphase, whereas motor neurons to synergistic muscle groups were active in phase. Levators of the trochanter were in phase with flexors of the tibia, and depressors of the trochanter were in phase with extensors of the tibia. The frequency of the observed rhythm was an approximately linear function of the concentration of pilocarpine, and saturated at about 0.2 Hz for a bath concentration of 10^{-4}M. This rhythm was thus slower than that underlying walking. The rhythms expressed by the left and right halves of a ganglion were not necessarily of the same frequency. Some lateral coupling between hemiganglia was observed, however, as depressors of the trochanter on one side were generally in phase with levators of the trochanter on the other.

The preparation we describe will be used to study sensory-motor interactions, since the walking rhythm can be induced reliably in a completely isolated preparation. Supported by the NIH and a Searle Scholarship to GL.

630.7

"HOT SPOTS" OF SPIKE-EVOKED CALCIUM ENTRY IN THE MAJOR NEURITES OF CRAB STOMATOGASTRIC NEURONS. K. Graubard, N. Lasser-Ross, D. Baldwin and W.N. Ross, Dept. of Zoology, Univ. of Washington, Seattle, WA 98195 and Dept. of Physiology, New York Medical College, Valhalla, NY 10595

Voltage-correlated changes in $[Ca]_i$ were examined in identified neurons of *Cancer borealis*, by using simultaneous electrical recording and high-speed imaging of Fura-2 fluorescence at 380 nm with a cooled CCD camera. The same somatic microelectrode was used to inject Fura-2, to inject current, and to record membrane potential. Sharp increases in $[Ca]_i$, time-locked to depolarizing potentials, were interpreted as due to entry through voltage dependent channels. Our results extend those previously obtained using arsenazo III and a photodiode array (Graubard and Ross PNAS *82*:5565-5569,1985; Ross and Graubard PNAS *86*:1679-1683,1989). Use of Fura-2 and the camera enabled us to estimate the magnitude of the changes in $[Ca]_i$ and to identify with greater spatial precision the sites of $[Ca]_i$ increase.

For all cells, bursts of intrasomatically evoked action potentials caused calcium entry into the soma and neuropil with distinctly higher levels in a small region of the main process (pre-axon) near the edge of the neuropil just before the axon entered a nerve. Cells with more than one axon had a "hot spot" on each pre-axon. During the spontaneous voltage oscillations and spike bursts of the pyloric rhythm, oscillations of $[Ca]_i$ were observed with the highest amplitudes at the "hot spots". In TTX saline, intrasomatic stimulation caused increases in calcium which were largest in the soma and decremented smoothly in the neuropil; no "hot spots" were seen.

We suggest that 1) during spontaneous bursting the mean variation in $[Ca]_i$ in the neuropil is small; 2) most spike-evoked synaptic transmission comes from the subset of neurites closest to the "hot spots"; 3) the calcium channel distribution along the largest neurites is roughly uniform; and 4) there are few calcium-permeant ion channels in the axon.

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630.8

REGIONAL DISTRIBUTION OF THE FINE NEURITES OF IDENTIFIED NEURONS OF THE CRAB STOMATOGASTRIC GANGLION. D. Baldwin and K. Graubard, Dept. of Zoology, University of Washington, Seattle, WA 98195.

The stomatogastric neuropil of crabs and lobsters can be divided into a central core containing the largest neurites, an intermediate region containing a mix of intermediate and fine neurites, and a peripheral neuropil containing mostly fine neurites (Baldwin and Graubard *Soc. Neurosci. Abstr.* **16**:724, 1990 and King *J. Neurocytology* 5:207-237, 1976). Regions containing fine neurites (fine neuropil) are the sites of intrinsic synaptic interactions and of extrinsic synaptic input (King, 1976). Neurons of the STG participate in two major motor patterns by using two interacting neural circuits. We have examined the regions of fine neurites in neurons that participate in one or both circuits to determine if the circuitry is reflected in a regional organization of the crab fine neuropil.

Identified stomatogastric neurons of *Cancer borealis* were injected with Lucifer Yellow, fixed and examined as whole mounts with a confocal microscope. In some cases, another dye was injected into a second neuron. For each cell, the neuropil was divided into radial arcs and the serial images scored based on the presence of fine neurites within each arc. This representation of the three-dimensional spread of the fine neurites of each neuron was compared with those of the other neurons. Examinations of PD and LP, purely pyloric neurons, and VD, a mixed pyloric/gastric neuron, show small differences in the distribution of regions of fine neurites.

Supported by N.I.H. Grants NS15697 and NS25505 to K. Graubard.

630.9

ELECTROPHYSIOLOGICAL PROPERTIES OF LAMPREY SPINAL NEURONS. J.T. Buchanan. Department of Biology, Marquette University, Milwaukee, WI 53233.

To provide information for locomotor network modeling, the following six classes of previously identified neurons were studied in the isolated spinal cord of adult *Ichthyomyzon unicuspis* with intracellular microelectrodes in normal Ringers: motoneurons (MN), lateral interneurons (LIN), cells with crossed, caudal axons (CC), excitatory interneurons (EIN), edge cells (EC), and giant interneurons (GI). The classes were generally not significantly different from one another in their resting potentials [for all cells (n=58) mean ± S.D. = -77.4±4.3mV], thresholds (-55.6±4.4mV), or action potentials (106.1± 8.5mV). The late after-spike hyperpolarizations were also similar except for the ECs which occurred earlier and were about twice as large as those in other cells. Cell classes varied considerably in their mean input resistances, consistent with known variation in soma sizes. CCs (71±47MΩ) and EINs (72±62MΩ) had the highest means and the most variance.

Most cells fired spikes throughout a 400ms depolarizing pulse with some tendency to adapt. MNs showed more adaptation than the interneurons. LINs and ECs had 20% higher peak first-interval frequencies (100Hz) than other cell types. When input current was normalized to rheobase, CCs had the largest range of firing (10X rheobase). ECs were unusual in their tendency to fire in doublets.

Overall, most resting and firing properties of these classes of lamprey spinal neurons were similar, except for ECs which are intraspinal stretch receptors. Within classes, CCs and EINs showed the most variation, probably indicating further functional diversities.

630.10

THE EFFECTS OF STRYCHNINE ON FICTIVE SWIMMING IN THE LAMPREY. D.R. McPherson and J.T. Buchanan. Department of Biology, Marquette University, Milwaukee, WI 53233.

Previous studies demonstrated that strychnine speeds fictive swimming in the isolated lamprey spinal cord, presumably by reducing glycinergic inhibitory synapses involved in rhythmogenesis. In contrast to these experimental findings, computer simulations of a proposed lamprey locomotor network display slowing of cycle rate when all inhibitory connections are reduced. The effect is dependent on cell type: reduction of only CC synapses (cells with crossed, caudal axons) speeds the rate, while reduction of only lateral interneuron (LIN) synapses slows the rate. This suggests that LIN ipsps may be insensitive to strychnine. This hypothesis is rejected as present results in silver lampreys show that strychnine abolishes the ipsps produced by LINs upon CC interneurons, while bicuculline has no effect.

The effect of strychnine on fictive swimming has therefore been re-examined. Confirming earlier reports, strychnine accelerates the swim rate in adult silver lampreys (*Ichthyomyzon unicuspis*), but in adult sea lampreys (*Petromyzon marinus*), low concentrations (0.5μM) of strychnine produce a slight speeding, while higher concentrations (2μM) elicit extremely slow alternating bursting (10% of control rate). The slow rate appears to developed as a progressive fusing of fast bursts into longer ones.

Thus, there appears to be a species difference in the response to strychnine during fictive swimming, and these results may indicate a need to revise the proposed locomotor network.

631.1

Effects of Ibotenate (IBO) and Quisqualate (QUIS) Lesions of the Nucleus Basalis Magnocellularis (NBM) on Cortical EEG. R.J. Radek, M.W. Decker, D.J. Anderson, M.J. Majchrzak. Neuroscience, Pharmaceutical Products Division, Abbott Laboratories, Abbott Park, IL. 60064

Lesions of the NBM produce deficits in various learning and memory tasks. The several lesion methods used to generate damage result in similar depletions of the cholinergic marker choline acetyltransferase (ChAT), but do not produce similar behavioral effects. For example, IBO lesions impair performance in the standard Morris water maze task, whereas QUIS lesioned rats are relatively unaffected. The purpose of this study was to compare the effects of these two NBM lesions on cortical EEG. Unilateral lesions were made with either IBO (0.06 M) or QUIS (0.12 M) in Long-Evans rats at coordinates (in mm); AP, -0.6, DV 7.5, Lat 2.8. Frontal-parietal EEG was recorded bilaterally and FFT calculated power spectrum analysis is expressed as percent change from the non-lesioned hemisphere. IBO lesioned rats had a significant 51% ($n=6$, $p<0.05$) increase of alpha (8-13 hz) power in the ipsilateral frontal-parietal cortex. Sham ($n=5$) and QUIS ($n=7$) rats had no significant increases or decreases of alpha power, although several QUIS rats did have moderate EEG changes in the ipsilateral cortex. ChAT activity in frontal-parietal cortex was decreased 34% ($p<0.05$) and 45% ($p<0.05$) with IBO and QUIS lesions, respectively. These data may indicate that IBO and QUIS produce differential basal forebrain injury and may be distinguished on the basis of cortical electrical activity.

631.2

COHERENT 40-HZ RESPONSES DURING AUDITORY PROCESSING IN THE HUMAN BRAIN.
U.Ribary, R.Llinas, F.Lado, A.Mogilner, R.Jagow* and L.Lopez. Center for Neuromagnetism, Department of Physiology and Biophysics, New York University Medical Center, New York, N.Y 10016, USA.

A 14- and a 37-channel MEG system (BTi) were used, in order to analyze the spatial and temporal organization of magnetic 40-Hz activity during auditory processing. MEG data was recorded over the right cerebral hemisphere (from 35-37 positions) of 5 normal humans (20-35 years old), before and during an auditory stimulation. The stimuli comprised frequency modulated tones starting at any frequency between 50-350 Hz. This modulation swept randomly upwards or downwards within this range. Responses were averaged and analyzed using various band-pass filters (5-15, 15-25, 25-35, 35-45 and 45-55 Hz). MEG data indicated synchronized activity at around 40-Hz over the entire hemisphere during auditory processing. This activity was coherent over large cortical areas and demonstrated a phase shift from the frontal to the occipital pole. This spatio-temporal pattern, which consisted of a positive/negative field, showed a coherent rostro-caudal sweep lasting for 4-8 msec, repeating every 12.5 msec. Similar magnetic field patterns were also observed in non-averaged single epochs using the 37-channel system. As in the averaged records, the pattern was absent when filtering above or below the 40-Hz band.

Our MEG data suggests that the 40Hz sweep is a major mechanism in the global organization of brain activity, supported by cortico-thalamic pathways, with a focus on the activated sensory area.

631.3

NETWORK MODULATION OF AN INTRINSIC DELTA (0.5-4 HZ) RHYTHM IN THALAMIC NEURONS. M. Steriade, R. Curró Dossi and A. Nuñez. Lab. Neurophysiol., Sch. Med., Univ. Laval, Quebec, Canada G1K 7P4.

Two questions have been addressed in this study: could slowly (0.5-4 Hz) oscillating single thalamocortical neurons become synchronized?; and, what are the potentiating and suppressing modulatory systems of this slow oscillation? Intra- and extracellular recordings of identified cortical-projecting cells were performed in cats under urethane anesthesia. At the resting Vm (-60 mV) cortical stimulation induced spindles (7-14 Hz), whereas at Vm more negative than -65 mV the same stimuli induced delta oscillations (low-threshold spikes and after-hyperpolarizations recurring rhythmically at 0.5-4 Hz). Auto- and crosscorrelograms of neuronal pairs revealed that unrelated cells became synchronized after a series of corticothalamic volleys, with both neurons displaying rhythmic (1-2 Hz) spike bursts. This effect probably involved inhibitory inputs from cortical-driven GABAergic thalamic cells. Stimulation of either mesopontine cholinergic nuclei or specific sensory channels blocked the delta oscillation by depolarizing thalamic cells, thus bringing them out of the voltage range of this slow rhythm.

We postulate a progressive hyperpolarization of thalamic cells with the deepening of quiet sleep, which would account for spindling during early stages and delta waves during late stages. By contrast, brainstem and/or sensory depolarizing inputs would explain the delta blockage at the transition from slow-wave sleep to either arousal or REM sleep. Supported by MRC grant MT-3689.

631.4

DELTA OSCILLATIONS IN PERIGENICULATE (RETICULAR) THALAMIC NEURONS. F. Amzica, A. Nuñez and M. Steriade. Lab. Neurophysiol., Sch. Med., Univ. Laval, Quebec, Canada G1K 7P4.

Cortical inputs potentiate a hyperpolarization-activated slow thalamic rhythm (0.5-4 Hz) and synchronize oscillatory relay neurons that are otherwise uncoupled (Steriade et al., this meeting). Since these facilitatory/synchronizing actions were exerted by volleys arising in cortical areas that were not directly related to the explored thalamic nucleus, we hypothesized that they were due to the conjunction properties of the interposed reticular nuclear complex. We have recorded extra- and intracellularly perigeniculate (PG) thalamic cells of cats under urethane anesthesia. A subpopulation of physiologically-identified PG cells oscillated spontaneously with spike bursts recurring at delta frequencies (between 0.5 and 4 Hz), most often at 3-4 Hz. The oscillation was voltage-dependent. Indeed, it was blocked by depolarizing current pulses or changes in the ambient light eliciting tonic firing, and by bringing the membrane potential below -90 mV. PG neurons displayed delta oscillation despite the fact that they did not possess the I_h current which is required for this oscillation in thalamocortical cells (see Curró Dossi et al., this meeting). As opposed to the barbiturate-induced blockage of delta rhythmicity in thalamocortical cells (Nuñez et al., this meeting), the slow oscillation in PG cells was not sensitive to barbiturates. These data support the hypothesis that PG or other reticular thalamic cells are involved in the cortical-induced potentiation of thalamic delta oscillation. Supported by MRC grant MT-3689.

631.5

SPINDLE OSCILLATIONS PREVENT DELTA OSCILLATIONS IN INTRACELLULARLY RECORDED THALAMOCORTICAL CELLS OF CAT. A. Nuñez, R. Curró Dossi and M. Steriade. Lab. Neurophysiol., Sch. Med., Univ. Laval, Quebec, Canada G1K 7P4.

The interaction between spontaneous spindle (7-14 Hz) and delta (0.5-4 Hz) rhythmic activities of thalamic neurons was studied intracellularly in antidromically identified cortical-projecting cells of cats under urethane anesthesia. Thalamic spindling was induced by cortical stimulation or by barbiturate administration. In other experiments *cerveau isolé* (collicular-transected) preparations were performed to induce spontaneous spindling in the absence of anesthetics. Oscillations within the frequency range of EEG delta waves were present at Vm between -68 to -90 mV, appearing as rhythmic low-threshold spikes alternating with afterhyperpolarizing potentials. These voltages could be reached by inward current injection or after removal of related cortical areas. In both cases the occurrence of spontaneous as well as barbiturate- or cortical-induced spindle sequences prevented delta oscillations, the effect being accompanied by an increase in membrane conductance.

This competitive interaction between spindle and delta oscillations may explain the prevalent appearance of the former and the latter EEG rhythmicity during early and late sleep stages, respectively. Supported by MRC grant MT-3689.

631.6

ELECTROPHYSIOLOGY OF A SLOW (0.5-4 Hz) INTRINSIC OSCILLATION OF CAT THALAMOCORTICAL NEURONS IN VIVO. R. Curró Dossi, A. Nuñez and M. Steriade. Lab. Neurophysiol., Sch. Med., Univ. Laval, Quebec, Canada G1K 7P4.

An intrinsic oscillation in the frequency range of sleep EEG delta waves (0.5-4 Hz) has been studied in cat thalamocortical neurons. Antidromically identified cortical-projecting cells have been recorded intra- and extracellularly in most major thalamic nuclei under urethane or nitrous oxyde-halothane anesthesia. About 80% of cells with intact cortical connections displayed the slow (delta) oscillation as their membrane potential was brought between -68 and -90 mV by DC. The oscillation consisted of rhythmic low-threshold spikes and after-hyperpolarizations, and it was not dependent upon the occurrence of fast action potentials.This rhythm, involving an interplay between the hyperpolarization-activated cation current (I_h) and the low-threshold transient Ca++ current (I_t), was similar to that reported by McCormick & Pape (1990) in a subpopulation of dLG cells in vitro. The oscillatory activity was spontaneously present in virtually all neurons anatomically or functionally disconnected from related cortical areas (Vm > -65 mV), in which case it could be blocked by DC depolarization. Under these experimental conditions focal delta waves built-up during epochs with synchronized rhythmic multiunitary firing. Only anterior thalamic neurons did not display a delta oscillatory capability.

We hypothesize that this intrinsic phenomenon contributes to switch the activity of thalamocortical neurons toward slow delta waves, as their membrane potential progressively hyperpolarizes during late stages of sleep. Supported by MRC grant MT-3689.

631.7

TOPOGRAPHICAL ORGANIZATION OF THE AFFERENT CONNECTIONS TO THE CAUDAL SUBDIVISION OF THE LP-PU THALAMIC COMPLEX. M.L. Rodrigo-Angulo and F. Reinoso-Suárez. Departamento de Morfolgía. Facultad de Medicina. Universidad Autónoma de Madrid. Spain.

The caudal subdivision of the lateral posterior-pulvinar thalamic complex (LP-Pu) has been defined histochemically and cytoarchitectonically in the cat, as formed by the lateralis posterior medialis (LPM), lateralis posterior lateralis (LPL), posterior part of the pulvinar (Pu) and lateralis medialis (LM) nuclei. In this study we have distinguished LPM, LPL and Pu on the retrograde cell labeling techniques. Small amounts of a horseradish peroxidase solution (20-40 nl) were injected in LPM (4 cats), LPL (4 cats) and posterior Pu (2 cats) by means of a glass micropipette. After injections in LPM, cortical neurons averaged 70% of the total labeled neurons in the brain and were homogeneously distributed in areas 17, 18, 19, 20, 21, posteromedial lateral suprasylvian (PmLS) and splenial visual (Sp); subcortically labeled neurons were located mainly in the superficial layers of the superior colliculus (SCs) and in the reticular thalamic and ventral lateral geniculate nuclei (RT and GLV), while only scattered neurons were found in locus coeruleus, parabrachial nuclei and oral pontine reticular nucleus. After injections in LPL, cortical labeled neurons averaged 93% and were very abundant in area 17, less numerous in areas 18, 20, 21, posterior suprasylvian (Ps), dorsal lateral suprasylvian, ventral lateral suprasylvian, PmLS and Sp, and scarce in area 7; subcortically labeled neurons occupied mainly the RT and GLV, and the SCs, beeing just a few in the pretectal region (Pt). After injections in posterior Pu a large amount of labeled neurons (89%) was detected in the cortex, mainly in area 7; fewer were found in areas 18, 19 20, 21, Ps, and in all visual areas associated to the suprasylvian sulcus and gyrus, as well as in Sp, and some appeared in areas 35 and 36; subcortically labeled neurons were located in RT, GLV, Pt and SCs. These results confirm that the nuclei of the caudal subdivision of the LP-Pu show a distinct and topographically organized linkage with different visual cortical areas and subcortical structures.

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631.8

THALAMIC CONNECTIONS OF THE ORBITOFRONTAL CORTEX OF MACAQUES RELATED TO ACETYLCHOLINESTERASE ACTIVITY PATTERNS. C. Cavada, T. Company*, and F. Reinoso-Suárez. Dept. Morfología, Fac. Medicina, Univ. Autónoma de Madrid, Spain.

The thalamic labeling resulting from axonal transport of tracers injected in the orbitofrontal cortex of macaques was compared with the patterns of staining present in the thalamus of adjacent sections processed to reveal AChE activity. Eleven experiments were performed using single or multiple labeling strategies. Aggregates of labeled neurons or terminals were present in the poorly stained anteromedial and paratenial nuclei, and were characteristically in register with the AChE-poor domains in the mediodorsal nucleus (MD). Labeling was also present in a moderately dense AChE zone in dorsal MD. By contrast, the most intensely AChE-stained zone of the ventral anterior nucleus, surrounding the mammillo-thalamic tract, showed a dense aggregation of labeled neurons and terminals. Also the ventromedial part of the paracentral nucleus, which exhibits very rich and uniform AChE staining, was labeled following the orbitofrontal injections. Other nuclei exhibiting high AChE activity and containing labeled neurons included the parafascicular, limitans, and the paraventricular, reuniens, and central (densocellular, latocellular, superior, intermediate, and inferior divisions) midline nuclei. In the medial pulvinar nucleus, labeling was assembled both within the medial part of the AChE-poor matrix, and in close register with patches of moderate AChE staining located in the mediodorsal part of the nucleus. These findings indicate that the thalamic connections of the orbitofrontal cortex are organized according to differential histochemical patterns. This feature must be considered, together with cytoarchitectonic and connectional attributes of the various thalamic nuclei, in understanding the thalamic connections of the prefrontal cortex.

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631.9

LATERALITY OF INPUT FROM CORTICALLY PROJECTING EXTRATHALAMIC NUCLEI. S.E. Choe*, C. Geula and M-M. Mesulam. Harvard University, Boston, MA.

The laterality of cortical projections from several extrathalamic nuclei which give rise to widespread corticopetal fibers was investigated in the rat. Horseradish peroxidase was injected into two primary sensory cortical areas (somatosensory cortex and visual cortex/area 17) and two limbic areas (dorsal hippocampus and amygdala). Observations in the basal forebrain (Ch1-Ch4), hypothalamus, ventral tegmental area, raphe and locus coeruleus revealed labelled neurons predominantly ipsilateral to the injection site, with varying degrees of contralateral labelling. Overall, the raphe showed the highest percentage of contralaterally labelled neurons (35% of all labelled neurons), while the basal forebrain cholinergic nuclei showed the lowest percentage (less than 1%). In the locus coeruleus, contralateral labelling was considerably higher after limbic cortical injections than after sensory cortical injections. The other nuclei examined in this study did not show consistent differences of laterality in their projections to limbic vs sensory cortex. The laterality of extrathalamic corticopetal projections thus varies from one nucleus to another and, in the case of the locus coeruleus, also as a function of the target projection site.

631.10

PROJECTIONS FROM THE LATERODORSAL NUCLEUS OF THE THALAMUS TO THE LIMBIC AND VISUAL CORTICES. T. van Groen and J.M. Wyss. Dept of Cell Biology, University of Alabama, Birmingham, AL 35294

Previous studies suggest that the laterodorsal nucleus (LD) of the thalamus projects to both retrosplenial cortex and adjacent visual cortical area 18b. In this study we used the very selective, anterograde tracer *Phaseolus vulgaris* leucoagglutinin (PHA-L) to characterize the projections from LD in detail. A large injection of PHA-L into the middle part of LD labels axons and terminals in caudal infraradiata (IRβ), area 18b, retrosplenial agranular (Rag), retrosplenial granular b (Rgb), retrosplenial granular a (Rga), postsubicular, presubicular and parasubicular cortices. Small terminal fields also are present in the precentral agranular, entorhinal and perirhinal cortices. Smaller injections demonstrated that the LD projection is topographically organized. The projections to IRβ arise from caudoventral parts of LD, predominantly from the medial regions. Rgb receives projections from caudomedial parts of LD, but Rga receives projections only from the rostromedial part of LD. Area 18b is projected upon by medial caudoventral parts of LD. Postsubiculum receives projections from caudal parts of LD. Presubiculum and parasubiculum receive projections from medial parts of LD. Further, the terminations of LD have a distinct laminar pattern in each cortical region. In the visual, Rag, and postsubicular cortices the LD axons end in layers I and III/IV, in Rgb and Rga the terminals are confined to layer I, whereas in presubiculum and parasubiculum and the adjacent entorhinal and perirhinal cortices the terminals are distributed in the deep layers (i.e., layers IV-VI). These results demonstrate that LD projects to retrosplenial, hippocampal, and visual cortices, and that these projections are topographically organized.

631.11

DISTRIBUTION OF CALRETININ IMMUNOREACTIVITY IN MONKEY CEREBRAL CORTEX. D.A. Lewis, J.S. Lund, M. Akil, and D.M. Jacobowitz. Depts. of Psychiatry and Behav. Neurosci., Univ. of Pittsburgh, Pittsburgh, PA 15213 and Lab. of Clinical Science, NIMH, Bethesda, MD 20892.

The calcium binding proteins, parvalbumin (PV) and calbindin D-28k (CB), are present in morphologically distinct subpopulations of nonpyramidal neurons in monkey neocortex (*PNAS* 83:2093; *Exp Br Res* 76:476, 1989; *JCN* 293:599, 1990). In this study, we used immunohistochemical techniques to characterize the distribution of immunoreactivity for a related calcium binding protein, calretinin (CR), in cerebral cortex of infant and adult rhesus (*Macaca mulatta*) monkeys. Most CR-immunoreactive (IR) neurons were small nonpyramidal cells with a variety of morphologies; many of these neurons had vertically oriented, oval cell bodies which gave rise to a single ascending and/or descending dendrite. In contrast to CB, CR immunoreactivity was not detected in pyramidal neurons. The greatest density of CR-IR neurons was in layers II-superficial III; a lower density band of labeled neurons was present in deeper cortical layers. Many association regions of cortex contained a high density of radially arrayed CR-IR processes. The morphology and laminar distribution of these fibers appeared to be distinct from that of CB-containing axon fascicles. In some regions, CR-IR axon terminals formed clusters that outlined the somata and proximal dendrites of a subpopulation of pyramidal neurons; the distribution of these pericellular arrays differed from that of PV-IR pericellular baskets. These findings suggest that the role of CR in primate cortical circuitry may be distinct from that of other calcium binding proteins.

631.12

LOCAL CIRCUIT NEURONS OF THE PRIMATE PREFRONTAL CORTEX: AREAS 9 AND 46. **J.S. Lund, K.M. Oeth, and D.A. Lewis, Dept. of Psychiatry and Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15261**

Human neuropsychiatric disorders, such as Alzheimer's disease and schizophrenia, appear to be disturbances of complex behaviors that may reflect disruption of neural circuitry in the prefrontal areas of the cerebral cortex. We have used Golgi and immunocytochemical approaches to characterize morphology, laminar distribution and biochemical specialization of nonpyramidal neurons of rhesus monkey prefrontal cortex. In Golgi impregnations, these neurons can be divided into categories on the basis of their axon distributions: vertically oriented (often a column no more than 100-150um wide), local (innervating 1-2 laminae, with an arbor 200-300um wide) and laterally spreading (up to at least 1mm). Within each of these categories, very different morphological types are found, often with their cell bodies broadly distributed in cortical depth. Matches between classes of Golgi impregnated and immunocytochemically labelled neurons have been made in some instances. For example, neurons making local beaded arbors (found in layers 2-5) may contain calretinin; neurons making local complex pericellular arbors (layers 4-6) and neurons with vertically oriented axon trunks with short curved collaterals (layers 2-4) may both express cholecystokinin; chandelier neurons (layers 2-5) express parvalbumin and corticotropin releasing factor (Lewis and Lund, '90). Probably most of these classes are also GABAergic. These findings begin to reveal the diversity of non-pyramidal cell types in prefrontal cortex with some suggestion of the post-synaptic components with which they may interact. This work is supported by MH45156 and MH00519.

631.13

CHOLECYSTOKININ-IMMUNOREACTIVE NEURONS IN MONKEY VENTRAL MESENCEPHALON PROJECT TO PREFRONTAL CORTEX BUT DO NOT CONTAIN TYROSINE HYDROXYLASE. K.M. OETH AND D.A. LEWIS. Depts. of Behavioral Neuroscience and Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA, 15213.

In rodents, cholecystokinin (CCK)-immunoreactive (IR) neurons in the ventral mesencephalon (VMC) are known to contain tyrosine hydroxylase (TH) and to project to prefrontal cortex (*J Comp Neurol* 279:397, 1989; *J Comp Neurol* 279:415, 1989). In primate VMC, some CCK-IR neurons do not contain TH (*PNAS* 87:8427, 1990). We have observed that the distribution of these CCK-positive/TH-negative cells in monkey (*Macaca fascularis*) VMC follows a rostrocaudal gradient. For example, at very rostral levels, CCK-IR cells are present medially in an area that is almost totally devoid of TH-positive neurons. In more caudal regions of the VMC, the location of CCK-containing neurons overlaps with the position of TH-IR cells, although CCK-positive/TH-negative neurons are still observed. We have previously found that CCK-containing cells in monkey VMC project to prefrontal cortex; however, it is not known if these neurons belong to the class of CCK-positive/TH-negative neurons. Using combined retrograde transport techniques and immunohistochemistry, we found that CCK-containing cells in the VMC which project to prefrontal cortex do not contain TH. These observations were confirmed in double labeling studies in prefrontal cortex which also demonstrated a lack of colocalization of CCK and TH in fibers or terminal fields. These findings demonstrate that a population of CCK-containing neurons in the VMC has the capacity, independent of dopamine, to directly influence prefrontal cortical function.

631.14

DOPAMINERGIC (DA) AND NORADRENERGIC (NA) INNERVATION OF THE RAT FRONTAL CORTEX. F.Condé (1), B.Onteniente (2), M.Geffard (3) and F.Crépel (1). 1:CNRS URA 1121, Bat.440, Univ. Paris XI,91405-Orsay, 2: INSERM U 161, Hôpital Henri Mondor, 94010-Créteil, 3: INSERM CJF 88-13, Univ. Bordeaux II, 33076 Bordeaux, France.

In this study we compared on serial adjacent sections the distribution of NA and DA innervation of the rat frontal cortex, as revealed with respectively polyclonal and monoclonal antibodies directed against NA- or DA- glutaraldehyde-protein conjugates (Geffard et al. Brain res., 1986, 363:395-400; Chagnaud et al., J. Neurochem., 1987, 49:487-494). Our results confirmed and extended previous results obtained with different techniques, and emphasized neuroanatomical differences between DA and NA innervation. Regional differences included medio-ventral orbital cortex, in which density of DA and NA fibers was lower than in prelimbic area (PL) and ventro-lateral orbital area in which density of DA fibers is weak whereas NA fibers are numerous; Laminar differences were observed in PL where NA tangential fibers mostly run in the middle part of layer I, whereas DA tangential fibers are mostly in the outer part of layer I. These results suggest morphofunctional differences for NA and DA in frontal areas. A computerized quantitative study of density of NA and DA fibers will be further attempted.

631.15

CHOLINERGIC SYNAPTIC ARRANGEMENTS IN THE PRIMATE PREFRONTAL CORTEX. L. Mrzljak*, C. Leranth and P.S. Goldman-Rakic. Sect. of Neurobiology and Dept. of Obstetrics and Gynecology, Yale Univ. Sch. of Med., New Haven, CT 06510.

The prefrontal cortex (PFC) contains neural circuits engaged in working memory and it is of interest to determine how the cortical cholinergic innervation may influence this circuitry. Therefore, cholinergic synapses in areas 9 and 46 of the PFC of three adult rhesus and two cynomolgus monkeys were visualized using a monoclonal antibody against choline acetyltransferase (ChAT; provided by Dr B. Wainer). In addition, a double labeling electron microscopic (EM) method was used in which immunoperoxidase reactive cholinergic synapses were visualized in contact with immunoferritin labeled GABAergic nonpyramidal neurons. Cholinergic synapses in contact with morphologically identified pyramidal neurons were also characterized at the EM level.Cholinergic synapses were found in all layers of the PFC and all synaptic contacts were symmetric. ChAT positive profiles were in synaptic contact with both pyramidal and GABAergic neurons. The predominate postsynaptic targets on pyramidal neurons were apical and basal dendritic shafts and less frequently dendritic spines. Cholinergic terminations on GABA-immunoreactive neurons were restricted to dendritic shafts. These results are the first demonstration of cholinergic synapses in the primate association cortex and are in accord with physiological data showing that cholinergic axons influence principal neurons in the cortex both by direct excitation and indirectly via feedforward inhibition by GABAergic interneurons. These findings elucidate an anatomical substrate for acetylcholine's modulatory role in cortical function. Supported by MH 44866 and NS 26068.

631.16

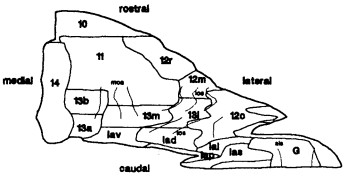
SINGLE UNIT ACTIVITY IN PREFRONTAL CORTEX AND MEDIODORSAL THALAMUS IN THE RAT: BEHAVIORAL CORRESPONDENCE AND EFFECTS OF ETHANOL. Kosobud, A.E., and Chapin, J.K. Hahnemann University, Philadelphia, PA, 19102, USA.

Using chronically implanted microwire electrodes, it is possible to obtain repeated recordings simultaneously from multiple neurons in awake, behaving animals. In the present experiments, multiple bundles containing 4-6 microwire electrodes were chronically implanted in MD thalamus and various sites in medial frontal and prefrontal cortex female rats, 250-300 g. Prefrontal and frontal cortex play a role in planning and organization of behavior, suppression of competing impulses, and in short-term task-related memory. These areas may be important sites for ethanol action, especially its disinhibiting and rewarding properties. Initially, animals were trained to walk on a treadmill with an intermittent cycle. Onset of treadmill movement was signalled by a fixed time interval or a tone cue. In subsequent experiments, rats were trained to press a lever to terminate treadmill movement. A videocamera was used to record behavior throughout experimental sessions. Firing rates were analyzed for individual cells, and in addition, factor analysis was used to assess ensemble properties of multiple neurons. In general, cells developed activity related firing rates, with higher rates of firing either during locomotion (treadmill on) or rest (treadmill off) in the course of an experimental session. Factor analysis suggested that activity levels among the ensemble of cells anticipated onset of the treadmill, and coded initiation and cessation of movement. In the initial 10-20 minutes of intoxication, EtOH consistently reduced or eliminated the difference in firing rate between locomotion and rest. In later stages, cells generally regained their original pattern of firing, but in one case, a cell switched from a higher rate during treadmill on to a higher rate during treadmill off. Supported by grants NS26722, AA06965, K02-AA00089 and AFOSR 90-0266

631.17

ORBITAL PREFRONTAL CORTEX IN THE MONKEY: STRUCTURALLY DISTINCT AREAS WITH SPECIFIC INPUTS CONNECTED IN PARALLEL NETWORKS. S.T. Carmichael and J.L. Price, Dept. Anat. & Neurobiol., Wash. Univ. Sch. Med. St. Louis, MO 63110

An analysis of Nissl, myelin, AChE and immunohistochemical staining patterns of the orbital cortex and rostral insula of Macaca fascicularis indicates that these two regions encompass 17 distinct areas, as seen in the following unfolded map.



Thirty-five injections of anterograde and retrograde tracers placed within these areas in fourteen monkeys indicate that each area receives a distinct set of limbic, sensory-related or multisensory connections. The limbic input is provided by the amygdala to the agranular insula areas (lav, lad, lal, lap, las) and 12o, 12m and 12l laterally and 11, 13a, 13b and 14 medially; and by the subiculum to areas 11, 13a, 13b and 14. Three areas receive sensory-related inputs: 12m somatic sensory input from SI, SII and 7b; 12l visual input from TE; and lav olfactory input from piriform cortex. Two areas receive a combined sensory input: 13l receives olfactory, gustatory and somatosensory input and 13m receives olfactory, somatosensory and possibly visceral input. lad and 12o receive multimodal input from the dorsal bank of the superior temporal sulcus. These areas then participate in a network of connections within the PFC, with specific projections to the medial wall, area 11 and the principal sulcus. Supported by NIH grant DC00093.

631.18

FUNCTIONAL CORRELATES OF NEGLECT PRODUCED BY UNILATERAL LESIONS OF MEDIAL AGRANULAR CORTEX IN RATS. J.V. Corwin, V. King, D. Paulson*, R.T. Watson*, K. Heilman* and R.L. Reep. Dept. of Psychology, Univ of New Orleans, New Orleans, LA 70148; and Depts. of Neurology and Physiol. Sciences, Univ. of Florida, Gainesville, FL 32610.

Our previous work has demonstrated that unilateral destruction of the medial agranular cortex (AGm), in rodents produces severe multimodal neglect. The present study examined the physiological correlates of severe neglect through the use of 14[C]2-deoxyglucose methodologies.

The functional correlates of neglect were examined in rats which received unilateral lesions of either the left AGm or the left lateral agranular cortex (AGl). Unlesioned controls were also examined. The physiological procedures were performed 4-6 days postop., after the rats were tested for the presence of neglect. Glucose utilization was determined by optical densitometry.

The results indicated that glucose utilization was significantly altered in rats with severe neglect produced by unilateral AGm lesions. Glucose utilization was significantly reduced ipsilesionally in the posterior striatum, the superior colliculus, and thalamus in the nuclei MD and ventralis lateralis. In the cortex glucose metabolism was significantly reduced in the AGl and retrosplenial cortices. These results generally support the pattern of glucose utilization found following physiological studies of frontal neglect in primates.

Funded by LEQSF grant RD-A-17 to JVC.

631.19

POSTERIOR PARIETAL CORTEX IN RATS: BEHAVIORAL ATTRIBUTES AND THALAMIC AND CORTICAL CONNECTIONS. H.C. Chandler*, R.L. Reep, V.R. King and J.V. Corwin. Depts. of Physiol. Sciences and Neurosurgery, Univ. of Florida, Gainesville, FL 32610; and Dept. of Psychology, Univ. of New Orleans, New Orleans, LA 70148.

Our previous anatomical work has demonstrated that a posterior area of parietal cortex lying just lateral to the cingulum bundle is reciprocally connected with medial agranular cortex and ventrolateral orbital cortex. Unilateral lesions of either of these latter two areas produces contralateral multimodal neglect. We now report that severe contralateral neglect also results from unilateral lesions selective to this posterior parietal area.

To delineate the connectivity of this posterior parietal field, rats received microinjections of three different fluorescent tracers, spaced in the anteroposterior or mediolateral direction. Within the a-p series, all cases had cortical labeling in medial agranular and orbital cortex. Thalamic labeling involved the ventral posterolateral, lateral dorsal/lateral posterior, lateral and medial geniculate nuclei. More rostral injections produced heavier labeling in vpl and none in lgn, typical of somatic sensory cortex, while more caudal injections resulted in the reverse, typical of visual areas; all three injection locations yielded labeling in ld/lp. Within the m-l series, more medial injections produced thalamic labeling which included the anterior thalamus, typical of retrosplenial cortex. Middle and lateral placements did not differ in thalamic and cortical labeling patterns. We conclude that a discrete posterior parietal area exists in the rat, based on the behavioral findings related to multimodal neglect, and on a combination of cortical and thalamic labeling patterns.

Funded by Univ. of Florida College of Veterinary Medicine (RLR) and Dept. of Neurosurgery (HCC), and grant LEQSF RD-A-17 (VRK, JVC).

631.20

CORTICAL SENSORY AND LIMBIC INPUTS TO DISCRETE AREAS OF THE SUPERIOR TEMPORAL SULCUS IN THE RHESUS MONKEY. B. Seltzer and D.N. Pandya. Depts. of Psych. and Neurol. and Anatomy, Tulane Univ. Sch. of Med., New Orleans, LA 70118 and V.A. Hosp., Bedford, MA 01730.

Afferent cortical connections of individual sectors of the superior temporal sulcus (STS) were studied using retrograde tracers. The rostral portion of area TPO in the upper bank receives input from the rostral superior temporal gyrus (STG), circular sulcus, perirhinal cortex, and rostral parahippocampal gyrus (area TH). Mid-TPO receives input from the mid-STG, caudal-most inferior parietal lobule, cingulate gyrus (area 23), and retrosplenial cortex. Caudal TPO has afferent connections with the caudal STG, caudal insula and circular sulcus, caudal parahippocampal gyrus (areas TF,TH,TL), lower bank (area LIP) and rim of the intraparietal sulcus, parietal operculum, areas 23 and 24, retrosplenial cortex, medial parietal cortex, and medial extrastriate areas. Cortex in the rostral lower bank of the sulcus (areas TEa and TEm), by contrast, receives input from inferotemporal cortex and the parahippocampal gyrus. Thus discrete subdivisions of the STS have distinct inputs from cortical sensory and paralimbic areas.

631.21

IS THE CORTEX OF CAT ANTERIOR ECTOSYLVIAN SULCUS A POLYSENSORY AREA? H.R. Clemo, M.A. Meredith, M.T.Wallace and B.E. Stein, Depts. Physiology and Anatomy, Medical College of Virginia, Virginia Commonwealth Univ. Richmond, Va 23298.

The anterior ectosylvian sulcus (AES) has been considered a polysensory/multisensory cortical region where convergence of different sensory inputs onto individual neurons occurs. However, three different modality-specific regions have been identified in the AES, suggesting that the degree of multisensory convergence may be less extensive than expected. Therefore, the AES cortex of 9 cats was examined to determine the extent of multisensory convergence. The vast majority (93%, 499/537) of sensory neurons encountered proved to be unimodal and visual, auditory, and somatosensory neurons were segregated from one another: somatosensory in the rostral dorsal bank, visual on the caudal ventral bank, auditory on the caudal dorsal bank which corresponded closely to the identified regions of SIV, EVA and Field AES. Multisensory neurons constituted only about 7% (38/537) of the population, and were found primarily near the borders of adjoining modality-specific fields. Given the paucity of multisensory convergence here it seems inappropriate to retain the designation 'polysensory' cortex. Rather, the AES is best regarded as a composite of three different unimodal areas flanked by strips where sensory convergence sometimes occurs.

Supported by BNS 8719234, NS 22543, NS 08902.

631.22

INTEGRATING THE DIFFERENT SENSES IN NEURONS FROM CAT ASSOCIATION CORTEX (ANTERIOR ECTOSYLVIAN SULCUS) M.A.Meredith, M.T.Wallace and B.E.Stein, Depts. Anat. & Physiol. Medical College. Va., Va. Commonwealth Univ., Richmond, VA.

The rules by which neurons integrate information from different senses have been identified in the superior colliculus (Meredith and Stein, J.Neurophysiol 56:640 '86) and their role in multisensory orientation behaviors have been demonstrated (Stein et al., J.Cognit.Neurosci 1:12 '89). However, their general applicability across the neuraxis is not known. The present experiments examined this issue in single neurons in the cortex of cat anterior ectosylvian sulcus. Among the multisensory neurons encountered (n=24/156), all combinations of visual, auditory and somatosensory inputs were identified. When stimuli from different modalities were combined, the responses were dramatically enhanced or depressed. These interactions were often multiplicative and were dependent on the same factors evident in the superior colliculus: receptive fields for the different modalities were spatially aligned; combined stimuli presented within their respective receptive fields evoked response enhancement; a stimulus falling outside its receptive field either depressed or had no effect on an effective stimulus from another modality; the temporal window for integration far exceeded the duration of the unimodal discharge trains; maximal levels of enhancement occur when stimulus onset is adjusted to overlap the peak periods of the discharge trains. These data indicate that the rules governing multisensory integration are independent of structure and apply to neural and behavioral processes throughout the CNS. Supported by NS 22543.

LEARNING AND MEMORY—PHARMACOLOGY: OTHER II

632.1

LEARNING INDUCED CHANGES IN mAChRs IN RAT BRAIN REVEALED BY IN-VIVO (125 I)-4-IODODEXETIMIDE BINDING Ş.Pöğün,U.Scheffel, A.A.Wilson*,M.Stathis*,C. Stainert*,R.F.Dannals*,and H.N.Wagner,Jr.*.Dept.of Physiology,Ege University,İzmir 35100,Turkey and Dept. of Radiology,The Johns Hopkins Medical Inst.,Baltimore MD 21205

The central cholinergic system has been implicated in learning and memory processes. I-125-4-Iododexetimide (I-125-DEX) is a high affinity muscarinic cholinergic receptor (mAChR) antagonist which has been shown to bind to central mAChRs in-vitro and in-vivo. The present study was undertaken to study in vivo I-125-DEX binding to mAChRs in rat brain after learning trials. 11 male Long-Evans rats were trained in a T-maze for a two choice spatial discrimination task;5 controls were handled only. Of tha 11 rats, 6 made more correct choices in more than 55% of the total trials, 5 scored lower. Accordingly, the animals were grouped into good and poor performers. After w2 day learning trials, the rats were injected i.v. with 10 uCi. I-125-DEX and sacrificed 30 min. later. There was a significant difference in I-125-DEX binding to mAChRs in the hippocampus between the three groups (p 0.037,ANOVA) with the good performers showing the highest binding, the controls the lowest. There was also significantly higher I-125-DEX binding in the hippocampus(p 0.001), striatum (p 0.0006), frontal (p 0.002), parietal (p 0.002) and temporal (p 0.026) cortices in good performers when compared to controls (t-test) Our results indicate that learning causes increased mAChR activity in the rat brain and that this is most pronounced in the hippocampus.

632.2

AGE AND SEX RELATED DIFFERENCES IN MORRIS WATER MAZE PERFORMANCE AND BRAIN AChE LEVELS IN RATS. S.Demirgören, S.Temuçin*, S.Amado*, and Ş.Pöğün. Dept. of Physiology, Ege University, İzmir, 35100 Turkey

Young (4 months old) and old (24 months old) male and female Sprague-Dawley rats (n=8 for each group) were trained in the Morris water maze for nine days. There were active controls for each group. 24 Hours after the last trial, the animals were decapitated, their brains removed, dissected and acetylcholinesterase (AChE) levels were assayed in the cerebellum, striatum, hippocampus and cortical areas. Analysis of variance revealed a significant interaction between sex and age in Morris water maze learning performance; young male and old female rats had better scores. Although there was no difference in hippocampal AChE levels in the control groups, experimental groups showed significant variations - higher levels in young males and old females (ANOVA). There was also correlation between performance and hippocampal AChE levels in these groups. In the control groups, the younger animals had higher AChE levels in most of the brain regions studied. In the experimental groups, however, sex differences were observed:young males and old females had higher enzyme levels post-training in some brain regions. Our results imply the sexually dimorphic involvement of the cholinergic system with aging in water maze performance: male rats apperad to be more sensitive to cholinergic impairments as reflected in learning deficits with aging processes.

632.3

MORRIS WATER MAZE PERFORMANCE IN 3 WEEK OLD RATS - SEX DIFFERENCES AND HORMONE EFFECTS.

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It has been demonstrated that adult male rats outperform females on spatial tasks, including the radial arm maze and the Morris water maze. In addition, this sex difference can be modified with early postnatal administration of testosterone. Testosterone treated females perform better than control females, and in some cases, as well as control males. In human studies of spatial ability, gender differences are not often found in prepubertal populations. It is not known when sex differences for spatial tasks first appear in rats, and whether the administered hormones exert their effects early in life or in a delayed manner. In order to determine whether such sex differences exist in very young rats, male and female rats, both testosterone treated and controls, were trained and tested in the Morris water maze at a young age.

Sprague-Daley rats were treated with 250 ug testosterone proprionate on postnatal days 2 and 4. Controls were given only the oil vehicle. At three weeks of age, these rats were trained and tested on 10 consecutive days in a Morris water maze. The task consisted of finding and remembering the location of a submerged hidden platform in milky water. Sex differences and effects of the testosterone are described in terms of time required to find the platform over trials, as well as time spent in each quadrant on probe trials.

The understanding of the time course of development of sex differences in spatial performance is critical if the anatomical and physiological bases for these sex differences are to be determined.

632.4

EFFECTS OF ZOLPIDEM ON SCHEDULE CONTROLLED BEHAVIOR IN A RADIAL MAZE MODEL OF FORAGING.

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The effects of the hypnotic imidazopyridine zolpidem on behavior of rats were studied in an effort to discriminate between drug-induced performance deficits and disruptions of schedule controlled behaviors involving memory. Delivery of food was controlled by fixed interval schedules of reinforcement, randomly assigned to each of 8 arms of a radial maze, ranging from 55 to 759 sec. Zolpidem (0.33-3.0 mg/kg ip), administered to well-trained rats immediately prior to test sessions, produced dose-dependent decreases in measures of performance at doses above 0.67 mg/kg, but the overall patterning of responding was altered only at intoxicating doses. The proportion of responses and proportion of time spent in each of the arms relative to the proportion of rewards obtained were relatively unaffected below 2.0 mg/kg. Zolpidem produced performance decrements consistent with its hypnotic actions, but no evidence was obtained to suggest impairment of memory processes required to perform the eight alternative concurrent schedule in effect in the radial maze.

632.5

EFFECTS OF BENZODIAZEPINE RECEPTOR LIGANDS ON THE PERFORMANCE OF AN OPERANT DELAYED MATCHING TO POSITION TASK IN RATS.

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The effects of a series of benzodiazepine (BDZ) receptor ligands, ranging from full agonists through antagonists to partial inverse agonists on short term working memory in the rat were investigated. The behavioral paradigm used was discrete trial, operant delayed matching to position, with delays of 0, 5, 15 and 30 s. These delays generated an orderly 'forgetting' curve in control rats. Diazepam and lorazepam (BDZ agonists) both produced a delay dependent impairment in matching accuracy at certain doses, but delay independent effects at higher doses, which were accompanied by behavioral indices of sedation, including lengthening of reaction times. The BDZ receptor partial inverse agonist FG 7142 also produced a delay dependent impairment in matching accuracy and lengthened reaction times. In contrast, the BDZ receptor antagonist ZK 93426 produced a slight, delay dependent increase in matching accuracy, but also lengthened reaction times. Taken together, these results suggest that an optimal level of GABA activity is necessary for accurate performance of delayed matching to position in rats, and that both increases and decreases in GABA function, effected through BDZ receptor ligands, can impair performance.

632.6

AMNESIC EFFECTS OF CHLORDIAZEPOXIDE IN THE RADIAL ARM MAZE.

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The benzodiazepine (BZ) chlordiazepoxide (CDP) produces amnesia in humans but has been reported to produce variable effects in rats on performance in the radial arm maze in tasks used to measure memory. Chronic pre-trial administration of saline or CDP (5, 10, 20 mg/kg, ip) to food-deprived male Sprague-Dawley rats produced impairment of performance over a 21-day training period. The number of trials required to attain a criterion of 2 or fewer errors on 3 consecutive days and the percentage of animals reaching that criterion was: saline, 7.4 $\pm$ 1.2 (100%); 5 mg/kg, 10.0 $\pm$ 1.6 (87.5%); 10 mg/kg, 11.0 $\pm$ 2.9 (50%); and 20 mg/kg, 12.0 $\pm$ 1.0 (25%). Measures of accuracy (number of errors and task efficiency) were impaired by 10 and 20 mg/kg CDP. Measures of motor responsiveness (latency and rate of response) were unaltered at 10 mg/kg CDP but reduced at 20 mg/kg CDP. In a second experiment, pretreatment with the BZ antagonist flumazenil (5 mg/kg, ip) partially reversed the accuracy impairments of 10 mg/kg CDP, although flumazenil produced no effect on its own. These results are in contrast to the effects in highly trained animals in which CDP (0.3-18 mg/kg, ip) produced no significant alteration in measures of accuracy and a progressive impairment of motor responsiveness in a cumulative dose-effect curve. Therefore, tasks of acquisition appear to be more sensitive than previously acquired tasks to disruption by CDP. Supported by DA 05186.

632.7

GABAERGIC AND CHOLINERGIC INTERACTION IN THE MODULATION OF MEMORY.

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The aim of this experiment was to study the interaction of GABAergic and cholinergic systems in memory processes. Male Wistar rats (250-350 g) were trained with 2.5 mA, on a passive avoidance task and tested 24 hr later. The rats were assigned to one of 19 groups: two groups were injected 5 min post-training (ip) with scopolamine (SCP) (4 or 8 mg/kg); six groups with picrotoxine (PX) (0.007, 0.015, 0.062, 0.5, 1.0 or 2.0 mg/kg) five groups received 8 mg/kg of SCP in combination with PX (0.007, 0.015, 0.062, 0.25, or 1.0 mg/kg); threee groups received SCP (4mg/kg) with 0.5, 1.0 or 2.0 mg/kg of PX, and three control groups, one injected with isotonic saline, an intact group and a group with no shock. . SCP induced amnesia in a dose dependent way; in contrast PX did not modify performance. Except the group with the smaller dose of PX, all combinations of PX and SCP reversed the the SCP-induced amnesia.

632.8

AGE-DEPENDENT MODULATION OF CORTICAL ACETYLCHOLINE RELEASE BY BENZODIAZEPINE LIGANDS.

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Administration of benzodiazepines produces bidirectional effects on cognition in animals and humans. Benzodiazepine receptor (BZR) agonists, such as chlordiazepoxide (CDP), impair performance while selective inverse agonists, such as ZK 93 426, enhance performance. These performance changes have been attributed to GABA-cholinergic interactions. Given age-related declines in cognitive processes, we also determined whether these transmitter interactions vary with aging. We have utilized in vivo microdialysis in freely moving animals to determine whether these BZR agents have similar bidirectional effects on cortical acetylcholine (ACh) release.

Fischer-344 rats (4 & 18 mo old) were implanted with unilateral guide shafts into the frontoparietal cortex. Three days later they were equipped with dialysis probes and treated every 2 days, in counterbalanced order, with CDP (3, 5, 10 mg/kg) or ZK 93 426 (1, 5 mg/kg). Baseline ACh release was higher in young (.78 $\pm$ .23 pmol/min) than in old rats (.32 $\pm$ .05 pmol/min). ZK 93 426 increased ACh release in a dose-dependent manner with old rats being more sensitive than young animals. However, old animals were less able than young rats to sustain this increased release. Curiously, CDP did not systematically affect ACh release, suggesting that BZR agonists and selective inverse agonists do not bidirectionally affect cortical ACh release. These data reveal that BZR selective inverse agonists enhance cortical ACh release but the sensitivity and temporal dynamics of this effect vary with age.

632.9

SPATIAL LEARNING IN THE MORRIS WATER TASK AFTER MEDIAL FRONTAL CORTEX LESIONS IN THE RAT: EFFECTS OF DIAZEPAM.
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Diazepam chronically disrupts recovery from sensorimotor asymmetries caused by unilateral anterior medial cortex lesions if administered continuously during a sensitive 12-96 hour postoperative period (Schallert, Hernandez & Barth, 1986). Diazepam appears to exaggerate secondary degeneration in the striatum which may contribute to the disruption of recovery (Jones & Schallert, 1991). In the present study, bilateral medial frontal cortex lesions significantly impaired spatial learning ability in Whishaw's learning-set version of the Morris water task. Unexpectedly, recovery was greatly facilitated in animals treated post-operatively with diazepam. Preliminary results indicate that diazepam may allow sparing in the CA1 and CA2 regions of the hippocampus. Such a finding suggests that spatial deficits produced by medial frontal cortex lesions may result from secondary damage at the hippocampal level. Depending on the neurological test, diazepam may retard or promote recovery of function after medial frontal cortex lesions.

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632.10

EFFECTS OF PERIPHERAL AND CENTRAL INJECTIONS OF GABA AGONISTS ON SPONTANEOUS ALTERNATION PERFORMANCE. C. M. GREY, W. K. K. LAM*, and P. E. GOLD. Neuroscience Graduate Program and Department of Psychology, U. Virginia, Charlottesville, VA 22903.

Peripheral, intraventricular and medial septum injections of glucose attenuate memory deficits caused by opiate agonists and cholinergic antagonists. GABA neurons are associated with cholinergic-opioid interactions in the medial septum, and injection of GABA agonists into the medial septum impair memory (Chrobak *et al*, 1989; Brioni *et al*, 1990). The present experiments examined the efficacy of peripheral and medial septum injections of glucose in attenuating spontaneous alternation deficits produced by GABA agonists.

Mice injected (IP) with either a GABA-A receptor agonist, muscimol (0.5 mg/kg) or a GABA-B receptor agonist, baclofen (5.0 mg/kg) 30 min before spontaneous alternation tests exhibited significantly impaired alternation performance (percent alternation scores: 62.7 ± 2.7 and 58.1 ± 2.6, respectively, vs. saline 69.7 ± 1.6). This deficit was not reversed by co-administration (IP) of glucose (10, 100 or 300 mg/kg). Muscimol injections directly into the medial septum (1 nmole/0.5 ul over 1 minute) impaired alternation performance (42.8 ± 7.3 vs saline 69.0 ± 3.8); this deficit was not attenuated by peripheral (100 mg/kg) or septal (3.3 ug/0.5 ul over 1 minute) injections of glucose.

With parallel findings that glucose does not enhance memory in rats with lesions of the medial septum, the results suggest that glucose effects on memory require the participation of GABA neurons contained in the medial septum, which may contribute to the modulation of memory processing via regulation of opioid and cholinergic systems. [Supported by ONR (N0001489-J-1216),NIA (AG 07648), and NSF (BNS-90122391)].

632.11

COGNITION ENHANCING PROPERTIES OF THE BENZODIAZEPINE RECEPTOR SELECTIVE INVERSE AGONIST MDL 26,479. M. Sarter, L.-A. Holley*, and P. Dudchenko. Dept. of Psychology, The Ohio State University, Columbus, OH 43210.

The triazole MDL 26,479 has been shown to displace the cortical binding of Ro15-1788, to reduce the effects of GABA on the activity of Purkinje cells, and to facilitate LTP (Dudley et al. 1990; Sorensen et al. 1990a). In contrast to benzodiazepine receptor inverse agonists (BZRIA), however, MDL 26,479 does not exhibit convulsive effects (Sorensen et al. 1990b), suggesting BZR-selective inverse agonism (BZRSIA; Sarter 1990). We have found that MDL 26,479 (0.1-6.25 mg/kg; 60 min pretest) attenuates the effects of scopolamine (0.03, 0.1 mg/kg (30 min pretest) on performance in a delayed alternation task (0-32 sec). Furthermore, the effects of MDL 26,479 on the acquisition of a conditional visual discrimination task in rats with ibotenic acid-induced (0.06 M) basal forebrain lesions were examined. Sham-lesioned or lesioned rats were treated with vehicle or MDL 26,479 (5 mg/kg; i.p.; daily; 60 min pretest). In comparison to sham-lesioned controls, learning in lesioned rats was drastically impaired. MDL 26,479 did not improve the performance of sham-lesioned animals. However, MDL 26,479-treated, lesioned rats acquired the task as rapidly and accurately as controls. While the extent to which the behavioral effects of the ibotenic acid-induced lesion depended on the destruction of cholinergic neurons remains unclear, we speculate that the beneficial behavioral effects of MDL 26,479 were mediated via an increase in cortical acetylcholine release (as has been previously proposed for other BZRSIAs; Sarter et al. 1990). This hypothesis is supported by the finding that MDL 26,479 stimulates [^{3}H]-hemicholinium-3 binding in cortical but not hippocampal membranes (Miller & Chmielewski 1990). These properties of MDL 26,479 suggest that it represents a potent BZRSIA and a promising therapeutic treatment for the cognitive symptoms that are associated with cortical hypocholinergic functions.

632.12

NMDA LESIONS OF THE AMYGDALA BLOCK DIAZEPAM INFLUENCES ON MEMORY. H. Dickinson-Anson, C. Tomaz and J.L. McGaugh. Center for the Neurobiology of Learning and Memory and Department of Psychobiology, University of California, Irvine, CA 92717.

It is well known that systemically administered benzodiazepines (BZ) induce anterograde amnesia in a variety of tasks. BZ effects are mediated through the GABA-A complex by enhancing GABA-induced synaptic inhibition. Extensive evidence suggests that GABAergic influences on memory are mediated through the amygdaloid complex. As the GABAergic system in the amygdala is a site of action for the anxiolytic effects of BZs, such findings suggest that BZ's may also influence memory through the amygdala. The present experiment was designed to examine this implication.

Male rats received either sham or bilateral amygdala lesions using N-methyl-D-aspartic acid (NMDA). NMDA (8µg/0.8µl phosphate buffer) was injected at a rate of 0.2 µl/min, with injection needle left in place 5 min following injection. One week later, animals were trained to criterion on a continuous multiple-trial inhibitory avoidance task (CMIA) and tested 48-h later (step-through latency). In this task, whenever a rat entered the dark compartment, a footshock was given until the rat escaped to the starting compartment. The number of trials required before the rat remained in the starting compartment for 100 sec in one trial was recorded. Diazepam (DZ) (1.0 and 2.0 mg/kg, ip) or vehicle was injected 30 min prior to acquisition. The results confirmed previous findings: amygdala lesions impair acquisition, systemic DZ impairs retention. The major finding of importance was that the DZ-induced retention deficit was blocked in rats with amygdala lesions. Our results strongly suggest that the amnestic effects of the benzodiazepine, diazepam, are mediated, at least in part, through the amygdaloid complex.

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632.13

INTERACTION OF NORADRENERGIC, MUSCARINIC AND GABAERGIC SYSTEMS IN THE AMYGDALA IN REGULATING MEMORY STORAGE. I.B. Introini-Collison, C. Dalmaz*, J. Salinas* and J.L. McGaugh. Center for the Neurobiology of Learning and Memory and Department of Psychobiology, University of California, Irvine, CA 92717.

Previous results from our laboratory have indicated that several neurochemical systems in the amygdala are involved in the modulation of memory storage. These experiments examined the interaction of the amygdala noradrenergic system with muscarinic cholinergic and GABAergic systems. Posttraining intra-amygdala administration of clenbuterol produced a dose-dependent enhancement of retention of an inhibitory avoidance task. The enhancing effects of clenbuterol (3.0 ng) were completely reversed by concurrent administration of atropine (1.0 µg), which by itself had no effect on retention.

In addition, intra-amygdala administration of a low dose of propranolol (0.3 µg), which alone did not affect retention, shifted the dose-response curve of the GABAergic receptor agonist muscimol (0.01, 0.03 and 0.1 ng) toward the left, i.e. lower doses of muscimol were necessary in the presence of propranolol to obtain similar effects.

These findings are consistent with other evidence suggesting that noradrenergic influences interact with GABAergic influences within the amygdala in regulating memory storage and that these influences are mediated through effects involving a muscarinic cholinergic mechanism.

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632.14

CL 275,838, A NOVEL COMPOUND SHOWING ANTIDEPRESSANT ACTIVITY AND BENEFICIAL EFFECTS ON MEMORY DEFICITS IN THE RAT.
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CL 275,838, 4,5-dihydro-4-{{4-(phenylmethyl)-1-piperazinyl}acetyl}-7-{3-(trifluoro-methyl)phenyl}pyrazolo{1,5a}pyrimidine-3-carbonitrile, reduced rats' immobility in the forced swimming test (5 mg/kg i.p., three injections in 24 h) and counteracted the impairment of rats' performance caused by 1 mg/kg scopolamine in a passive avoidance task (20 mg/kg i.p. CL 275,838 administered before the training trial and the retention test). Similar treatments with oral doses of 40 and 160 mg/kg CL 275,838 respectively reduced rats' immobility in the forced swimming test and antagonized the decrease in retention latency caused by aging (26-28 months old rats) or 5 mg/kg i.p. diazepam in the passive avoidance task. The compound blocks the neuronal uptake of serotonin, increases dopamine release in the mesolimbic system, has moderate affinity and low intrinsic activity for benzodiazepine receptor binding and facilitates the transmission of glutamate on NMDA receptor. These effects can be involved in the favourable effects of CL 275,838 on depression and memory deficits, but clear evidence of their role is not yet available.

632.15

DuP 996 IS A NEUROTRANSMITTER RELEASE ENHANCER WITH COGNITIVE ENHANCING PROPERTIES. V. J. DeNoble, V.J. Nickolson,* K. F. DeNoble, K. W. Rohrbach, S. W. Tam, L. Cook, G. F. Steinfels, M. J. Myers,* and R. M. Scribner.* The Du Pont Merck Pharmaceutical Company, P.O. Box 80400, Wilmington, DE 19880-0400.

DuP 996 (3,3,-bis-C4-pyrindinylmethyl)-1-phenylindolin-2-one) enhances the K^+-stimulated release of acetylcholine, dopamine, and serotonin *in vitro*. DuP 996 is essentially inactive in binding to muscarinic and nicotinic receptors and in inhibiting cholinesterase activity. Behaviorally, DuP 996 was compared to Physostigmine (PH), and Tetrahydroaminoacridine (THA) for its ability to 1) prevent hypoxia-induced amnesia in rats, 2) increase lever press acquisition in rats, and 3) prevent hypoxia-induced disruption of visual recognition in squirrel monkeys. In these tests DuP 996 was found to be superior in that it either produced greater efficacy, was more reliable, and/or had a broader therapeutic window with a better safety margin (Table 1).

Table 1

	Activity in mg/kg		
	DuP 996	Ph	THA
Test			
Hypoxia Passive Avoidance (SC)	0.01-0.1	0.001-0.1	0.3-5.0
Lever Press Acquisition (PO)	0.1-1.0	IA*	IA
Monkey Visual Recognition (PO)	0.3, 0.6	0.1	IA
Tremor Rat (SC)	10	0.3	6.0
Monkey (PO)	5	0.5	3.0**

* IA-Inactive ** Emesis

These results suggest DuP 996 may have therapeutic value in the treatment of diseases which result in cognitive dysfunction.

632.16

[^{3}H]DuP 996 LABELS A NOVEL RECEPTOR SITE INVOLVED IN ENHANCEMENT OF STIMULUS-INDUCED ACETYLCHOLINE, DOPAMINE AND SEROTONIN RELEASE. D. Rominger and S. W. Tam. Central Nervous System Diseases Research, The Du Pont Merck Pharmaceutical Company, Wilmington, DE 19880-0400.

DuP 996 [3,3-bis(4-pyrindinylmethyl)-1-phenylindolin-2-one] is a novel cognitive enhancer which has been shown to improve learning and memory in rodents and primates. DuP 996 and structural analogs enhance the K^+-stimulated release of acetylcholine, dopamine and serotonin in brain slices without effect on basal release. A novel receptor site labeled by [^{3}H]DuP 996 has been identified with a K_d of 19 nM and a B_{max} of 102 fmol/mg protein. Binding to this site is specific, saturable, reversible, time-, pH-, and temperature-dependent. Specific binding is decreased by treatment with trypsin and not affected by phospholipase C. Specific binding is inhibited by Ca^{2+} and increased by Mn^{2+} but not affected by Na^+, K^+, or Mg^{2+}. The [^{3}H]DuP 996 binding sites are heterogenously distributed in brain with striatum and hypothalamus having highest density and cerebellum lowest. The [^{3}H]DuP 996 binding site is novel because the [^{3}H]DuP 996 binding could not be displaced by a broad variety of standard pharmacological agents and neuropeptides. Physiological significance of this binding site is suggested by the excellent correlation between the binding affinity to this site and the potency to enhance K^+-stimulated release of acetylcholine for a series of DuP 996 analogs. Ligands for this receptor site may have therapeutic potential for the treatment of cognitive deficits and neurodegenerative diseases.

632.17

[^{3}H]DuP 996 LABELS A NOVEL BINDING SITE IN RAT BRAIN INVOLVED IN THE ENHACEMENT OF STIMULUS-INDUCED ACETYLCHOLINE, DOPAMINE AND SEROTONIN RELEASE: AUTORADIOGRAPHIC LOCALIZATION STUDIES. E. B. De Souza, B. L. Rule* and S. W. Tam. Central Nervous System Diseases Research, The Du Pont Merck Pharmaceutical Company, Wilmington, DE 19880.

DuP 996 [3,3-bis(4-pyrindinylmethyl)-1-phenylindolin-2-one] enhances K^+-stimulated release of acetylcholine, dopamine and serotonin in brain slices without effects on basal release. DuP 996 has significant "cognitive-enhancing" effects in a variety of animal models of learning and memory. A novel high-affinity binding site for [^{3}H]DuP 996 which fulfills many of the criteria of a membrane receptor has recently been identified in homogenates of rat brain. In the present study, we have used in vitro labeling light microscopic autoradiography to localize [^{3}H]DuP 996 binding sites in rat brain. Preliminary experiments were carted out to optimize the binding parameters and to characterize the pharmacology of the [^{3}H]DuP 996 binding site in slide-mounted sections of rat brain. Overall, [^{3}H]DuP 996 binding sites were discretely localized with highest densities present in brain areas (cerebral cortex, hippocampus and amygdala) involved in cognitive processes. A laminar distribution of [^{3}H]DuP 996 binding sites was evident in the cerebral cortex and binding was highly localized in the CA1 to CA3 pyramidal cell layers of the hippocampus. Moderate densities of binding sites were present in the olfactory bulb, olfactory tubercle and amygdala. Lower levels of binding were present in striatum, thalamus and hypothalamus. The localization of [^{3}H]DuP 996 binding sites in brain areas implicated in memory processes and affected in Alzheimer's disease suggest that ligands for this binding site may have therapeutic potential for the treatment of cognitive deficits seen in dementia.

632.18

The Electroencephalographic (EEG) Effects of DuP 996, a Compound with Cognitive Enhancing Properties. G. P. ALBERICI, L. Cook, J.-L. GASKILL and G. F. STEINFELS. The Du Pont Merck Pharmaceutical Company, Wilmington, DE 19880-0400 USA.

Using EEG as a measure of brain electrical activity we examined the effects of DuP 996 on the EEG of 3 animal species; rat, rabbit and dog. Frontal-parietal, bipolar recordings were made from rat and dog, and multi-lead (16 electrodes) recordings were made in the rabbit for topographic analysis. EEG activity was collected online (125 Hz) and transformed into power spectra via a fast Fourier transform. The results were then grouped into the following EEG bandwidths: *delta* 1-5 Hz; *theta* 5-9 Hz; *alpha* 9-13 Hz; and *beta* 13-35 Hz for rabbit, *beta-1* 13-25, *beta-2* 25-50 for rat and dog. DuP 996 in a single dose of ≥ 0.085 mg/kg (sc) in rats and ≥ 0.0085 mg/kg (sc) in dogs decreased relative spectral power (RP) in the *delta* EEG band , and concomitantly increased RP in the *beta-1* EEG band. Topographical (rabbit) EEG results (0.85 mg/kg, sc) displayed the following: for mean frequency, a total brain increase in *beta* and a total brain decrease in *theta*. For relative spectral power, an occipital region increase in *beta*, a frontal region increase in *theta* and a frontal region decrease in *alpha*, and for absolute spectral power, a total brain decrease in *delta* and *alpha* and an occipital region increase in *beta*. In some cases these changes parallel those reported in clinical EEG studies of DuP 996 (Saletu, 1989).

632.19

ELECTROPHYSIOLOGICAL EFFECTS OF DUP 996 ON HIPPOCAMPAL CA1 NEURONS. B. W. Lampe and B. S. Brown*, CNS Diseases Research, The Du Pont Merck Pharmaceutical Co., Wilmington, DE 19880

DuP 996 (3,3-bis(4-pyridinylmethyl)-1-phenylindolin-2-one) enhances K^+-stimulated release of several neurotransmitters in various regions of rat brain *in vitro*. To explore its mechanism of action, conventional intracellular recording techniques were used to study the effects of DuP 996 on electrophysiological characteristics of CA1 pyramidal cells in rat hippocampal slices. DuP 996 (5-100 μM) enhanced the slow cholinergic epsp, reduced spike frequency adaptation (SFA), delayed repolarization of action potentials elicited by brief, threshold-level depolarizing pulses and increased the frequency of spontaneous action potentials, but had no consistent effect on resting membrane potential, input resistance, or inward rectification in response to hyperpolarizing current pulses. The effect of DuP 996 on epsp was blocked by atropine. The effect on SFA was not atropine-sensitive nor was it accompanied by a reduction of the cumulative slow afterhyperpolarization. Both facilitation of the cholinergic epsp and reduction of SFA occurred with all tested concentrations of DuP 996. These two effects may be due to a common mechanism, possibly the attenuation of certain K^+ conductances, and may provide the basis of DuP 996-enhanced neurotransmitter release.

632.20

DuP 996, A NOVEL NEUROTRANSMITTER RELEASER, BLOCKS VOLTAGE-ACTIVATED POTASSIUM CURRENTS IN CULTURED NEOCORTICAL NEURONS. J.M. Frey, P.A. Murphy* and B.S. Brown*, CNS Diseases Research, The Du Pont Merck Pharmaceutical Co., Wilmington, DE 19880

DuP 996 [3,3-bis(4-pyridinylmethyl)-1-phenylindolin-2-one] enhances the K^+-stimulated release of a number of neurotransmitters from rat neocortex, hippocampus and striatum *in vitro* without affecting basal release. To determine if increased release could be related to blockade of potassium channels, we examined the ability of DuP 996 to inhibit voltage-activated potassium currents in rat cultured neocortical neurons using the whole cell patch clamp.

In the presence of both Na^+ and Ca^{++} channel blockers (3 μM TTX and 4-5 mM Co^{++}/5 mM EGTA or BAPTA, respectively), depolarizing voltage commands (V_C) above -50 mV evoked outward currents with an early 4-aminopyridine-sensitive transient component (I_A) and a late tetraethylammonium-sensitive component (I_K). DuP 996 blocked both I_A and I_K nonselectively in a concentration-dependent manner above 30 μM; the half maximal inhibitory concentration (IC_{50}) was approximately 300 μM.

When Ca^{++} channels were not blocked by Co^{++}, evoked outward currents were ≈1.5-fold larger (at V_C=25mV) than those observed in the presence of Na^+ and Ca^{++} channel blockers. Under these conditions, 10 and 30 μM DuP 996 significantly blocked outward currents and the IC_{50} was < 100 μM. Our results indicate that DuP 996 is a more potent blocker of voltage activated, Ca^{++}-dependent outward currents than of I_A or I_K in cultured cortical neurons. Whether DuP 996 blocks Ca^{++}-dependent outward currents directly, or indirectly through a block of calcium channels is presently under investigation.

632.21

GLUCOSE FAILED TO REVERSE SCOPOLAMINE-INDUCED DEFICITS IN A WATER-ESCAPE TASK IN RATS. R.D. Holsten* and L.W. Means. Department of Psychology, East Carolina University, Greenville, NC 27858.

Thirteen Sprague-Dawley male rats were trained to escape in a two-choice water maze, where the choice section containing the escape platform was reversed on each trial following a correct choice. Rats were given 1 reference and 10 test trials/day with the trials separated by intertrial intervals of 1-2 min. Incorrect choices were punished by retaining the rat in the incorrect choice section for 30 sec. The rats attained a performance level of 9.2 correct choices/session (CC/S). Injections (s.c.) of .5, 1.0 or 2.0 mg/Kg scopolamine hydrobromide 30 min prior to testing significantly ($p<.01$ in each case) reduced the number of CC/S to 6.4, 6.8 and 7.0, respectively, whereas .5 mg/Kg methylscopolamine failed to affect CC/S (9.5). In a subsequent experiment using the same rats, no injection, injections with .5mg/Kg scopolamine alone, or in combination with 100, 250 or 500 mg/Kg glucose (i.p.) resulted in 9.6, 8.6, 7.9, 8.5 and 8.2 CC/S, respectively. The rats performed significantly ($p<.05$ in each case) worse after all injections than when tested after no injection. Thus, the rats were impaired on the task by the CNS activity of scopolamine, and glucose failed to ameliorate the scopolamine-induced impairment.

632.22

PARADOXICAL SLEEP IS SELECTIVELY SENSITIVE TO GLUCOSE IN AMNESIC RATS. W.S. STONE AND P.E. GOLD. Dept. of Psychology, U. of Virginia, Charlottesville, VA 22903.

Poor memory is highly correlated with impaired paradoxical sleep under several conditions. We determined here whether glucose administration, which enhances memory in rodents and humans, also enhances sleep In three amnesic populations: amygdala-kindled, atropine-treated, and old rats. Sleep records (3-hr EEGs) were obtained before and after rats received daily kindling stimulation (biphasic square waves, 1 msec, 60 Hz, 250 uA, 1 sec train) for 4-5 weeks or a single injection of atropine (1 mg/kg, IP). Later, spontaneous alternation performance was assessed after saline or glucose (100 mg/kg, IP) administration. EEGs were also obtained from aged (26 mo) Fischer 344 rats which received saline or glucose (100 mg/kg). These rats were then trained on an inhibitory avoidance task (1 ma, 1 sec) and later tested for generalization of the learned response.

The results were similar despite different designs and subject populations. Glucose selectively increased the duration of paradoxical sleep bouts - and improved memory - in amnesic but not in respective control populations, including within individual rats in these populations which do not exhibit sleep or memory impairments. These results show that paradoxical sleep measures predict the ability of glucose to attenuate memory deficits in 3 amnesic populations, and more generally, they show that the brain becomes significantly more sensitive to the effects of glucose administration in several amnesic populations. [Supported by ONR (N0001489-J-1216), NIA (AG 07648), and NSF (BNS-9012239)].

632.23

URINARY INCONTINENCE AND MEMORY IMPAIRMENT IN RATS WITH BASAL FOREBRAIN LESIONS AND IMPROVEMENT BY TREATMENT WITH A CEREBRAL ENERGY METABOLISM ENHANCER ASSOCIATED WITH RECOVERY OF LCGU. M. Miyamoto, Y. Nagai*, T. Nakayama* and A. Nagaoka*, Biology Res. Lab., Res. and Dev. Div., Takeda Chem. Ind. Ltd., Osaka 532, Japan.

Effects of chronic administration of idebenone on urinary incontinence and learning and memory impairment in rats with basal forebrain (BF) lesions were studied. BF lesions were made by injection of ibotenic acid (24 nmol/0.4 μl/side) into the bilateral BF involving cholinergic neurons projecting to the cerebral cortex. The BF-lesioned rats showed a significant urinary incontinence assessed by area saturated with urine, which lasted for about 4 weeks. Chronic treatment with idebenone (10 and 30 mg/kg/day, p.o.), started 4 days after BF lesions when the score of urinary incontinence became maximum, improved the urinary incontinence in a dose-dependent manner. The drug also ameliorated the impairment of water maze learning, as demonstrated by shortening of the latency to find the platform submerged in the water tank, and decrease in swimming distance until the rat found the platform. After completion of behavioral tests, effects of chronic treatment with idebenone on local cerebral glucose utilization (LCGU) were examined. The BF-lesioned rats showed a significant decrease in LCGU, especially in the cerebral cortex, such as frontoparietal cortex and temporal cortex, striatum and habenula, when compared to sham-operated rats. Idebenone provided a dose-dependent recovery of the decreased LCGU in rats with BF lesions. These results indicated that urinary incontinence and memory impairmment in the BF-lesioned rats may be related to the decrease in cerebral energy metabolism, and suggest that treatment with idebenone improves the deficits in the rats, associated with recovery of LCGU.

INVERTEBRATE LEARNING AND BEHAVIOR IV

633.1

LONG-TERM (24 HR) MORPHOLOGICAL CHANGES INDUCED BY cAMP IN PLEURAL SENSORY NEURONS OF *APLYSIA* ARE DEPENDENT ON *DE NOVO* PROTEIN SYNTHESIS. F. A. NAZIF, J. H. BYRNE and L. J. CLEARY. Dept. Neurobiology and Anatomy, Univ. Texas Med. Sch., Houston, TX 77225

Previous studies have shown that long-term sensitization is correlated with morphogical changes in the sensory neurons mediating a defensive withdrawal reflex (Bailey and Chen, 1988). Some of these changes can be mimicked by the intracellular injection of cAMP (Nazif *et al.*, 1991). Since long-term sensitization and long-term facilitation require protein synthesis (Montarolo *et al.*, 1988; Castellucci *et al.*, 1989), we were interested in examining the role of protein synthesis in the induction of these morphological changes by cAMP.

Pleural ganglia of *Aplysia* were preincubated in either anisomycin (10 μM) or its inactive derivative deacetylanisomycin (10 μM) for one hr. cAMP was then iontophoretically injected into sensory neurons from right and left pleural ganglia. One hr after injection the ganglia were perfused with fresh culture medium. About 22 hr after injection the same neurons were pressure injected with HRP and incubated for another 2 hr. After histochemical processing, varicosities in axonal branches within the pleural neuropil were counted. The number of varicosities was greater in neurons whose ganglia were bathed in deacetylanisomycin than in neurons from contralateral ganglia, which were bathed in anisomycin (50 ± 10 SEM vs 23 ± 7 SEM; $P<0.05$; $n=6$).

These data suggest that the morphological changes induced by cAMP are dependent on the production of new proteins during and/or immediately following the injection of cAMP. This conclusion is consistent with the hypothesis that cAMP-dependent regulation of gene expression results in the synthesis of new proteins. Consequently, several mechanisms are affected, including those regulating neuronal morphology.

633.2

CHANGES IN PROTEIN PHOSPHORYLATION IN PLEURAL SENSORY NEURONS OF *APLYSIA* VARY DEPENDING ON THE DURATION OF SEROTONIN TREATMENTS. R. Homayouni, R. Zwartjes, J.H. Byrne, and A. Eskin. Dept. Biochem., Univ. of Houston, Houston, TX 77204. Dept. Neurobiol. & Anat., Univ. of Texas Medical School, Houston, TX 77225.

The facilitatory effects of serotonin (5-HT) on tail sensory neurons (SNs) in pleural ganglia of *Aplysia* are due in part to activation of cAMP-dependent protein kinase. The phosphorylation of a number of proteins in pleural SNs was found to be increased both following 2 min 5-HT and 24 h after 2 h 5-HT (Sweatt et al.,1989). The increase in phosphorylation 24 h after treatment did not occur when 5-HT was given with anisomycin, a protein synthesis inhibitor. To study the events responsible for the transition between short-term and long-term facilitation, we investigated the phosphorylation of proteins at the end of 2 min, 25 min, and 1.5 h treatments of 5-HT.

For each experiment 6 pedal-pleural ganglia and their matched controls were isolated and incubated in ^{32}P for 20.5 h. Experimental ganglia were treated during the end of labeling. After treatment, clusters of SNs were removed and the incorporation of ^{32}P into proteins was examined using 2D-PAGE. The phosphorylation of approximately 9 proteins was increased and 1 protein was decreased following either 2 min, 25 min, or 1.5 h 5-HT treatments. Only one protein was affected by all 3 treatments. Different sets of proteins were phosphorylated by different durations of 5-HT. This variation in protein phosphorylation may contribute to the transition between short-term and long-term facilitation.

We examined the effect of anisomycin alone and together with 1.5 h 5-HT on the phosphorylation of proteins. Anisomycin alone altered the phosphorylation of 5 proteins, some of which were the same as those affected by 5-HT. The effect of 1.5 h 5-HT on phosphorylation of one protein, whose phosphorylation was not affected by short 5-HT treatments or anisomycin alone, was blocked by anisomycin. This protein may play a key role in the induction of long-term facilitation.

633.3

EFFECTS OF ANISOMYCIN ON THE LATE CHANGES OF PROTEINS IN PLEURAL SENSORY NEURONS OF *APLYSIA* PRODUCED BY AN *IN VITRO* ANALOGUE OF SENSITIZATION TRAINING. F. Noel, J.H. Byrne, A. Eskin. Dept. of Neurobiol. & Anat., Univ. of Texas Med. Sch., Houston, TX 77225, & Dept. of Biochem. & Biophys. Sciences, Univ. of Houston, TX 77204.

An *in vitro* analogue of sensitization training in *Aplysia* produces early and late changes in the incorporation of label into specific proteins in pleural sensory neurons. Proteins were increased both at the end of (early) and 24 hr after (late) the training procedure. To determine whether the late changes require translation, we studied the effect of anisomycin applied during the training on the incorporation of labeled amino acid into proteins 24 hr after the training.

Experimental pleural-pedal ganglia were exposed to anisomycin (10^{-5} M) for a 3 hr period. One hour after the beginning of anisomycin, peripheral nerves of experimental ganglia were electrically stimulated with 4 trains of shocks for a 1.5 hr period. Twenty-two hours after the last electrical shock the control (C) and experimental (E) ganglia were exposed to ^{35}S methionine for a 2 hr period. The sensory neuron clusters were surgically removed and processed for 2D-PAGE. Control experiments included treatment with anisomycin without electrical stimulation and electrical stimulation without anisomycin.

Anisomycin by itself had a general effect on protein synthesis as well as an effect on specific proteins. The overall ^{35}S methionine incorporation into proteins was significantly decreased both in the presence (Ratio E/C = 0.65, n=12, $p<0.05$) and in the absence (0.69, n=10, $p<0.05$) of the training procedure, whereas it was not affected by the training procedure alone (0.83, n=8, p=0.12). In addition, the incorporation of label into 21 proteins were either increased or decreased by application of anisomycin alone. Most of the late proteins affected by training were also affected by the anisomycin treatment itself. However, the effect of training on one protein which was not affected by anisomycin alone, was blocked by anisomycin. These results indicate that at least one of the late changes was due to increased translation of a specific protein. They also emphasize the importance of controlling for the possible effects of anisomycin alone.

633.4

CALMODULIN SYNTHESIS AND mRNA LEVEL ARE REGULATED IN *APLYSIA* NEURONS BY TREATMENTS PRODUCING LONG-TERM FACILITATION. R. Zwartjes, F. Noel, J. H. Byrne, and A. Eskin. Dept. Bioch., Univ. of Houston, 77204, and Univ. Tex. Med. Sch., Houston, TX 77225.

Both translation and transcription inhibitors block the long-term effects induced by serotonin (5-HT) on cultured sensory neurons of *Aplysia* (Montarolo et al., 1986). Also, the induction of long-term facilitation is blocked by the injection of an oligonucleotide containing the cAMP response element (Dash et al., 1990). A number of studies have shown that changes in the synthesis of specific proteins occur as a consequence of behavioral sensitization training, an *in vitro* analogue of the training procedure, or application of 5-HT (Castellucci et al., 1988; Noel et al., 1989, 1990; Barzilai et al., 1989; Eskin et al., 1989). These results indicate that transcription of new messenger RNA, as well as the production of new proteins, may be involved in long-term changes in sensory neurons associated with sensitization.

To examine whether levels of mRNA change during induction of long-term facilitation, we used *in vitro* translation of RNA isolated from pleural-pedal ganglia, with 2-D PAGE of the labeled protein products. The level of mRNA is proportional to the amount of label incorporated into a protein. We previously reported that *in vitro* incorporation of ^{35}S-methionine into 4 proteins was increased following a 1.5 hr treatment of ganglia with 5-HT (Zwartjes et al., 1990). We have now identified one of the 4 proteins reported above as calmodulin (MW 17,000, pI 3.9). Purified chicken calmodulin comigrates with the *Aplysia* protein on 2-D PAGE, and a rat calmodulin antibody specifically labels this protein on Western blots of pleural-pedal proteins.

Is the increase in calmodulin mRNA accompanied by an increase in protein in stimulated pleural-pedal ganglia? We examined changes in ^{35}S-methionine incorporation into proteins of pleural sensory neurons produced by 1.5 hr electrical stimulation of peripheral nerves. Incorporation of amino acid into calmodulin was consistently increased 24 hr after stimulation (N=4). These findings suggest that calmodulin is transcriptionally regulated during the induction of sensitization and, moreover, calmodulin may be important in the induction or maintenance of long-term sensitization.

633.5

IDENTIFICATION OF PLEURAL NEURONS THAT INHIBIT TAIL SENSORY NEURONS OF *APLYSIA*: CORRELATION WITH FMRFAMIDE IMMUNOREACTIVITY. Y.Xu, L.J.Cleary and J.H.Byrne. Dept. of Neurobiology and Anatomy, University of Texas Medical School, Houston, TX 77225.

Sensory neurons (SNs) in the pleural ganglion are hyperpolarized by mechanical stimulation of the body outside their receptive field. FMRFa is a peptide that has inhibitory effects on SNs, and FMRFa immunoreactive (IR) neurons and processes are present in the pleural ganglion. No FMRFa IR neurons that inhibit the tail SNs have been identified, however.

A cluster of small cells in the right pleural ganglion had inhibitory effects on the SNs. Intracellular stimulation of these cells produced hyperpolarization of SNs. Identified inhibitory cells were injected with Lucifer yellow (LY) and labelled with rabbit anti-FMRFa antibody followed by goat anti-rabbit antibody conjugated to Texas Red. The hyperpolarization in the SNs produced by FMRFa IR cells developed slowly, and upon removal of the stimulation, diminished slowly, but the hyperpolarization produced by non-FMRFa IR cells diminished more rapidly. LY injection demonstrated that the FMRFa IR cells projected out into the pleural-cerebral connective (3 of 3), while most non-FMRFa IR cells (9 of 11) did not. Both FMRFa and non-FMRFa IR cells received strong synaptic inputs from the pleural-abdominal connective and peripheral nerves P8 and P9. The nerve-evoked EPSPs in FMRFa IR cells were always followed by a slow, long-lasting IPSP, whereas this was not always observed for the non-FMRFa IR cells. In one case, the FMRFa cell also received excitatory inputs from the SN it hyperpolarized. Preliminary immunohistochemical staining indicated that myomodulin, another peptide transmitter that inhibits SNs, is not contained in non-FMRFa IR cells.

These results suggest that inhibition of the SNs is produced by a family of inhibitory transmitters, one of which is FMRFa. Further studies are necessary to identify other inhibitory transmitters as well as the physiological role of inhibitory neurons in the mediation and modulation of the reflex responses for which the tail SNs comprise the afferent limb.

633.6

A NETWORK MODEL OF THE TAIL-WITHDRAWAL CIRCUIT IN *APLYSIA* J.A. White, L.J. Cleary, I. Ziv, and J.H. Byrne Dept. of Neurobiology and Anatomy, Univ. of Texas Medical School, Houston, TX 77225

The tail-withdrawal reflex is mediated at least in part by a monosynaptic circuit with properties that may be modulated by several forms of learning. The strength of the synaptic connection between sensory neurons (SNs) and motor neurons (MNs) is correlated with strength of the reflex response. However, it is difficult to explain how brief (< 1 s) trains of monosynaptic EPSPs from SNs to MNs can drive extended (> 10 s) activity in MNs and how observed changes in the amplitudes of these EPSPs produce changes in the duration of MN responses. To examine the determinants of intensity and duration of MN responses, we used a simulation program SNNAP (Ziv et. al., this volume) to construct a computer model representing the interactions among neurons of the circuit. Membrane conductances of model neurons are described by H-H type equations. Parameters were derived from electrophysiological and, in some cases, voltage-clamp studies.

Simulations indicate that monosynaptic connections from a number of SNs to a single MN contribute only to the first 1 s of the MN response, even when synaptic efficacies are greatly enhanced. In order to simulate the duration of the MN response, it is necessary to include elements representing recently described excitatory interneurons (INs) that elicit slow, decreased-conductance EPSPs in MNs. Disynaptic connections via as few as two INs can lead to extended (~20 s) response durations (RDs) in MNs at average spike rates (SRs) of about 2 sp/s. Changes in cellular properties of SNs and INs lead to changes in both RDs and SRs in MNs. Enhancement of EPSP amplitude by 100% via spike broadening in SNs leads to a 12% increase in SR and an 18% increase in RD. Synaptic depletion in SNs to 50% of control levels reduces SR by 31% and RD by 11%. Spike broadening in the INs is even more effective than that in SNs in increasing both SR and RD. Effects of spike broadening in SNs and INs are cumulative. We conclude that a network like the one described can account for the relationship between spike width and synaptic depletion in SNs and duration of responses in MNs. The simulations predict that changes in the properties of the INs would prove very effective in modulating the tail-withdrawal response.

633.7

SIMULATIONS OF ACTION POTENTIALS, TRANSMITTER RELEASE, AND PLASTICITY OF SENSORIMOTOR SYNAPSES IN *APLYSIA*. C. C. Canavier, D. A. Baxter, J. W. Clark*, and J. H. Byrne. Dept. of Electrical and Computer Engineering, Rice University, Houston, TX 77251 and Dept. of Neurobiology and Anatomy, The University of Texas Medical School, Houston, TX 77225.

A Hodgkin-Huxley type membrane model of an *Aplysia* sensory neuron has been combined with a model of transmitter mobilization and release, as well as a model of the synaptic coupling with a follower motor neuron. The membrane currents in the model, as well as their modulation by 5-HT, were largely based on voltage-clamp data from the sensory neuron soma. In addition, material balances on cAMP and Ca^{2+} in the terminal were included. Release was modeled as a cubic function of Ca^{2+} current and a linear function of available transmitter. The population of transmitter vesicles was compartmentalized into pools. In addition to its effects on membrane currents, a facilitatory effect of 5-HT on transmitter mobilization was modeled. The parameters of the transmitter mobilization and release model were adjusted by fitting the simulated EPSP to published data recorded during presynaptic voltage-clamp pulses of varying duration (Hochner et al., 1986). The model of synaptic coupling included transmitter accumulation and removal from the cleft, as well as kinetics associated with the transmitter-gated current in the postsynaptic membrane. Simulations of voltage-clamped sensory neurons produced a good fit to the waveforms and maximum amplitudes of EPSPs under normal, depressed, and facilitated conditions. Simulations of action potentials also accurately predicted the resultant spike-induced EPSP. Moreover, modulation of K^+ channels and mobilization simulated empirically observed 5-HT-induced spike broadening and enhancement of transmitter release. Our goal is to incorporate this model of a sensory neuron and its synaptic terminal into a realistic network in order to simulate the modification of reflex circuits by simple forms of learning.

633.8

SEROTONIN- AND PKC-INDUCED SPIKE BROADENING IN TAIL SENSORY NEURONS OF *APLYSIA*. S.Sugita, D.A.Baxter and J.H.Byrne. Dept. of Neurobiology and Anatomy, Univ. of Texas Med. Sch., Houston, TX 77225.

Serotonin-induced spike broadening in the tail sensory neurons is due to both cAMP-dependent and cAMP-independent mechanisms (Baxter and Byrne, 1990). Since 5-HT translocates protein kinase C (Sacktor and Schwartz, 1990), we have begun to examine the possible role of PKC in the cAMP-independent aspect of broadening.

Application of phorbol 12,13-diacetate (PDAc, 3μM, n=9) led to a 26% increase in the duration of the action potential recorded in ASW (15°C), where spike width was measured as the time from the peak to 10% of the amplitude of the peak. 12-deoxyphorbol 13-isobutyrate (1-2μM, n=6) had similar effects. This spike broadening, like that induced by 5-HT, developed slowly and reached a maximum in 10-20 min. PDAc-induced broadening was blocked by a PKC inhibitor, calphostin C (3μM, n=4). Moreover, inactive phorbols (3μM, n=3) did not produce broadening nor interfere with the ability of 5-HT to induce broadening. Thus, the phorbol ester-induced broadening seems to be specific and due to the activation of PKC. Coapplication (n=8) of PDAc (3μM) and 8-bromo cAMP (1.5-5mM) produced the same level of broadening (25%) as PDAc alone.

We also examined the relationship between 5-HT- and phorbol ester-induced broadening. Application of 5-HT (40μM) after phorbol esters produced further broadening (increase from 25% to 53%, n=15), but was less than the maximal broadening produced by 5-HT alone (82%, n=8). In contrast, application of PDAc after 5-HT did not produce further broadening. These results suggest that spike broadening produced by 5-HT and PKC have some convergent sites or some common mechanism.

633.9

ATTENUATION OF SYNAPTIC STRENGTH PRODUCED BY β-BAG CELL PEPTIDE IS NOT DEPENDENT ON SPIKE NARROWING IN TAIL SENSORY NEURONS OF *APLYSIA*. J.R. Goldsmith and J.H. Byrne. Department of Neurobiology & Anatomy, University of Texas Medical School, Houston, TX 77225.

An extract of the bag cells inhibited the baseline tail-siphon withdrawal reflex and selectively suppressed long-term sensitization of the reflex (Goldsmith and Byrne, 1990). In addition, using an *in vitro* analogue of the reflex, we identified a possible neural site of action for the inhibition of the baseline reflex as well as three bag cell peptides (BCPs) which may produce the effect. β-BCP, ELH and γ-BCP were found to attenuate the synaptic strength of the sensory-to-motor connection underlying the tail withdrawal component of the reflex. The present experiments were designed to explore the possible role of presynaptic spike narrowing in this modulation. The effects of β-BCP, the BCP which produced the most robust attenuation of the EPSP, were examined.

Testing consisted of eliciting a single action potential in a tail sensory neuron (SN) by a brief intracellular depolarizing pulse and recording the monosynaptic EPSP elicited in a tail motor neuron. Ganglia were perfused with ASW containing 100 mM TEA (to facilitate detection of changes in duration of the action potential) and low Na^+ (to reduce spontaneous synaptic activity). Five action potentials were elicited in each of two test periods, the pretest and posttest, at an interstimulus interval of 5 min. β-BCP (1-10 μM) (n=14) or artificial seawater (ASW) (n=14) was bath applied 2.5 min after the last stimulus in the pretest. The posttest was begun 2.5 min after the bath application. Motor neurons were hyperpolarized by 30 mV during each test.

β-BCP produced a small but statistically insignificant narrowing of the SN action potential. However, there was an attenuation of the monosynaptic EPSP produced by β-BCP which became significant at the last trial of the posttest. These results suggest that β-BCP does not attenuate the monosynaptic EPSP by modulating membrane channels that regulate the duration of the SN action potential.

633.10

EFFECTS OF FMRFAMIDE ON INPUTS TO PERIPHERAL SIPHON MOTOR NEURONS IN *APLYSIA*. J. Cooper* and G. A. Clark. Program in Neuroscience, Princeton University, Princeton, NJ 08544.

The connections of *Aplysia* LE siphon sensory cells onto central motor neurons in the abdominal ganglion undergo presynaptic inhibition in response to the neurotransmitter FMRFamide. However, the siphon sensory cells also synapse onto a group of peripheral siphon motor neurons, and the effects of FMRFamide at peripheral connections were unknown. As an initial step toward investigating whether presynaptic inhibition, like presynaptic facilitation, can be synapse-specific in these cells, we have examined the effects of FMRFamide on excitatory postsynaptic potentials (EPSPs) elicited in peripheral siphon motor neurons by stimulation of either individual sensory neurons or the siphon nerve. As with previous work on FMRFamide at central synapses, we found that application of 10^{-6} M FMRFamide strongly inhibited the monosynaptic EPSP elicited in peripheral motor neurons by intracellular sensory neuron stimulation (97% ± 2% decrease, $p < .001$). However, in contrast to this inhibition, we also found that FMRFamide produced facilitation of the EPSP evoked by stimulation of the siphon nerve, which contains the axons of the LE sensory neurons as well as other cells (144% ± 39% increase, $p < .02$). As yet, we do not know whether this facilitation is due to an increase in the number of afferent fibers activated by the extracellular stimulus, or to an increase in synaptic efficacy *per se* (presumably at connections of low-threshold, non-LE cells). Nonetheless, because synaptic facilitation by FMRFamide has not been previously described, these results point to a possible new function for this transmitter, and further suggest that the same neuromodulatory substance can produce both inhibition and facilitation at different inputs onto a common postsynaptic target.

633.11

SIMPLE NEURAL NETWORK MODELS PROVIDE HEURISTIC TOOLS FOR UNDERSTANDING THE POSSIBLE ROLE OF COMMAND-LIKE NEURONS CONTROLLING BEHAVIORS IN *APLYSIA*. I. Kupfermann, D. Deodhar* and K.R. Weiss. Center for Neurobiolgy and Behavior, and Dept. of Physiology and Biophysics, Columbia U. School of Medicine and Mt. Sinai School of Medicine, New York, N.Y.

Feeding behavior in *Aplysia* involves two different types of responses: a highly stereotyped bite-swallow, and a more diverse orienting response. Bite-swallows are elicted by food stimuli applied to the medial region of the lip-tentacles. Orienting turns are elicited by intermediate or far lateral stimulation of the lip-tentacle region, and the magnitude of the response is a function of the distance of the stimulus from the mouth. If a far lateral stimulus is followed by a more medial stimulus, the response is appropriate to the more medial stimulus. No turns are elicited during the execution of a bite-swallow. The above input-output characteristics was modelled by means of a simple neural network consisting of an input layer (sensory neurons) of 5 units (medial, left and right intermediate and lateral receptive fields); an output layer (motor neurons) of three units (bite, left turn, right turn); and a hidden layer of 5 units. The network was repeatedly presented with 12 combinations of inputs and target outputs, and the connections were adjusted until the circuit produced the desired output for each of the possible inputs. Different networks were generated by varying the intitial conditions and transfer functions for the units. The functional role of individual units was analyzed by "stimulating" and "ablating" individual hidden units. The results indicate that simple tests done on individual neurons can provide misleading information on the actual role of the neuron in generating behavior.(MH 35564 & GM 320099)

633.12

FMRFamide INDUCES AN INCREASE IN Na^+ AND K^+ CONDUCTANCE IN THE LFS SIPHON MOTOR NEURONS OF *APLYSIA*. K.J. Belkin and T.W. Abrams. Dept. of Biol. and Inst. of Neurological Sci., Univ. of Pennsylvania, Phila., PA 19104

The molluscan neuropeptide FMRFamide has an inhibitory effect on at least two sites in the neural circuit mediating siphon withdrawal in Aplysia -- siphon sensory neurons (Piomelli et. al., 1987) and the LFS motor neurons (Belkin and Abrams, 1990). We have analyzed the ionic basis of the response of LFS neurons to FMRFamide. A puff of 2 uM FMRFamide onto the cell body causes a biphasic response: a transient inward current lasting 2-5 sec, followed by a prolonged outward current. The early phase of the FMRFamide response, which involves an increase in membrane conductance, is reduced in low $[Na^+]_o$ saline and is not blocked by 100 uM TTX. The early inward current inactivates with repeated application of FMRFamide. As previously reported, the prolonged outward current is carried by K^+. 2mM 4-AP blocks the late outward current. Neither 100 mM TEA nor the reduction of $[Ca^{++}]_o$ affect this outward current. In the presence of 4-AP, a third component of the FMRFamide response, a small prolonged inward current, is unmasked. Our pharmacological observations indicate that the K^+ current modulated by FMRFamide is distinct from $I_{K\text{-}S}$, which is increased by FMRFamide in the presynaptic sensory neurons of this circuit (Belardetti, et. al., 1987), as well as from $I_{K\text{-}V}$ and $I_{K\text{-}Ca}$.

633.13

cAMP AND ARACHIDONIC ACID INDUCE OPPOSITE MORPHOLOGICAL CHANGES WITH LONG-TERM PRESYNAPTIC FACILITATION AND INHIBITION IN THE SENSORY NEURONS OF *APLYSIA*. P.G. Montarolo*, D.L. Glanzman, E.R. Kandel & S. Schacher. Ctr. Neurobiol. & Behav., Columbia P&S, NYSPI, & HHMI, N.Y., N.Y. 10032.

The long-term changes in synaptic effectiveness with presynaptic facilitation of sensorimotor synapses in culture produced by 5-HT, and with long-term presynaptic inhibition produced by FMRFamide, can be mimicked by their respective second messengers. We now asked: Can cyclic AMP and arachidonic acid also produce structural changes in the sensory cells similar to those produced by 5-HT and FMRFamide? Iontophoretic injection of cAMP (200mM) into sensory cells evoked an increase of 70% ± 11 (N = 8), in the amplitude of the EPSP that was detectable 24 hrs later. This increase in synaptic strength was accompanied by a significant increase of 42% ± 18 in the number of sensory cell varicosities. Similarly, bath incubation (2 hr) of arachidonic acid (10 μM) evoked a significant decrease of -29% ± 3 in the amplitude of the EPSP and a decrease of -19% ± 2 (N = 8) in the number of sensory cell varicosities after 24 hr. Control injection of 5' AMP (200mM) into sensory cells (N = 8) or control incubation of sensorimotor cultures (N = 8) with eicosenoic acid (25 μM) had no significant effect on the EPSP or on the number of sensory cell varicosities (-10% ± 5 or 3% ± 2 for EPSP; -7% ± 3 or 4% ± 4 for varicosities). These results support the idea that the number of synaptic varicosities can be regulated in both directions by the appropriate modulatory transmitters and second messengers involved in presynaptic facilitation and presynaptic inhibition.

633.14

CYPROHEPTADINE BLOCKS 5-HT-INDUCED SPIKE BROADENING BUT NOT 5-HT-INDUCED ANTI-ACCOMODATION: EVIDENCE FOR MULTIPLE 5-HT RECEPTORS IN *APLYSIA* SENSORY NEURONS. Mercer, A.R.* and Carew, T.J. Dept of Psychology, Yale University, New Haven, CT 06520

Serotonin (5-HT) acts as a neuromodulator of pleural sensory neurons (SNs) in *Aplysia*. Two known effects of 5-HT are spike broadening and increased excitability (anti-accomodation). We examined whether these 5-HT-induced effects could be blocked by the 5-HT antagonist cyproheptadine (CYP). Bilateral pairs of pleural ganglia were used: one was tested with 5-HT alone, the other was treated with CYP 10 min prior to and during 5-HT application. SPIKE BROADENING: Brief pulses (2ms, 5nA) were used to elicit single spikes from SNs. 5-HT (5μM) significantly broadened spikes in control cells ($\bar{x}$ =19% increase, $p<.01$, N=5), whereas in the presence of CYP (200μM) SNs showed no significant spike broadening ($\bar{x}$ =-0.7% increase, N=6). A between-group comparison showed that CYP caused a significant reduction in 5-HT-induced spike broadening ($p<.01$). CYP itself had no significant effect on spike duration ($\bar{x}$ =-0.8% increase). 5-HT-induced spike broadening in pleural SNs involves reduction of IKv (Baxter and Byrne, 1990). In preliminary voltage clamp studies, we find that CYP can antagonize 5-HT-modulation of IKv. We are currently examining the effects of CYP on other currents (eg: IKs , Siegelbaum et al, 1982). ANTI-ACCOMODATION: In the same cells as above, 200ms (0.5nA) pulses were used to elicit spikes from SNs. 5-HT produced a significant increase in spike number both in 5-HT alone ($\bar{x}$ increase=200%, $p<.02$, N=4) and in 5-HT+CYP ($\bar{x}$ increase=200%, $p<.02$, N=5) . Thus CYP does not block the 5-HT-induced increase in excitability.

Our results indicate that some of the actions of 5-HT on pleural SNs are mediated by receptors differentially sensitive to CYP. This 5-HT blocker now provides a valuable tool with which to analyze the relative contributions of different receptors to neuromodulation associated with sensitization in *Aplysia*.

633.15

SENSORY NEURON SPIKE BROADENING INDUCED BY TAIL NERVE STIMULATION IN *APLYSIA* IS BLOCKED BY CYPROHEPTADINE. Marcus, E.A., Mercer, A.R.*, Emptage, N. J.* and Carew, T.J. Departments of Biology and Psychology, Yale University, New Haven, CT 06520.

Tail shock-induced sensitization in *Aplysia* is known to involve spike broadening in pleural sensory neurons (SNs). This effect on spike duration is mimicked by serotonin (5-HT) suggesting that tail shock-induced sensitization is mediated, at least in part, by 5-HT. We have tested this hypothesis directly by using the 5-HT antagonist cyproheptadine (CYP), which blocks 5-HT-induced spike broadening in SNs (Mercer and Carew, this volume).

Brief pulses of depolarizing current (2ms, 5nA) were used to elicit single spikes from pleural sensory neurons. Modulatory input to SNs was activated by stimulation of the tail nerve P9 (3 sec train of 50 ms pulses at 10Hz). Spike duration was measured before and after P9 stimulation. Ganglia from one side of the animal served as controls; those from the opposite side were treated with CYP (200 μM). P9 stimulation significantly increased spike duration in control SNs ($\bar{x}$ =16%, p<.05, N=6), whereas SNs treated with CYP showed no significant spike broadening ($\bar{x}$ =3%, N=6). Thus, CYP blocks spike broadening induced in pleural SNs by tail nerve stimulation.

Consistent with earlier findings that CYP selectively blocks 5-HT-induced spike broadening without affecting 5-HT-induced changes in excitability (Mercer and Carew, this volume), we have also found that CYP does not affect increases in excitability produced by P9 nerve stimulation. Specifically, in 5 experiments, P9 stimulation in normal sea water produced significant anti-accomodation ($\bar{x}$ increase in spike number=880%, p<.03) that was unchanged in CYP ($\bar{x}$ increase=880%, p<.03).

Our results complement previous work by providing direct evidence that 5-HT acts as a neuromodulator in tail SNs of *Aplysia*. Furthermore, our data suggest that endogenous 5-HT acts at different receptor sites in producing different forms of plasticity.

633.16

THE DUAL PROCESSES OF PRESYNAPTIC FACILITATION PRODUCED BY SEROTONIN ARE INHIBITED BY CYPROHEPTADINE IN SENSORY NEURONS OF *APLYSIA*. J.P. Pieroni and J.H. Byrne. Dept. of Neurobiology and Anatomy, Univ. of Texas Med. Sch., Houston, TX 77225

Serotonin (5-HT)-induced facilitation of transmitter release from sensory neurons mediating defensive reflex responses in *Aplysia* is due to at least two processes, spike broadening and a second process termed mobilization (Gingrich *et al.*, 1985, 1988; Hochner *et al.*, 1986). Since Mercer and Carew (this volume) demonstrated that cyproheptadine, a serotonin antagonist, blocks 5-HT-induced spike broadening in pleural sensory neurons, we were interested in determining whether it also affects 5-HT-induced enhancement of mobilization.

Abdominal ganglia were pretreated with TEA (100 mM) and TTX (250 μM) and connections between LE sensory neurons and follower cells (LFS or other similarly sized cells in the same vicinity) were monitored. In this preparation, application of 5-HT leads to an increase in spike duration and an increase in the amplitude of the monosynaptic EPSP which is independent of spike broadening (Pieroni and Byrne, 1989, 1990). Confirming Mercer and Carew, we found that cyproheptadine (200 μM) inhibited 5-HT-induced spike broadening. Moreover, it inhibited 5-HT-induced facilitation of the EPSP. Cyproheptadine did not appear to block spike broadening or facilitation induced by small cardioactive peptide B.

The inhibitory effects of cyproheptadine were seen when 5-HT was applied at either 5 or 25 μM. Preliminary analyses indicate that cyproheptadine may block spike broadening to a greater degree than it does enhancement of the EPSP, especially at the higher concentration of 5-HT. These results lend further support to the hypothesis that spike broadening and other mechanisms involved in the enhancement of the EPSP can be dissociated. Moreover, just as the facilitatory processes of spike broadening and anti-accommodation seem to be mediated by separate 5-HT receptors (Mercer and Carew, this volume), our results indicate that this dissociation may extend also to other aspects of facilitation, such as mobilization.

633.17

ANNEXINS ARE EXPRESSED DIFFERENTIALLY IN CNS, INCLUDING SENSORY NEURONS, AND EYE OF *APLYSIA*. L.J. Cleary, A. Eskin, J.H. Byrne. Dept. of Neurobiology and Anatomy, Univ. Texas Med. School, Houston, TX 77225.

In pleural sensory neurons and the eye, 5HT affects the synthesis of several proteins. One such protein from eye has been partially sequenced and shown to be a member of the annexin family (Raju et al., '90). An interesting property of the annexins is their high affinity binding to membrane phospholipids in the presence of Ca^{2+}, suggesting that they could play a role in regulating neuronal function. Using affinity-purified polyclonal antibodies against the consensus sequence peptide (αCS; Kaetzel & Dedman, '89), we examined the distribution of annexins in CNS and eye.

αCS-immunoreactivity (IR) was selectively, but not exclusively, expressed in sensory neurons throughout the CNS. In whole mounts, αCS-IR appeared to be present in all neurons in the pleural sensory cluster. Larger medial cells were not labeled. In the abdominal ganglion, neurons in the LE and RE clusters were labeled as well. Only a fraction of neurons in the sensory J cluster of the cerebral ganglion were labeled, and the sensory S cluster of the buccal ganglion was unlabeled. Other labeled cell groups include three medial clusters on the ventral surface of the cerebral and a cluster of 5-7 neurons, including B1, on the caudal surface of the buccal ganglia. In the eye, αCS-IR was present in the rhabdom, neuropil and optic nerve, but seemed to be excluded from the pigment cell layer. The distribution of labeling was clearly different from that of 5HT, which labels only the neuropil, or FMRFa and myomodulin, which each label a subpopulation of small cells in the photoreceptor layer. Western blots of homogenized pleural ganglia revealed one major cross-reactive protein of ~40 kD, and several minor bands.

The differential expression of annexins within the CNS might be related to the functional role of the IR neurons. One common feature of sensory neurons and the eye is sensitivity to 5HT. It is conceivable that annexins contribute to the cellular response to modulatory transmitters through alterations of intracellular calcium.

633.18

CLASS III ß-TUBULIN STAINING OF PERIPHERAL NEURONAL STRUCTURES IN APLYSIA. I.Steffensen*, M.Anctil, D.H.Vandorpe* and C.E.Morris. Biol. Dept., Univ. Ottawa & Neurosciences, Loeb Inst., OCH, Ottawa, Ont. K1Y 4E9.

Aplysia mechanosensory inputs from the tail region make monosynaptic connections with motor neurons in the pedal ganglia. Although synaptic changes associated with learning and memory have been extensively studied at the sensory-motor synapse, the peripheral mechanosensitive terminals have not been characterized. Class III ß-tubulin, a tubulin isotype expressed at the onset of neuronal differentiation in vertebrates, is neuronal-specific. Because it also appears to be neuronally-specific in Aplysia, we have been able to use it here to trace peripheral neuronal structures. The structures were visualized by immunofluorescence, using a monoclonal antibody against class III ß-tubulin on frozen cryostat sections from the tail region and from tentacles (tentacles have been described at the EM level: Emery & Audesirk 1977 J Neurobiol. 9:173). Neuronal structures in the tail region were comparable to those of the tentacles' mechanosensory exterior but not to those in its chemosensory groove. A variety of other ultrastructural and neuronal tracing techniques (eg. 2-deoxyglucose, lipophilic dyes, EM) to reveal mechanosensory endings in the tail region are also being explored. Supported by the Canadian NCE and NSERC.

633.19

SENSITIZATION IN JUVENILE APLYSIA IS AFFECTED BY DIET. J.M.Flinn,S.Kurtz,C.Hong,S.West*,Dols,S.* George Mason Univ,Fairfax,VA; EPA,Gulf Breeze,FL.

Juvenile Aplysia were raised from 50 days post-hatch on algae treated to be low in protein & tryptophan (LO), low in protein but high in tryp. (T), low in tryp. but high in protein (P) and high in both protein & tryp., (HI). Animals were tested for sensitization over a 3 wk period beginning 110 days post-hatching. At the beginning of testing the mean wts. in gms were .09 (LO), .10 (T), .42 (P), .37 (HI). Sensitization was examined by measuring escape behavior following tail shock and comparing control (C) and sensitization (S) groups. Preliminary analyses showed that the greatest sensitization level was seen in the T group at 130+ days post-hatch. The sensitization in this group increased over the 3 wk period and at 130+ days was +235% (S) vs -29% (C). Sensitization in the HI group also increased over the 3 wks and at 130+ days was +69% (S) vs -31% (C). The LO & P groups decreased in sensitization over 3 wks and did not show sensitization at 130+ days. The data suggests that a tryptophan-rich, protein-poor diet produced greater sensitization than a diet rich in both. HPLC analyses will be performed to examine the levels of serotonin and other neurotransmitters.

633.20

INDIRECT MODULATORY EFFECTS ON ANTERIOR FOOT MOVEMENTS BY THE C-PR NEURON IN *APLYSIA*. T. Nagahama, K. R. Weiss and I. Kupfermann. Center for Neurobiol. & Behav., Columbia U. and NYS Psychiat. Inst., New York, N.Y. 10032, and Dept. of Physiol. and Biophysics, Mt.Sinai School of Med., New York, N.Y. 10029

The cerebral neuron C-PR may function to elicit various manifestations of a food arousal state in *Aplysia*. We previously reported that C-PR firing produces polysynaptic EPSPs in neck motor neurons, and polysynaptic IPSPs in anterior foot motor neurons (AFs). We now report that the C-PR firing produces polysynaptic EPSPs in motor neurons innervating the middle and posterior foot. These results are consistent with the suggestion that the C-PR may mediate the appetitive feeding posture in which the animal lifts its head and expands the anterior foot while supporting its body with the middle and posterior foot. C-PR also has indirect modulatory effects on the anterior foot movements produced by the AFs. We previously showed that these effects may be caused by pedal modulator neurons (P1Rs) which receive monosynaptic EPSPs from the C-PR. There appear to be two types of P1R neurons: those (P1R-E) that enhance (or initially reduce and then enhance) contraction, and those (P1R-R) that reduce contraction. P1R-E neurons enhance (or reduce and enhance), whereas P1R-R neurons reduce the size of the synaptic currents produced by AFs. Preliminary data indicate that the P1R-E neurons are immunoreactive to pedal peptide (PEP) (antibody supplied by P.E. Lloyd). PEP applied arterially predominantly enhanced anterior foot movements evoked by AFs and increased the size of synaptic currents produced by AFs. These results suggest that the P1R-E neurons may contain PEP, and that PEP may contribute to the enhancement effect of these neurons. The enhancement effects of PEP and of P1R-E are completely blocked when the muscle is bathed in ASW containing high divalent cations, suggesting that PEP is either acting on a peripheral nerve net or is altering the excitability of the nerve terminals of the AFs.

633.21

Two patterns of buccal nerve activity that distinguish ingestion from rejection can be replicated in a reduced preparation in *Aplysia*. D. W. Morton[1] and H. J. Chiel[2,1], Departments of [1]Neuroscience and [2]Biology, Case Western Reserve University, Cleveland, OH., 44106.

We are studying the neural basis of feeding in *Aplysia californica*. Our approach has been to associate *in vivo* patterns of neural activity with specific behaviors and then develop a reduced preparation which produces similar patterns of activity and is suitable for intracellular recording. Using *in vivo* cuff electrode recordings of buccal nerve 2 (BN2) and the ipsilateral radula nerve (RN) with a simultaneous video record, we have associated two patterns of large unit activity with two types of consummatory feeding behavior. Pattern I is associated with ingestion (biting or swallowing), and is characterized by a burst of large units on the RN occurring together with a burst of large units on BN2. Pattern II is associated with rejection, and is characterized by the RN activity preceding the BN2 activity. The burst of RN activity occurs with retraction in pattern I and protraction in pattern II. To investigate the neural basis of these patterns, we have developed a reduced preparation producing two patterns of large unit activity, termed *in vitro* patterns, that are qualitatively similar to the *in vivo* patterns. Using this preparation, we have identified a buccal ganglion neuron that closes the radula when stimulated, appears as a large unit in RN extracellular recordings, and is active during the RN burst in both *in vitro* patterns. These results suggest that this radula closer motor neuron is active with retraction during ingestion and protraction during rejection, consistent with observations of radula closure during these behaviors. Studying this motor neuron's pre-synaptic cells during both *in vitro* patterns could provide insights into the neural control of radula closure during ingestion and rejection in *Aplysia*. Support: BNS-8810757 and 5T32GM07250.

633.22

Characterization of Pattern Triggering Neurons from *Aplysia's* Buccal Ganglion *In Situ* and in Culture. H. J. Chiel[1,2] and Jingtao You[1], Departments of [1]Biology and [2]Neuroscience, Case Western Reserve University, Cleveland, OH, 44106.

Pattern triggering neurons have been described in the buccal ganglion of *Aplysia* (Susswein and Byrne, J. Neurosci. 8:2049, 1988). Do these neurons induce bursting due to their intrinsic properties or by their connections to other neurons? To answer this question, we have studied these neurons in the buccal ganglion and in isolated cell culture. Cells were identified using the criteria of Susswein and Byrne. The only neurons which we found that triggered patterns also generated action potentials, and thus might correspond to B33, 35 or 37. In the intact ganglion, cells were injected with lucifer yellow and their morphology was visualized. Of 8 cells filled in 8 different ganglia, 5 showed a single axon which crossed the midline of the ganglion via the buccal commissure; the remaining 3 had axons projecting via peripheral nerves (buccal nerves 2, 3 or the cerebral buccal connective). In the intact ganglion, low calcium solutions blocked the ability of the neurons to trigger patterns, but caused spontaneous bursts to occur in B4. High divalent cation solutions greatly reduced the ability of the neurons to trigger patterns, but fast IPSPs and slow EPSPs from these neurons to B4 were clearly observed. Maps of ganglia were drawn with a camera lucida, pattern triggering cells were identified physiologically, and cells were removed for isolated cell culture. In 10 experiments (one in which the cell was cultured in isolation, and 9 in which it was co-cultured with B4), pattern triggering neurons showed irregular firing in response to large depolarizing currents *in vitro*, but did not burst. These results support the hypothesis that the pattern-triggering neurons induce bursting in the buccal ganglion by means of synaptic connections to other neurons. Supported by NSF grant BNS-8810757.

633.23

THE RELATIVE INFLUENCE OF OLFACTORY AND TACTILE STIMULI ON THE FEEDING BEHAVIOR OF THE NUDIBRANCH, *MELIBE LEONINA*. W.H. Watson III and C.M. Chester*. Dept. of Zoology, University of New Hampshire, Durham, N.H. 03824

The nudibranch, *Melibe leonina*, feeds by rhythmically sieving small crustaceans from the water with its large oral hood. Previous studies demonstrated that the frequency of feeding movements is proportional to the concentration of prey. The purpose of this study was to determine what qualities of prey are important in modulating the frequency of *Melibe* feeding.

During a typical experiment 4 animals were placed in an aquarium and exposed to one of the following treatments: 1)*Artemia* at a concentration of 1500/ml; 2) *Artemia*-conditioned seawater; 3) small (350 μm) Sephadex beads in clean, filtered seawater or; 4) Sephadex beads soaked in *Artemia*-conditioned water. The feeding frequency of each animal was then monitored for 20 min.

Both conditioned water (olfactory stimuli) and Sephadex beads (tactile stimuli) caused appetitive behavior (orientation of the oral hood) and a significant increase in the rate of feeding. When both were added together, they produced a larger increase than either did alone, but still not as great a response as the intact *Artemia*. Thus, we conclude that *Melibe* detects the presence of prey using at least three different signals: smell, touch, and perhaps movement of live prey. This study was supported by the U.N.H. BRSG program.

633.24

OPTICAL MEASUREMENT OF ACTIVITY IN THE MELIBE LEONINA BUCCAL GANGLION. Larry B. Cohen, Win Watson, Jim Trimarchi, Chun X. Falk and Jian-young Wu. Dept. of Physiology, Yale University School of Medicine; Dept. of Zoology, University of New Hampshire; and Marine Biological Laboratory.

Molluscan buccal ganglia have been used to study the neural basis of feeding behavior. However, detailed information about the underlying neural circuits is lacking for most buccal preparations because of their complexity. The buccal ganglion of Melibe leonina, with only 40-50 neurons, is relatively simple and transparent. Optical recordings from this ganglion might be able to monitor all the action potentials in all of the neurons. Using ganglia stained with a merocyanine (JPW1124) or an oxonol (RH155) dye we tested the completeness of the recording. In one preparation we were able to detect activity in more than 90% of the cells. In a recording made during rhythmic output from the ganglion we detected activity in about 50% of the neurons. However, in many preparations the measuring light led to a photo-stimulation. We plan to try reduced dye concentration and additional dyes to avoid this effect. Supported by NIH grant number NS08437 and a UNH Summer Faculty Scholarship.

633.25

ACQUISITION PHASE OF FEEDING BEHAVIOR IN THE PTEROPOD MOLLUSC CLIONE LIMACINA. T.P. Norekian and R.A. Satterlie. Dept. of Zoology, Arizona State Univ., Tempe, AZ 85287-1501 and Friday Harbor Laboratories, Friday Harbor, WA 98250.

Two types of acquisition responses have been observed in Clione. (1) Buccal cone eversion can be triggered by direct contact with prey. The threshold for such a "fast strike" is very high, and an unsuccessful strike results in immediate withdrawal of the buccal cones. (2) Perfusion of prey extract across the head of a Clione, placement of an animal in the vicinity of feeding animals, or injection of serotonin into the hemocoel of a non-feeding animal triggers buccal cone eversion without direct contact with a prey item. In these cases, buccal cones remain extended for prolonged periods (hunting behavior).

Three classes of cerebral motoneurons have been identified that are directly involved in prey acquisition. One group (A neurons) initiates lip retraction, buccal cone eversion and general contraction of head musculature. A neurons inhibit the other two classes of neurons which retract the buccal cones (B neurons) and close the lips (C neurons). Serotonin activates A neurons and thus favors buccal cone eversion. Dopamine depolarizes both A and B neurons, but the effect is much more powerful in B neurons. Dopamine thus favors buccal cone retraction. Superfusion of GABA produces simultaneous inhibition of B neurons and excitation of A neurons. The difference between a fast strike and hunting behavior may hinge, at least in part, on the interplay between several modulators/transmitters which regulate the excitability of feeding neurons.

634.1

DEPLETION OF SOMATOSTATIN BY CYSTEAMINE SELECTIVELY INHIBITS DARK-ONSET FEEDING IN RATS. D. Feifel and F.J. Vaccarino, Department of Psychology, University of Toronto, Toronto, Canada M5S 1A1.

In a previous study we demonstrated that blockade of endogenous GRF by injections of GRF antiserum into rat hypothalamus, selectively inhibited dark-onset feeding, but not feeding at other circadian time points, This suggested that endogenous GRF plays a role in the circadian organization of feeding. We have also reported that inncreased somatostatin activity is an important output for GRF-induced feeding. This study sought to determine if endogenous somatostatin, like GRF, also plays a role in circadian-specific feeding.

Male Wistar rats were housed under a 12:12 light-dark photoschedule and tested for their feeding response to subcutaneous injections of cysteamine (300 mg/kg) (known to deplete body somatostatin). Results indicated that cysteamine injections selectively inhibited food intake (51%) at dark-onset but not at other time point tested.

These findings suggest that somatostatin, like GRF, is involved in the mechanism which organizes the circadian pattern of feeding in rats. This work was supported by NSERC grant 35036 to F.J.V. D.F. was supported by a MRC Postgraduate Scholarship.

634.2

THE EFFECT OF Nle^4,D-Phe^7-[α-MSH] AND ITS FRAGMENTS ON CORE TEMPERATURE IN RATS. L.H. Raible*[1] and M. Camacho-Ochoa[2]. Kalamazoo College, Kalamazoo, MI 49007[1], The Upjohn Company, Kalamazoo, MI 49001[2].

The research of Lipton and coworkers indicates that α-melanocyte stimulating hormone (MSH) exerts antipyretic and hypothermic actions in the rabbit. Research on MSH and temperature in the rat has utilized peripheral or ventricular injections and has yielded inconclusive findings. More direct application of MSH or an analog of MSH might produce more conclusive results. The present experiments examined the effects of a potent MSH analog (Nle$_4$, D-Phe$_7$-[α-MSH]; NDP-MSH) and its fragments (NDP-MSH$_{1-7}$, NDP-MSH$_{4-10}$, NDP-MSH$_{7-13}$; all provided by Dr. Tomi Sawyer, The Upjohn Company) on temperature when injected into the anterior hypothalamic/medial preoptic area (AH/POA). Results indicated that rats receiving .5 pmol of NDP-MSH/.5 µl NaCl displayed significantly greater temperatures than those receiving the vehicle, $F(5,30)= 5.00$, $p<.002$. The least amount of variance was observed in those subjects receiving 5 pmol NDP-MSH. The second experiment indicated that AH/POA injections of 5 pmol/.5 µl NDP-MSH and its 4-10 and 7-13 fragments significantly elevated temperature while injections of vehicle and of the 1-7 fragment did not, $F(4,52)=12.72$, $p<.0001$, a finding consistent with other assays. Studies designed to eliminate the possibility of a pyrogenic contaminant in the effective solutions are currently underway.

634.3

CENTRALLY INJECTED BOMBESIN ELICITS GROOMING IN DEVELOPING RAT PUPS.
H. Piggins[1] and Z. Merali[1,2], [1]Psychology & [2]Pharmacology, University of Ottawa, Ottawa, Canada, K1N 6N5.
In adult rats intracerebroventricular (i.c.v.) injection of the tetradecapeptide bombesin (BN) elicits a scratching form of grooming behaviour. In this study, we aimed: (1) to ascertain if i.c.v. BN would elicit grooming behaviour in developing rats and (2) to characterize the sequential nature of this response. Briefly, anaesthetized pups of 1-20 days of age were injected i.c.v. with BN (0.01-1.0 µg) or saline (n=7/dose/age) and the videotaped responses scored for grooming and other behaviours. BN dose-dependently induced grooming in rat pups up to 20 days of age. The 20 day olds were the most and 1 day olds the least sensitive to i.c.v. BN. Non-contact scratching activity was prevalent from postnatal day 1 and was replaced in the sequence of grooming behaviour with contact scratching activities by postnatal day 20. Scratching activities appeared to form a subsystem connected to but nonetheless separate from washing behaviours and this was particularly distinguishable at postnatal day 20. Changes in BN-induced grooming reflected the maturation of the motor capabilities of the developing rat. Further, these results indicate that BN binding sites in the developing rat central nervous system are pharmacologically functional from an early stage in ontogeny.

(Supported by MRC)

634.4

EFFECT OF SYSTEMICALLY ADMINISTERED CERULETIDE ON MOUTH MOVEMENT IN CHRONIC FLUPHENAZINE TREATED RATS. T.Saito, T. Ashizawa, E.Hashimoto, H. Ikeda, H.Ozawa, N.Takahata. Dept. Neuropsychiatry, Sapporo Medical College, Sapporo 060 Japan

The effect of repeated administration of ceruletide (100 ug/kg/day, 1P, 3days) on mouth movement and SCH 23390 binding to striatal membrane were examined in chronic fluphenazine enanthate (FPZ)treated rats(25mg/kg, IM, every 3 weeksx10) and sesame oil treated (control) rats. Three weeks after final FPZ injection, mouth movement (teeth chattering, chewing, licking, opening of mouth, perioral tremor) and an increase in SCH 23390 binding to striatal membrane were observed in the FPZ-treated rats. High amplitude EMG discharge(8c/s),which correlated to perioral tremor was recorded from the masseter in the FPZ-treated rats. Repeated ceruletide injection suppressed mouth movement and normalized SCH 23990 bining to striatal membrane in the FPZ-treated rats. The effects of ceruletide on mouth movement and D_1 receptor continued for 6 days after final ceruletide injection. These findings indicate that systemically administered ceruletide affects the D_1 receptor and that an increase of D_1 receptor function may play an important role in the pathogenesis of tardive dyskinesia.

634.5

MIF-1 ALTERS PAVLOVIAN CARDIAC CONDITIONING, CONCOMITANT CORTICAL NEURONAL ACTIVITY, & DELAYS EXTINCTION IN RABBITS
Linda L. Hernández, Philip J. Tollison* & Charles M. Gibbs, Dept. VA Hospital & U. South Carolina, Columbia, SC 29201.

Rabbits received Pavlovian conditioning training in which a tone (CS+) was paired repeatedly with a mild eye-shock and another tone (CS-) was never paired with shock. Separate groups were treated with 10 or 100 µg/kg MIF-1 or saline vehicle (i.v.) prior to training and subsequent extinction testing. Tone-evoked bradycardia was acquired which was larger in response to the CS+ than the CS-; 10 µg/kg MIF augmented the magnitude of these learned responses, compared to saline, whereas 100 µg/kg MIF decreased conditioned response magnitude. Concomitant, tone-evoked multiple unit activity in medial prefrontal cortex (PFCm MUA) showed comparable changes; i.e. evoked PFCm MUA was larger in the group treated with the low dose of MIF, and was smaller in the group treated with the higher dose, than in saline-treated controls. During extinction, both MIF treatments delayed disappearance of the conditioned heart rate discrimination compared to saline; the higher dose reduced the magnitude of responses evoked by both CSs, whereas the low dose reduced the magnitude of responses evoked by the CS- and augmented those evoked by the CS+. These data show that MIF-1 can promote acquisition and delay extinction of conditioned autonomic responses and alter associated PFCm MUA during training. Supported by DVA Research funds and USPHS Grant RO1-DA06676.

634.6

EFFECTS OF POSTNATAL ADMINISTRATION OF ACTH AND NICOTINE ON REPRODUCTIVE PARAMETERS OF THE FEMALE RAT. S.E. Alves, H.M. Akbari, F.J. Antonawich, E.C. Azmitia and F.L. Strand. Department of Biology and Center for Neural Science, New York University, New York, NY 10003.

A previous study in our laboratory has shown that *prenatal* administration of ACTH 1-24 or nicotine decreases the lordosis response in female rats tested as virgins (Alves and Strand, 1990; Soc. for Neurosci., #114.8). This study was conducted to investigate whether *postnatal* treatment of these substances would affect the reproductive capacity of female rats. Sprague-Dawley rat pups were injected s.c. with either ACTH 1-24 (0.1mg/kg), nicotine hydrogen tartrate (0.25mg/kg) or saline vehicle once daily from postnatal day one (day of birth) to day 7. Starting at 30 days of age, the females were checked for vaginal opening as a sign of sexual maturation. At approximately 60 days of age, these animals were tested for sexual behavior as virgins. The sexual maturation of ACTH treated animals was significantly delayed when compared to nicotine treated and control animals ($p<0.01$). Animals from both treatment groups displayed decreased female sexual behavior, having significantly lower lordosis quotients and lordotic quality scores compared to control animals ($p<0.01$). Hypothalamic 5-HT fiber density was assessed using high affinity ^{3}H-5-HT uptake. 5-HT uptake was significantly increased in ACTH treated animals ($P<0.02$) but unchanged in nicotine treated animals compared to controls. This increase in hypothalamic 5-HT in ACTH treated animals could account for the decrease in female sexual behavior seen in these animals. Based on this study we suggest that sexual differentiation of the brain in the female rat is also susceptible to *postnatal* manipulation with ACTH or nicotine. We are currently investigating plasma sex steroid levels and immunohistochemistry for the neurotransmitters involved in female sexual behavior. This study was supported by the Council for Tobacco Research.

634.7

PEPTIDE T TREATMENT OF COGNITIVE IMPAIRMENT IN HIV+ INTRAVENOUS DRUG ABUSERS. M. I. Rosen*, C. VanDyck*, T. P. Bridge*, C. Duncan, S. S. O'Malley*, H. R. Pearsall*, B. L. Martini*, H. M. Thomas*, P. O'Connor*, H. Brett-Smith*, S. W. Woods, T. R. Kosten*. Yale University CMHC New Haven, CT 06519

Peptide T, an analogue of Vasoactive Intestinal Peptide, has improved cognitive function in patients with AIDS Dementia Complex in open clinical trials. We treated five methadone maintained, cognitively impaired HIV positive patients in a double blind cross-over study for four weeks with Peptide T, 5 Mg intranasally tid, and with placebo for four weeks. The five patients were impaired on at least two tests of neuropsychological function, and had been treated with AZT for at least one month prior to enrollment. The sample was 80% male, with a mean age of 37 and baseline WAIS of 84, verbal IQ of 86, performance IQ of 83.

Our patients showed an average improvement of .4 SD on the following five tests with Peptide T, compared with no change with placebo: Trails B (TMB), the Parker Verbal Learning Test (PVLT), Grooved Pegboard (GPB-N), Stroop Interference Test (STRP-CW), and the Paced Auditory Serial Addition Test (PASAT). Patients improved .7 SD on Peptide T compared with placebo for those tests they were originally impaired on. Our other data suggest improvement in constitutional symptoms with Peptide T.

Methodologic differences between our study and the previous open label trials include; different doses of Peptide T, different routes of administration (IN vs. IV), concurrent administration of AZT, and subject population (methadone maintained substance abusers vs. homosexual men). Further studies will be necessary to determine optimal parameters for Peptide T administration and selection of patients likely to respond to Peptide T.

634.8

EFFECTS OF MESENCEPHALIC INJECTIONS OF NEUROTENSIN$_{1-13}$ AND NEUROTENSIN$_{8-13}$ ON CENTRAL GRAY SELF-STIMULATION. P.P. Rompré[1] and A. Gratton[2]. [1]CSBN, Psychology Dept., Concordia University, Montreal, Quebec, H3G 1M8 and [2]Douglas Hosp. Res. Ctr., McGill University, Verdun, Quebec, H3H 1R3.

Results from anatomical, biochemical and electrophysiological studies suggest that mesencephalic dopamine (DA) neurons receive a direct excitatory input from nerve elements that release neurotensin (NT). In this experiment, we compared the effects of mesencephalic injections of NT1-13 and its C-terminal fragment, NT8-13, on central gray self-stimulation (SS). Rats were implanted with a stimulation electrode in the central gray and a guide cannula in the ventral or dorsal mesencephalon and subsequently trained to bar press to obtain a train of cathodal, rectangular pulses of a fixed intensity. Changes in the function relating the rate of bar presses to the stimulation frequency were measured immediately before, and after the injection of 3 nmoles of NT1-13, NT8-13 or its vehicle. Results obtained from thirteen rats with ventral cannula placement, show that the C-terminal fragment, NT8-13, is fully active and is as potent as NT1-13 in decreasing the frequency threshold for SS; the facilitation effect, however, was observed 15 minutes after the injection with NT8-13 compared to 35 minutes with NT1-13. No significant changes in threshold were seen when the peptides were injected in the dorsal mesencephalon.

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634.9

EVIDENCE FOR AN INTERACTION OF NEUROTENSIN WITH THE A-1 SUBTYPE OF ADENOSINE RECEPTORS. **F. B. Jolicoeur and D. Ménard. Depts. Psychiatry and Pharmac., University of Sherbrooke, Sherbrooke Qc. Canada J1H 5N4**

We have reported that pre-administration of the mixed adenosine receptor antagonist, theophylline, systematically abolishes the hypothermic and hypokinetic effects of neurotensin (Soc. Neurosci. Abstr. 1990) In order to better delineate this interaction, we examined the effects of pre-treatment of several adenosinergic drugs purported to act specifically at the A1 or A2 receptor subtype, either as agonists or antagonists. For each drug examined, a dose which was centrally inactive when given alone, was injected SC 15 min prior to the intracerebro-ventricular administration of several doses of neurotensin (0.03 - 50.0 µg). LPIA (A1 agonist) significantly enhanced both the hypothermic and hypokinetic effects of neurotensin. On the other hand, IBMX (A1 antagonist) significantly decreased the hypothermia but not the hypokinesia produced by neurotensin. Prior administration of CPCA and DMPX (A2 agonist and antagonist respectively) did not alter these effects of neurotensin. These results suggest that neurotensin induced hypothermia and possibly hypoactivity might be mediated via an interaction with adenosine A1 receptors. However, an interaction of the peptide with phosphodiesterase might also be of importance, since theophylline and IBMX are well known inhibitors of this enzyme.

634.10

THE EFFECTS OF INTRACEREBRAL INJECTIONS OF TWO NOVEL NEUROKININS (NEUROKININ A AND NEUROKININ K) ON THE EXPRESSION OF MALE RAT SEXUAL BEHAVIOR. Peter Malen*, Kevin Short*, and Wayne Dornan. Dept. of Psychology, Illinois Wesleyan University, Bloomington, IL. 61701

Substance P (sP), a member of the tachykinin group of neuropeptides, has been implicated in the neural control of male copulatory behavior. Recently two novel neurokinins, neurokinin K (neuropeptide K) and neurokinin A (substance K), derived from the same gene and precursor molecule (ß-preprotachykinin) as sP, have been identified in the mammalian CNS. At present, however, it is not known whether neurokinin K (NKK) or neurokinin A (NKA) has any role in the neural regulation of male rat copulatory behavior. Therefore, in a series of experiments we examined the effects of bilateral injections of different doses of NKK or NKA into the Medial Preoptic Area (MPOA) on male rat copulatory behavior. In the first experiment, bilateral injections of 10 or 100 ng of NKK into the MPOA had no effect on male copulatory behavior when compared to saline injected controls. Injections of 1 and 2 µg of NKK into the MPOA, however, produced a dramatic disruption in the expression of copulatory behavior in sexually vigorous male rats when compared to controls. In contrast, bilateral injections of 10 or 1000 ng of NKA into the MPOA failed to affect any parameters of male copulatory behavior. The results of the present study provide further support for a role of neurokinins in the neural regulation of copulatory behavior in the male rat. Taken together, these results suggest that at the level of the MPOA, sP and NKK, two neurokinins synthesized from the sP gene, play a differential role in the regulation of male copulatory behavior while NKA, another neurokinin derived from the same sP gene may not be involved in the neural regulation of male copulatory behavior.

634.11

CHRONIC ADMINISTRATION OF ANGIOTENSIN II AND DuP 753 INCREASES GROWTH OF EMBRYONIC RAT HEART CULTURED *IN OCULO*. R. Hunt, A. Torres, and D. C. Tucker. Dept. of Psychology, Univ. of Alabama at Birmingham, Birmingham, AL 35294.

Angiotensin II (AII) is implicated in the development of cardiac hypertrophy. AII may exert a direct trophic effect or act indirectly by potentiating sympathetic neural influences. Little is known about the effect of AII on developing cardiac tissue.

Embryonic rat heart (E-12) was cultured in the anterior eye chambers of male Sprague-Dawley hosts. One eye chamber of each host was sympathectomized by removal of the ipsilateral superior cervical ganglion. Eye chamber sympathectomy significantly decreased graft mass in control animals (4.72±0.71 vs. 1.54±0.81 mg) after 9 weeks *in oculo*.

Chronic administration of non-pressor doses of AII (60 or 120 ng/kg/hr) or the non-peptide AII-1 receptor antagonist DuP 753 (10 mg/kg/day, p.o.) did not alter graft growth in innervated eye chambers. In sympathetically denervated eye chambers, however, both AII and DuP 753 significantly increased graft mass (3.33±0.78 and 3.53±0.62 mg, respectively). Although both AII and DuP 753 caused similar growth in embryonic myocardium cultured in sympathetically denervated eye chambers, we hypothesize that the mechanisms by which this growth was achieved are different.

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634.12

INHIBITION OF CALCITONIN-INDUCED ANOREXIA BY ANTIDEPRESSANT DRUGS. R. de Beaurepaire*. Laboratoire de Pharmacologie, CHU Côte de Nacre, 14032, Caen, FRANCE.

Calcitonin is a peptide hormone secreted by the thyroid gland, which acts primarily on calcium homeostasis. When injected peripherally or centrally, calcitonin reduces food intake. The effects of chronic antidepressant treatment on calcitonin-induced anorexia were tested in rats.

Daily intraperitoneal injections of 9 different antidepressants, two anxiolytics, and one neuroleptic were made during three weeks, and the daily food intake of the animals were recorded every day during the last week. The drugs were tested in groups of 8 animals, and for each drug a control group of 8 animals was daily injected with saline. On day 21, the animals received an intraperitoneal injection of 40 units of calcitonin.

The results show that calcitonin reduces food intake by more than 90% in all control animals and animal treated by the anxiolytics (meprobamate, 100 mg/kg, clorazepate, 60 mg/kg), and the neuroleptic (chlorpromazine, 10 mg/kg), but not in animals treated with the antidepressant drugs. The most effective drugs were clomipramine (15 and 25 mg/kg), imipramine (25 mg/kg), amitriptyline (25 mg/kg), trimipramine (30 mg/kg), and maprotiline (25 mg/kg). Conversely, fluoxetine (15 mg/kg), fluvoxamine (30 mg/kg), viloxazine (30 mg/kg), and trazodone (100 mg/kg) inhibited the effects of calcitonin in some animals, but the results were not significant.

These results demonstrate that tricyclic antidepressants can have a specific inhibitory effect on a behavior induced by a centrally active peptide.

634.13

SELECTIVE ANTAGONISTIC PROPERTIES OF THE C-TERMINAL FRAGMENT OF CALCITONIN GENE-RELATED PEPTIDE, H-CGRP$_{8-37}$. D. Ménard, [1]A. Fournier and F. Jolicoeur. Depts of Psychiatry and Pharmacology, Univ. Sherbrooke, Sherbrooke Qc, Canada J1H5N4 and [1]INRS-Santé, Pointe Claire, Qc, Canada .

Results from our recent studies indicated that a C-terminal fragment of Calcitonin Gene-Related peptide, CGRP$_{8-37}$ could block certain in vivo and in vitro effects of h-CGRP (JPET 254:123,1990). In order to further characterize the antagonistic properties of this fragment, its activity against the following h-CGRP neurobehavioral effects were examined: hypokinesia, anorexia, analgesia, hyperthermia and catalepsy. These effects were obtained following intracerebroventricular (ICV) administration of 20 µg of the peptide. First, CGRP$_{8-37}$ alone injected ICV in several doses (10-80 µg) did not alter any neurobehavioral measurements. However, prior administration of the fragment at 40 µg significantly attenuated and at 80 µg systematically abolished the anorexia and analgesia produced by CGRP. The other neurobehavioral effects of CGRP were not affected. These results support the existence of heterogeneous receptors mediating central effects of CGRP. Follow-up structure activity studies, using various C-terminal fragments of the peptide, indicated that CGRP$_{9-37}$ was the minimal length required to obtain antagonistic activity.

634.14

MOLECULAR AND CELLULAR ORGANIZATION OF THE PEPTIDERGIC NETWORK UNDERLYING MALE COPULATORY BEHAVIOR IN A SIMULTANEOUS HERMAPHRODITE ANIMAL, *LYMNAEA STAGNALIS*. W.P.M. Geraerts, A.B. Smit* and K.W. Li*. Faculty of Biology, Vrije Universiteit, Amsterdam, The Netherlands.

Male mating behavior of the gastropod snail, *Lymnaea stagnalis*, consists of a series of flexible (non-cyclic) behaviors that are a prelude to copulation. To define the distribution of neurons that compose the network underlying male copulatory behavior, retrograde cobalt chloride fillings of the penis nerve were carried out. Several clusters of peptidergic neurons together with individual neurons and a serotonergic cluster ("I-cluster"), all located at the right side of the CNS, were identified.

We used recombinant DNA methodology and peptide chemistry to identify the structural organization of the prohormones and the bioactive peptides that are expressed in the neurons of the network. Large neurons in the lobus anterior of the right cerebral ganglion and several smaller clusters in other ganglia express a gene encoding the APGWa precursor. It contains 10 copies of APGWa and a 36 aa peptide, the C-terminal anterior lobe peptide (CALP). All 10 copies of APGWa and CALP are endoproteolytically processed from the prohormone. The gene is also expressed in the Ring Neuron that affects the egg laying controlling neuroendocrine caudodorsal cells. The lobus ventral neurons in the right cerebral ganglion, which form also part of the network, express the FMRFa gene (Linnacre *et al.*, 1990). Finally, a small cluster adjacent to the I-cluster in the right pedal ganglion expresses the peptide, myo-modulin. Currently, several additional peptidergic neuron clusters are in the process of analysis. All peptides as well as serotonin are transported to the penial complex. Sero-tonin and FMRFa, when applied *in vitro*, evoke contractions of the penis retractor muscle. APGWa antagonizes the serotonin-induced contractions. APGWa has, in addition, excitatory effects on several types of neurons, including those of the I-cluster. These data are sustained by whole-mount and light- and electron-microscopic immunochemical studies, showing intrinsic APGWa-positive axonal branching patterns and synapse-like structures that form connections between the neurons of the network.

We conclude that the male mating behavior of *Lymnaea* is controlled by a, mainly peptidergic, network. The organization of information processing of the network is complex and no doubt further studies of this network will reveal novel means of communication between (peptidergic) neurons that are part of complex networks.

634.15

DIFFERENTIAL EXPRESSION OF NEUROPEPTIDE mRNA AS RELATED TO AGE IN *APLYSIA*. M. Kindy*, M. Srivatsan*, and B. Peretz. Depts. Biochem. and Physiol. Univ. of Kentucky Med. Ctr., Lexington, KY 40536-0084.

With increased age the *Aplysia* nervous system manifests behavioral, physiological and morphological changes (Peretz et al., 1984; Peretz, 1989). The basis of these age related changes is unknown. Although environmental factors can influence the effects of age on the *Aplysia* nervous system (Zolman & Peretz, 1987), as yet the role of genetic factors has not been examined. Here we describe age-related differences in the mRNA expression of Phe-Met-Arg-Phe-NH_2 (FMRFamide) and egg-laying hormone (ELH) in the abdominal ganglion, a part of the *Aplysia* CNS. Northern blot analysis of RNA isolated in ganglia from young (ca 80 days old), mature (ca 140 days old) and aged (over 240 days old) animals revealed two mRNA species of 1.4 and 3.2 kb for FMRFamide mRNA and a single mRNA species of 0.8 kb for ELH. In young animals FMRFamide mRNA was exhibited at low levels, with a 1.5 fold increase in the mature group and a 2-3 fold decrease in old animals. In contrast, the ELH mRNA levels increased 2 fold from young to mature animals, but in old animals the increase was ca 25 fold. These studies show that age has a differential effect on mRNA of these two peptides in the abdominal ganglion, thus the differential effect of age on abdominal ganglion neurons reported earlier (Peretz et al., 1984) may well have a genetic basis. Our findings suggest that there is a genetic basis for the age-related change in behaviors mediated by ELH and FMRFamide in *Aplysia*.

634.16

CONOPRESSIN G SUPPRESSION OF THE SEROTONIN-SENSITIVE K^+ CURRENT IN APLYSIA SENSORY NEURONS. M. Martinez-Padron, J. Edstrom and K. Lukowiak. Neuroscience Research Group, University of Calgary, Calgary, Alberta T2N 4N1 Canada.

Single-electrode voltage clamp techniques were used to analyze the mechanism of action of the excitability and action potential shape changes caused by conopressin G on *Aplysia* siphon sensory neurons (Martinez-Padron and Lukowiak, 1989). Bath application of micromolar concentration of conopressin G reversibly decreases the total outward membrane current produced by depolarizing voltage commands to -20 mV. The threshold for this action is in the 10^{-8} M range. The reversal potential of the outward current is shifted in the positive direction by about 50 mV per 10-fold increase in the extracellular potassium concentration, indicating that at this range of membrane potentials, the outward steady-state current is carried mainly by potassium ions. changes in the extracellular concentration of sodium or chloride had little or no effect on the reversal potential. Intracellular injection of CsCl blocks the outward potassium current by about 75% and abolishes or greatly reduces the effect of conopressin G, suggesting that the peptide is acting to reduce the outward K^+ current.

The current is highly resistant to tetraethylammonium ions (Kd = 200 mM) and to 4-aminopyridine (Kd = 10 mM). The effect of conopressin G is reduced by prior application of 5-HT and forskolin. Taken together, these data suggest that conopressin G decreases the S-like current in abdominal siphon sensory neurons.

DRUGS OF ABUSE—PRENATAL ETHANOL

635.1

FETAL ETHANOL EXPOSURE ALTERS GLUCOCORTICOID AND ß-ENDORPHIN RESPONSES TO STRESS. J. Weinberg and C. Gianoulakis, Dept of Anatomy, Univ of British Columbia, Vancouver, BC V6T 1Z3; Douglas Hosp Res Center, Dept of Psychiatry, McGill Univ, Verdun, Quebec H4H 1R3.

Prenatal ethanol exposure alters development and response of the hypothalamo-pituitary-adrenal (HPA) axis and the pituitary ß-endorphin (ß-EP) system in the rat. The present study examined effects of fetal ethanol exposure (FEE) on habituation to repeated exposures to a stressor (60 min restraint, once daily).

Sprague-Dawley males and females from FEE, pair-fed (PF) and ad lib-fed (C) conditions were terminated prior to stress (basal) or immediately after 1, 5 or 10 exposures to restraint stress. Plasma levels of corticosterone (CORT) and ß-EP were elevated over basal levels in both males and females following single or multiple restraint periods. In females, CORTs in FEE and PF were higher than levels in C, while ß-EP levels did not differ among groups. In contrast, for males, CORT levels were similar among the 3 groups, whereas FEE males exhibited a greater and more sustained elevation of plasma ß-EP after multiple restraints. These data indicate little or no habituation of CORTS or ß-EP to repeated stress and suggest that prenatal ethanol exposure may differentially affect FEE males and females at different levels of the HPA-axis and ß-EP system. Supported by grants NIAAA AA07789 (JW); MRC MA-6923 (CG).

635.2

WITHDRAWN

635.3

EFFECTS OF PRENATAL ALCOHOL EXPOSURE AND ACOUSTIC TRAUMA ON THE RAT'S AUDITORY BRAINSTEM RESPONSE (ABR). M.W. Church and G.W. Overbeck*. Fetal Alcohol Research Center, Dept. Ob/Gyn, Wayne State Univ. Sch. Med., Detroit, MI 48201.

Sprague-Dawley rats were prenatally exposed to alcohol by giving liquid diets containing 35% ethanol-derived calories to pregnant dams (gestation days 8-22). Pair-fed and non-treated control groups were also used. One pup from each litter was exposed to acoustic trauma (118 dB alarm bell) during the critical period for noise-induced hearing loss (about 20 days of age). To evaluate auditory acuity, ABR latency-intensity profiles were developed for click and tone burst stimuli with intensities of 25 to 100 dB peSPL.

Regarding offspring not exposed to acoustic trauma, the alcohol group showed marked prolongations in ABR latencies as pups but normal ABRs as adults. This suggested that prenatal alcohol exposure delayed otoneurological development. Regarding offspring exposed to acoustic trauma, animals in the alcohol group had longer ABR latencies and higher ABR thresholds as adults. This indicated some degree of permanent hearing loss, suggesting that prenatal alcohol exposure increased the vulnerability to noise-induced hearing loss. (Supported by NIAAA grant AA07606 and NIDA grant DA05536).

635.4

SYNERGISTIC EFFECTS OF SHORT-TERM MATERNAL EXPOSURE TO ETHANOL AND EARLY SENSORY DEPRIVATION ON VISUAL DISCRIMINATION LEARNING IN RATS. M.H. Lee, A. Potempska*, and A. Rabe. NY State Institute for Basic Research in Developmental Disabilities, Staten Island, NY 10314

Ethanol exposure during cerebral cortical development has been shown to produce cortical thinning and disorganization, but not necessarily a detectable functional deficit. Nevertheless, the animal may have been rendered vulnerable to other adverse influences. Possible synergistic effects of ethanol exposure and light deprivation during development were tested. Offspring of Long-Evans rats who had received a total oral dose of 10g/kg of ethanol on gestation days 14 and 15 (E) and lab chow controls (C) were reared either in a dark room (D) or under a 12-hour light/dark cycle (L) from day 5 to 45. They were then trained in a shock-motivated two-choice box to discriminate between horizontal and vertical alternating black-and-white stripes.

The ED rats were impaired in learning the discrimination: they needed more trials than the CD rats to reach criterion. In contrast, the EL rats did not differ from the CL rats. The results thus indicate that acute maternal consumption of ethanol and adverse early postnatal rearing environment can synergistically produce a functional deficit.

635.5

DEVELOPMENTAL REGULATION OF GABA EXPRESSION IN THE SUPERIOR COLLICULUS OF SWISS WEBSTER MICE IS AFFECTED BY PRENATAL ALCOHOL EXPOSURE. C.S.Zajac and D.L.McGunagle*. Fetal Alc Res Ctr, Wayne State Univ., Detroit, MI 48201.

This study was undertaken to determine the developmental regulation of gamma amino butyric acid (GABA) expression in the superior colliculus (SC) of Swiss Webster mice and the effect of prenatal alcohol exposure (3g/kg) on that regulation. To that effect, newborn (NB) and gestation day (GD) 15 fetal mouse pups were sacrificed by decapitation into liquid nitrogen, lyophylized, and stored at -70°C. GABA levels were determined for lyophylized SC using the "GABAse" method. The SC of NB male pups prenatally exposed to alcohol had significantly greater levels of GABA than all other groups tested including NB females with the same treatment. There were no significant differences between other groups.

The superior colliculus plays an important role in coordinating reflex responses to visual, auditory, vestibular and somesthetic input. The significantly higher GABA levels in newborn male pups (but not in females) confirm other studies showing a sex related difference in the response of neurons to prenatal alcohol exposure.

635.6

SPONTANEOUS BEHAVIORAL ORGANIZATION IN RATS PRENATALLY EXPOSED TO ETHANOL

M. J. Renner[1], A. J. Bennett*[1], D. R. Widman*[2], & B. A. Blanchard*[3] [1]Department of Psychology, Memphis State Univ., Memphis, TN 38152 [2]Department of Psychology, State Univ. of New York, Albany, NY 12222 [3]Department of Pharmacology and Toxicology, Albany Medical College, Albany, NY 12208.

Prenatal exposure to alcohol is associated with deficits in learning, attention, and motor performance. This study was conducted to identify the characteristics of spontaneous behavior that may contribute to these deficits. Pregnant female rats were fed one of three diets from gestation days 6-20: a liquid diet in which 35% of calories were derived from ethanol (35%, $n=6$ pups); liquid diet without ethanol, fortified with sucrose for equivalent caloric intake to the 35% ETOH diet (0%, $n=8$ pups), and standard lab chow (LC, $n=7$ pups). After G20, mothers and pups were given standard lab chow. At 80 days of age pups were videotaped during 3 consecutive nights' access to a trapezoidal arena containing two objects. Tapes were scored for locomotion, general activities (e.g., grooming), and object interactions, using a standard protocol (Renner & Seltzer, *J Comparative Psychology*, 1991, in press). This method minimizes subject stress and maximizes expression of the subjects' behavioral repertoire. Objects with different stimulus characteristics were treated differently, consistent with other studies using these techniques. No effect of prenatal maternal diet was found for any molar-level measure of behavior, including interactions with objects. Microanalysis of behavior sequences used during object interaction, however, revealed increases in behavioral complexity over days for LC group and decreases for the 35% group; the 0% showed different effects for manipulable (decreases) and nonmanipulable (increases) objects. These results are consistent with the hypothesis that prenatal exposure to ethanol exerts its effects on information-processing capability through subtle behavioral and attentional changes.

635.7

THE EFFECT OF EMBRYONIC ETHANOL EXPOSURE ON SENSORY AND MOTOR NEURONAL DEVELOPMENT IN THE CHICK SPINAL CORD. B Mendelson. Dept. of Anatomy, U. of Ark. for Med. Sci., Little Rock, AR 72205.

Embryonic exposure to ethanol often produces motor dysfunction consisting of poor balance, and altered gait. To determine if abnormalities produced in spinal sensory and motor neurons contribute to these symptoms, chick embryos were exposed to physiological concentrations of ethanol during development. Subsequently, the effect of ethanol treatment on neural morphology was determined by retrogradely labeling identified populations of sensory and motor neurons with DiI. After diffusion of the dye, the arborization patterns of afferent collaterals and the cell body positions of the neurons were analyzed. The positions of the cells after ethanol treatment were appropriate for the nerve labeled. Sensory afferents that had peripheral processes in either the lateral femoral cutaneous (LFC) or the sartorius (Sart) nerves were located in appropriate dorsal root ganglia, and motoneurons that supplied the Sart muscle were positioned laterally in the lateral motor column of lumbosacral segments 1 and 2, as in normal embryos. However, LFC afferent collaterals arborized in an abnormal pattern. Normally at E12 (St 38), the vast majority of LFC collaterals enter the gray matter medially, within lamina 3 in the chick. The few fibers that enter laterally, remain lateral in laminae 1 and 2. In ETOH-treated E12 embryos, a large number of LFC collaterals enter the gray matter laterally and many proceed medially into lamina 3. The density of arborization in the medial and lateral regions was similar, whereas in normal E12 embryos the lateral region is almost devoid of processes. Therefore, ETOH-treatment appears to induce inappropriate sensory collateral growth that may lead to abnormal processing of sensory information in the spinal cord. Supported by BRSG 2 S07 RR 05350 29

635.8

BRAIN CATECHOLAMINE CONTENT DURING RECOVERY FROM STRESS IN FETAL ETHANOL-EXPOSED RATS. P.K. Rudeen and J. Weinberg. Dept of Anatomy & Neurobiology, Univ of Missouri, Columbia, MO 65212; Dept of Anatomy, Univ of British Columbia, Vancouver, BC. V6T 1Z3

Effects of fetal ethanol exposure on brain catecholamine (CA) content during recovery from restraint stress were examined. Sprague-Dawley male and female rats from fetal ethanol-exposed (FEE), pair-fed (PF) and ad libitum-fed (C) conditions were terminated prior to stress (basal), immediately after 60 min restraint, or following 15, 30 or 60 min recovery. Tissue CA were measured in hippocampus (HIP), hypothalamus (HY), and cortex (CX) by HPLC-EC. Restraint resulted in a reduction of HY norepinephrine (NE) content that persisted for at least 30 min following stress termination. NE levels returned toward basal levels by 60 min of recovery. A similar response was seen in CX NE but the decrease was smaller and longer lasting. Cortical NE was reduced overall in FEE and PF compared to C animals, whereas HY NE was reduced in FEE females but not males. There was a sex difference in HIP NE but no sex or treatment effects in epinephrine (E) or dopamine (DA) content of HY or CX. Reduction of neuronal CA content after restraint that continues throughout 30 min of the recovery period suggests that the effects of stress continue following the cessation of the stressor. The differential response seen in FEE animals may be related to the pituitary-adrenal hyperresponsiveness previously observed. (Supported by NIAAA grants AA05893 & AA00107 (PKR) & AA07789 (JW).

635.9

EFFECTS OF CROSS-FOSTERING ON THERMOREGULATION DEVELOPMENT AFTER PRENATAL ALCOHOL EXPOSURE.
B. Zimmerberg, A.A. Beliveau*, A.H. Furniss*, Williams College, Williamstown, MA 01267.

One critical variable in neonatal thermoregulation studies is the role of the mother. Prenatal alcohol-exposed (35% EDC) rat pups were reared by their biological dams or fostered to control (LC) dams at postnatal day 1. Control pups were also cross-fostered. On postnatal days 5, 10 and 15, pups were isolated and rectal temperatures taken hourly for 4 hours. Effects of cross-fostering were detected. Alcohol-exposed pups reared by 35% EDC dams had significant thermoregulatory deficits at 5 and 10 days of age compared to all control groups, but fostered alcohol-exposed pups were no different from pair-fed controls, although they did differ from both LC control groups. Both fostered and non-fostered alcohol-exposed pups gained less weight postnatally compared to controls, but LC pups fostered to 35% EDC dams also gained less weight than non-fostered LC pups. These results suggest that maternal behavior by 35% EDC dams should be considered in neonatal state regulation studies that use this animal model.
(supported by NIAAA #AA08605)

635.10

EVIDENCE THAT MODERATE ETHANOL CONSUMPTION DURING GESTATION DOES NOT ALTER THE METABOLOTROPIC OR IONOTROPIC COMPONENTS OF HIPPOCAMPAL QUISQUALATE RECEPTORS IN RAT OFFSPRING.
S.A. Queen, C.F. Sanchez,* S.R. Lopez,* L.L. Paxton* and D.D. Savage. Department of Pharmacology, Univ. of New Mexico School of Medicine, Albuquerque, NM 87131.

Prenatal exposure to a relatively high maternal blood ethanol concentration (BEC) decreases quisqualate metabolotropic stimulated inositol–1–phosphate (IP_1) accumulation in the hippocampal formation (HPF) of adult rat offspring (*Life Sci.* 45:803, 1989). We have reported reductions in the number of HPF N–methyl–D–aspartate (NMDA) and kainate subtypes of glutamate receptors in rats exposed to a more moderate BEC *in utero*. We investigated whether prenatal exposure to a more moderate maternal BEC would affect either the metabolotropic or ionotropic components of HPF quisqualate receptors in rat offspring.

Rat dams were fed a liquid diet containing 3.35% ethanol (17.5% ethanol–derived calories) throughout gestation. Consumption of this diet produced a peak maternal BEC of 40 mg/dL. Another group of dams was pair–fed a 0% ethanol diet isocalorically equivalent to the 3.35% ethanol diet. A third group of dams was fed lab chow *ad libitum*. Offspring from each group were sacrificed at 45 days of age and the brains processed either for IP_1 accumulation studies or *in vitro* ^{3}H–amino–3–hydroxy–5–methylisoxazole–4–propionate (AMPA) autoradiography.

Quisqualate metabolotropic agonist stimulation of IP_1 accumulation by either ibotenate or trans–1–amino–1,3–cyclopentanedicarboxylic acid (ACPD) was not altered in the HPF of prenatal ethanol exposed rats compared to pair–fed or *ad lib* controls. Furthermore, no differences were observed among the three experimental groups in specific ^{3}H–AMPA binding to the ionotropic quisqualate site in HPF or cerebral or cerebellar cortical regions. These results suggest a differential sensitivity among subtypes of HPF glutamate receptors to the effects of prenatal ethanol exposure. Higher maternal BECs are required to alter HPF metabolotropic quisqualate responses than the BEC required to alter HPF NMDA and kainate subtypes of glutamate receptors. Whether higher BECs are required to alter the ionotropic subtype of HPF quisqualate receptor remains to be established.
(Supported by AA06548 and RR08139)

635.11

ELECTROPHYSIOLOGICAL AND BEHAVIORAL FINDINGS IN RATS EXPOSED TO ALCOHOL PRENATALLY.
W.M. Kaneko*, E. Riley, S.L. Lopez* and C.L. Ehlers. Dept. of Psychology, San Diego State Univ., and Dept. of Neuropharm. Res. Inst. Scripps Clinic, La Jolla, CA 92037

Fetal alcohol syndrome (FAS) is one of the highest known causes of mental retardation in the western world. Previous research has shown behavioral and electrophysiological abnormalities in human infants born to alcoholic mothers, particularly hyperactivity. A rat model for FAS, which has several qualitative similarities to the human condition, has recently been developed. In the present study this rat FAS model was used to evaluate the effects of prenatal exposure on locomotor behavior and two paradigms for the generation of auditory event-related potentials (ERPs). Nineteen Sprague-Dawley adult male rats from two prenatal liquid diet treatment groups: alcohol exposed (35% ethanol-derived calories, 35% EDC) and nutritional control (0% ethanol-derived calories, 0% EDC), were stereotaxically implanted with electrodes aimed at dorsal hippocampus (DHPC), amygdala (AMYG) and frontal cortex (CTX). ERPs were recorded in response to an auditory "oddball" paradigm and an oddball plus "startle" paradigm. Evaluation of ERPs revealed that both paradigms produced consistent results. The prenatal alcohol exposed group had significantly longer P1 and N1 latencies than the control group for the rare tone in both paradigms ($p<0.005$ and $p<0.02$ respectively). Statistical analysis of these animals locomotor behavior evaluated in photocell cages revealed that the prenatal alcohol exposed rats also showed significantly more locomotor behavior (crossovers) in the first four hours of their activity cycle ($p<0.03$). These results suggest that this animal model displays electrophysiological and behavioral findings similar to human FAS and further suggests that this model may provide the substrate to investigate what brain mechanisms may be altered in FAS children.
(supported by AA 00098,06059)

635.12

EFFECT OF IN UTERO ETHANOL EXPOSURE ON THE POSTNATAL DEVELOPMENT OF BERGMANN GLIA IN RAT CEREBELLUM.
A.K. Shetty* and D.E. Phillips. Dept. of Biology, Montana State University, Bozeman, MT 59717.

Animals prenatally exposed to ethanol exhibit a delay in the postnatal migration of cerebellar neurons from the external granular layer to the internal granular layer. Since these neurons use the long processes of Bergmann glial cells across the molecular layer as guides during migration, we have examined the postnatal development of Bergmann glia following prenatal ethanol exposure. Pregnant rats were either fed with an ethanol containing liquid diet in which 37.5% of the total caloric content was ethanol derived or were pair fed with an isocaloric diet throughout gestation. Female offspring were perfused on postnatal day 15 and Bergmann glial fibers in the cerebellar vermis were stained immunohistochemically using an antibody against glial fibrillary acidic protein (GFAP). GFAP positive Bergmann glial fibers stretched from the Purkinje cell layer to the glial limiting membrane in both ethanol treated and control rats; however, the density of fibers per unit length was reduced in lobule I ($p<0.03$), lobule VII ($p<0.0001$) and lobule X ($p<0.1$) of ethanol treated rats. It appears that prenatal ethanol exposure retards the postnatal development of Bergmann glial cells in the cerebellum which may contribute to the delayed migration of granule cells.
Supported by NIAAA AA7042 and NSF EPSCoR RII-8921978.

635.13

THE EFFECTS OF PRENATAL OR POSTNATAL ETHANOL EXPOSURE ON SPATIAL LEARNING IN RATS.
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The purpose of this experiment was to investigate the effects of pre- and postnatal ethanol (ETOH) exposure on spatial learning in 30 & 60 day old Sprague-Dawley rats (Charles River). Pregnant rats were randomly assigned to one of 3 groups: liquid diet (BioServ) containing 35% ethanol derived calories (EDC), 0% EDC, or ad lib rat chow (Purina). The postnatal exposure group self administered either a 15% sweetened (Nutrasweet) ETOH solution or distilled water from postnatal day 13 to 17. Spatial learning performance was measured over 5 consecutive days beginning on either day 30 or 60, in a Morris swim maze. At both 30 & 60 days, there was a significant difference in performance between the pre- and postnatal exposure groups. At both ages, the postnatally exposed group showed greater deficits in learning. These data suggest that postnatal ETOH exposure produces greater deficits in spatial learning than prenatal ETOH exposure.

635.14

MORPHOLOGICAL AND ELECTROPHYSIOLOGICAL EFFECTS OF ETHANOL ON DEVELOPMENT OF DORSAL RAPHE TRANSPLANTS *IN OCULO*.
A-Ch. Granholm and C. Bäckman*. Dept. Basic Sci. Oral Res., Univ. Colorado HSC, Denver, CO 80262. A number of studies have demonstrated alterations in brain serotonin levels in animals after ethanol exposure *in utero*. To investigate the cellular basis for ethanol induced abnormalities in serotonergic neurons, we have transplanted fetal rat tissue of embryonic day 17 from nucleus raphe dorsalis to the anterior chamber of the eye in two groups of adult albino rats. The experimental group received 16% ethanol in the drinking water and the control group received water *ad libitum*. Immunohistochemical analysis revealed a disorganized serotonin immunoreactive innervation of surrounding host iris without axon bundles and with diminished outgrowth area in the ethanol group. Extracellular recording of monoaminergic neurons in grafts showed no difference in spontaneous activity or firing pattern between the two groups. Ethanol, superfused over the surface of grafts, elicited two different responses in both groups; low dose excitations and high dose inhibitions. However, monoaminergic neurons in the ethanol group showed a decreased sensitivity to locally applied ethanol. The dose-response curve was significantly shifted to the right in this group. These results suggest that chronic treatment with ethanol during early development disturbs the normal structure of brainstem serotonergic neurons, as well as their response to acute ethanol. Supported by Swedish MRC, grant 8650.

635.15

LACK OF RECOVERY FROM MEMORIAL AND NEUROBIOLOGICAL DEFICITS FROM HIGH-PEAK BEC RESULTING FROM EARLY POSTNATAL EXPOSURE TO ETHANOL P. L. Greene, J. L. Diaz-Granados & A. Amsel. Department of Psychology and Institute for Neuroscience, University of Texas, Austin, Texas, 78712

Electrolytic hippocampal lesions at 10-11 days of age (P10-11) (Lobaugh et al., 1989), postnatal hippocampal exposure to x-irradiation (Diaz-Granados et al., submitted) and postnatal exposure to ethanol from P4-10 that results in high daily peak blood ethanol concentration (BEC) (Greene et al., submitted) significantly retard the acquisition of patterned (single) alternation (PA), a form of memory-based learning, with 60-s but not 30-s intertrial interval (ITI) in rat pups tested on P17-18. We examined the effects on PA at P60-86 of early postnatal (P4-10) exposure to EtOH with high- (H) and low- (L) peak BEC. Artificially-reared pups in the H condition were fed a 10.2% EtOH-adulterated diet (control pups received an isocaloric control diet) on 4 consecutive hourly feedings each day and unadulterated diet on the remaining 20 feedings. L pups received a 1.7% EtOH-adulterated diet on all 24 feedings. H pups at P60-86 were impaired in PA learning relative to L and control pups at 60-ITI, the same ITI at which PA deficits were observed in infant rats. Group H brain weights were significantly reduced, relative to Group-L and control weights, at 86 days, mirroring the result at 21 days of age. Morphometric correlates of recovery from specific hippocampal cell damage seen in infants are presented. These results suggest incomplete recovery from the memorial and neurobiological teratologic effects of the high-peak ethanol exposure regimen in infancy. Supported by NIAAA grant AA07052.

DRUGS OF ABUSE—CELLULAR EFFECTS OF ETHANOL

636.1

MARKED INHIBITION OF DOPAMINE RELEASE: A COMMON NEUROBIOLOGICAL SUBSTRATE FOR ABSTINENCE FROM DRUGS OF ABUSE. Z.L. Rossetti, F. Melis, S. Carboni and G.L. Gessa. Dept. of Neuroscience, Univ. of Cagliari, Italy.

Behavioral and biochemical evidence indicates that the dopamine (DA) pathways mediating reward and pleasure are deeply involved in dependence from drugs of abuse. By using the microdialysis technique, we studied the changes in DA efflux from the mesolimbic system induced in rats by repeated treatment with morphine, alcohol, or cocaine, and after withdrawal from these drugs. *Ethanol*. In ethanol-dependent rats (5 g/Kg p.o. every 6 h for 6 days) withdrawal symptomatology was associated with a progressive decrease of DA extracellular levels. DA reached 20% of controls when abstinence symptomatology was fully established. Ethanol (5 g/kg p.o.) suddenly reverted both abstinence symptomatology and inhibition of DA outflow. *Cocaine*. In chronic cocaine-treated rats (15 mg/kg twice per day for 18 days) extracellular DA concentrations were decreased to 35% of controls for at least 5 days after the supension of the treatment. Cocaine (10 mg/kg i.p.) immediately reverted this effect. *Morphine*. In morphine-dependent rats, (s.c. 70-mg morphine pellets for 5 days) the gradual onset of abstinence symptomatology following by pellet removal was paralleled by a progressive decrease of DA levels. DA efflux reached a minimum (23% of controls) when opiate withdrawal symptomatology was clearly manifested. Morphine (100 mg/kg s.c.) suddenly reversed both abstinence symptomatology and inhibition of DA release. Thus, DA system mediating the hedonic and reinforcing actions of drugs of abuse is impaired in drug dependence. The inhibition of DA transmission in the mesolimbic system appears to be a common neurobiological substrate for abstinence from drugs of abuse.

636.2

A GENERAL CELLULAR MODEL FOR TOLERANCE AND DEPENDENCE. A.M. Babey and R.M. Palmour. Dept Biology and Psychiatry & Centre for Human Genetics, McGill Univ., Montreal Que H3A 1A1.

Symptoms exhibited upon withdrawal from various addictive substances differ mainly in magnitude of expression, rather than in basic character, and are unlikely to be explained by changes restricted to the receptor. In cultured murine neuroblastoma cells, chronic theophylline treatment results in an increased production of cAMP in response to adenosine receptor stimulation. A parallel increase in receptor affinity persists, but physiological hypersensitivity disappears by two weeks. Increased responsiveness to forskolin stimulation appears after 4 days of continuous theophylline exposure. Chronic ethanol elicits altered response to adenosine, prostaglandins and opiates; these phenomena are mediated by changes in both G proteins and receptors, and are expressed primarily through altered coupling. We propose that chronic exposure to an addictive or habit-forming substance proceeds sequentially: Changes first to the receptor, then to G proteins signal the onset of tolerance. The development of cellular dependence requires alterations in adenylate cyclase. Withdrawal of drug initiates biochemical rebound only after completion of this sequence. The time lag associated with true cellular tolerance mimics that of physiological tolerance, which rapidly leads to dependence. This model describes the cellular characteristics of a wide range of habit-forming drugs of very different chemical classes, and should provide a useful screening tool for testing the abuse liability of new drugs.

636.3

INFLUENCE OF ETHANOL ON NEUROBLASTOMA AND GLIOMA CELLS IN VITRO: GLYCOSPHINGOLIPIDS. *K.C. Leskawa and C.E. Gabe**. Dept. Anatomical Sciences & Neurobiology, University of Louisville, Louisville, KY

The reports of *in vivo* effects of ethanol on brain glycosphingolipids (GSLs) are conflicting. To pursue this, we have recently examined neuroblastoma (NG108-15) and glioma (C6) cells in culture using two experimental paradigms: acute (0.1% for 8 hr) and chronic (0.5% for 24 hr) ethanol exposure. The synthesis of neutral GSLs and gangliosides was analyzed by the addition of ^{14}C-serine to the media during the last 8 hr of exposure. Ethanol exposed NG108-15 cells demonstrated decreased ganglioside synthesis, with the decrease being greater under chronic ($p<0.01$) than acute ($p<0.05$) conditions. Ethanol had no effect on neutral GSL synthesis by NG108-15 cells. Conversely, when C6 cells were exposed to ethanol neutral GSL synthesis doubled ($p<0.01$), few differences were observed between acute and chronic conditions, and ganglioside synthesis was not altered. Further experiments demonstrated that these changes were not due to alterations in the size of the intracellular pool of the ^{14}C-serine precurser. Levels of intracellular free serine equilibrated rapidly (within 1 hr) and neither acute or chronic ethanol treatment had any effect. In addition, we studied the activity of the first enzyme in ganglioside catabolism, sialidase (N-acetylneuraminic acid), using ^{3}H-GM3 as a substrate. Sialidase activity in C6 cells was inhibited only after chronic ethanol exposure ($p<0.05$), whereas ethanol treatment of NG108-15 cells (either condition) had no effect on sialidase activity when compared to control cultures. The results suggest that effects of ethanol on GSLs varies greatly with cell type, and that the response is at the level of GSL synthesis rather than GSL catabolism.

636.4

SELECTIVE EFFECTS OF CHRONIC ETHANOL EXPOSURE ON BRADYKININ-MEDIATED INTRACELLULAR CALCIUM MOBILIZATION IN N1E-115 NEUROBLASTOMA CELLS. T.L. Smith, C. McCollum* and L. Hoerr*. Res.Service, Vet.Affairs Med.Cntr., Tucson, AZ 85723.

Chronic (7day) ethanol (E) exposure has been reported by this laboratory to selectively inhibit bradykinin (BK)-stimulated [^{3}H] inositol phosphate (InP) production in N1E-115 cells, while having no effect on this response to neurotensin (NT). In order to determine whether chronic E also produces selective effects on receptor-mediated increases in intracellular free calcium, [Ca^{2+}]i, N1E-115 cells were exposed to 100mM E for 7 days. In other studies, the effects of acute exposure to 100-400mM E on resting [Ca^{2+}]i were also determined. Confluent cells grown in DMEM with 10% fetal calf serum were incubated in DMEM containing 8μM fluo-3/AM for 30 mins. at 35 C. Aliquots of the washed cells ($5-8x10^{5}$) were resuspended in 3 ml HEPES buffered salt solution and [Ca^{2+}]i determined fluorometrically. Chronic E reduced by 43% the [Ca^{2+}]i response to BK, but had no effect on the same response to NT. Resting [Ca^{2+}]i values (nM) for control and chronic E treated cells were 64±15 and 70±20nM, respectively. Acute E (3-4 mins) as high as 400mM had no significant effects on resting [Ca^{2+}]i. It is concluded that BK-mediated [Ca^{2+}]i is reduced in parallel with InP production after chronic E. Furthermore, in contrast to synaptosomes and PC12 cells, N1E-115 neuroblastoma do not exhibit an increased resting [Ca^{2+}]i subsequent to acute E exposure. (Supported by the Dept.of Vet. Affairs).

636.5

ETHANOL-INDUCED CHANGES IN ^{3}H-GABA RELEASE FROM SUBSTANTIA NIGRA AND SUPERIOR COLLICULUS. Joanna Peris, Mia Coleman-Hardee*, and Amy Shawley*, Dept. Pharmacodynamics, Univ. Florida, Gainesville, FL.

An increase in neuronal transmission in the GABAergic striatonigral pathway and a decrease in GABAergic transmission in the nigrocollicular pathway both are correlated with decreases in seizure sensitivity. If the relation is causal, then the anticonvulsant effects of ethanol should have opposite actions on GABAergic transmission in SN and SC and the reverse should occur during ethanol withdrawal. We measured the effects of in vitro ethanol on pre- and postsynaptic measures of GABA transmission in SN and SC both in naive rats and in rats given ethanol in their drinking water for 24 days and then withdrawn for 24 hrs. While ethanol inhibited ^{3}H-GABA release from slices of SC at low concentrations (50-200 mM), 500 mM was required to inhibit release from SN. In fact, release was increased by low concentrations of ethanol in SN. We next measured these parameters in SN and SC from rats that had a mean ethanol consumption of 9.4 g/kg body weight/day. Ethanol inhibition of release in SN from ethanol-treated rats was greater than in the control rats whereas ethanol inhibition of release from SC was less in ethanol-drinking rats compared to control rats. When ^{35}S-TBPS binding was measured using quantitative autoradiography, there was a greater number of binding sites in striatum of ethanol-treated rats compared to controls. Thus when ethanol is acting as an anticonvulsant, it increases release in SN and decreases release in SC, but when animals are sensitized to seizures by ethanol treatment, release is decreased in the nigrostriatal pathway and increased in the striatonigral pathway. (Supported by PHS AA 08262)

636.6

EFFECTS OF CHRONIC AND ACUTE ETHANOL EXPOSURE ON CALCIUM CURRENTS IN PC12 CELLS. A.J. Grant, G. Koski[1], and S.N. Treistman. Worcester Foundation for Experimental Biology, 222 Maple Ave., Shrewsbury, MA 01545 and [1]Department of Anesthesia, Massachusettes General Hospital, Boston, MA 02114.

Previous studies, using biochemical assays, have indicated that there is an increase in depolarization-induced calcium influx into PC12 cells which have been chronically exposed to ethanol, as compared with cells not exposed to ethanol. To further elucidate the mechanisms involved, we describe here the effects of acute and chronic ethanol on whole cell calcium currents in PC12 cells using electrophysiological as well as biochemical methods. Undifferentiated pheochromocytoma (PC12) cells were chronically exposed to 200 mM ethanol for six days. Parallel cultures from the same parent passage were maintained, under the identical conditions, without ethanol. Following chronic exposure, whole cell calcium currents were recorded from the two groups using standard patch clamp techniques. Cells chronically exposed to ethanol had significantly larger voltage-gated calcium currents than those grown in the absence of ethanol. The inactivation kinetics of the currents, as well as the voltage protocols used to elicit these currents, suggest that calcium ions were passing primarily through L-type channels. Radiolabelled calcium uptake assays confirm that calcium influx is increased in chronically exposed cells from the same parent passage as those tested electrophysiologically. In contrast to the augmentation of current from chronically exposed cells, acute exposure to low ethanol concentrations greatly reduced the magnitude of the currents. Supported by Grant #AA05542.

636.7

THE EFFECT OF ETHANOL AND NITROUS OXIDE ON CEREBELLAR GRANULE CELLS AND PURKINJE CELLS. C.-F. Hsiao, R. Huang* and C. Huang. School of Basic Life Sciences, Univ. Missouri-Kansas City, Kansas City, MO 64110

Among the initial manifestations of ethanol intoxication are the delay in reaction times, the impairment of fine motor skills, and the deterioration of motor coordination and mental abilities. We previously showed that ethanol or nitrous oxide severely inhibited neuronal activities in the granule cell layer of the cerebellum in the cat. To further identify the effect of ethanol and nitrous oxide on specific cell types, we have combined the method of intracellular recording/injection with ethanol delivery (0.3g/Kg IV) or nitrous oxide anesthesia (66%). Ethanol inhibited the spontaneous discharges and auditory responses of most, but not all, granule cells. The spontaneous discharges of Purkinje cells were either suppressed or enhanced by ethanol. Occasionally, the amplitude of action potentials also seemed to be affected. On the other hand, nitrous oxide abolished the spontaneous discharges and auditory responses of all granule cells. Some Purkinje cells were excited, others were inhibited, and still others showed no effect. In conclusion, ethanol and nitrous oxide have significant but different effects on the spontaneous discharges and responses of most cerebellar granule cells and Purkinje cells. (Supported by PHS grant AA07643.)

636.8

PHENCYCLIDINE POTENTIATES ETHANOL-INDUCED ACTIONS VIA A β-ADRENERGIC MECHANISM IN CEREBELLAR PURKINJE NEURONS, BUT NOT IN PUTATIVE GOLGI NEURONS *IN VITRO*. M.R. Palmer, Y. Wang and R.K. Freund. National Defense Medical Center, Taipei, Taiwan, R.O.C., and Department of Pharmacology, University of Colorado Health Sciences Center, Denver, CO 80262.

Previous studies have established that ethanol (EtOH) inhibits firing of Purkinje neurons (PN) both *in vivo* and *in vitro*. It has been reported that β-adrenergic agonists potentiate inhibitory responses to GABA in these cells. Phencyclidine (PCP) has been found to increase catecholaminergic overflow, possibly by increasing catecholamine release and/or by decreasing uptake in various brain regions, including cerebellum. Data from this laboratory and others suggest that EtOH-induced inhibition of PN firing involves GABA mechanisms. In this study we found that PCP potentiated EtOH-induced depressions of spontaneous firing of PN's in cerebellar slices from Sprague-Dawley rats. (-)-Isoproterenol appeared to mimic the effect of PCP on the EtOH response. In preliminary experiments, timolol antagonized the potentiation by PCP of EtOH responses. By contrast to the EtOH response in PN's, the firing of putative Golgi neurons was markedly increased by EtOH, and this effect was attenuated by PCP. We conclude that PCP potentiates ethanol-induced inhibition via β-adrenergic mechanisms in cerebellar PN's, but not in Golgi cells.

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636.9

URETHANE POTENTIATES ETHANOL-INDUCED INHIBITION IN RAT BRAIN SLICES. R.K. Freund, Y. Wang, W.R. Proctor and M.R. Palmer. Department of Pharmacology, University of Colorado Health Sciences Center, Denver, CO 80262, and National Defense Medical Center, Taipei, Taiwan, R.O.C.

Ethanol (EtOH) has been found to inhibit firing of neurons in several different brain regions *in situ* (e.g., cerebellum, hippocampus, cortex, striatum). One potential complication regards the use of anesthetics in these studies. It is not well-established whether anesthetics may significantly modify the observed EtOH responses. In the present study the effects of urethane (ethyl carbamate), an anesthetic commonly used in animal research, was examined for its possible influence on the inhibitory action produced by EtOH on electrophysiological responses measured in slices from several brain areas. In cerebellar slices, EtOH-induced inhibition of the unit activity (spontaneous firing rate) was potentiated by 20 μM urethane. A similar urethane-induced potentiation of the depressant effects of EtOH was observed with evoked extracellularly-recorded population spike amplitudes from the somal layer in the CA1 region from hippocampal slices. These data indicate that urethane increases ethanol potency (but does not qualitatively alter the inhibitory response) in cerebellum and hippocampus, and thus, suggest that the electrophysiological effects of EtOH in the CNS observed in urethane-anesthetized animals are potentiated by the anesthetic.

Supported by USPHS grants AA05915 and AA00102. MP is supported by an ADMH Research Scientist Development Award.

636.10

EFFECTS OF ETHANOL ON THE SENSITIVITY OF RAT RETINAL GANGLION CELLS TO INHIBITORY NEUROTRANSMITTERS. H.H. Yeh. Dept. Neurobiology & Anatomy, U. Rochester Med. Ctr., NY 14642.

Evidence is accumulating that ethanol can modulate neuronal sensitivity to certain neurotransmitters. We report here our initial findings using the rat retina as a model to determine whether direct exposure of retinal ganglion cells to ethanol can influence their responsiveness to GABA and glycine, the major inhibitory neurotransmitters resident in the retina. This study was motivated in part by our recent demonstration that both $GABA_A$ receptor- and glycine-activated currents in ganglion cells are subject to modulation via interaction with other neuroactive substances.

Solitary ganglion cells, obtained following enzymatic treatment and acute dissociation of the intact rat retina, were selected for whole-cell patch-clamp recording. Current responses to brief pressure pulses of GABA (I_{GABA}, $\leq$20 uM) or glycine (I_{Gly}, $\leq$200 uM) were monitored before, during and after exposure to ethanol (15-150 mM). Toward the higher end of the range of concentration used in this study, a clear-cut potentiation of I_{GABA} could be observed during paired applications of ethanol and GABA. The potentiating effect was reversible, returning promptly toward control (pre-ethanol) levels of I_{GABA} upon termination of ethanol exposure. In contrast, ethanol did not potentiate I_{Gly}. In fact, the tendency was for I_{Gly} to be attenuated upon ethanol exposure. Such a differential effect on I_{GABA} and I_{Gly} could be demonstrated in the same ganglion cell.

Thus, our data suggest that ethanol may exert selective effects on signal processing involving the GABAergic and glycinergic pathways in the retina. The retina should be a useful model system for elucidating the mechanisms of ethanol action on central neurons and circuits.

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636.11

ETHANOL ENHANCES $GABA_A$ RECEPTOR-MEDIATED CHLORIDE CURRENTS IN CHICK CEREBRAL CORTICAL NEURONS. J.N. Reynolds and A. Prasad, Faculty of Medicine, Memorial University of Newfoundland, St. John's, Nfld., Canada A1B 3V6.

Primary cultures of cerebral cortical neurons were prepared from seven day old chick embryos. The effect of ethanol on GABA-activated membrane currents was examined using whole-cell voltage-clamp recording in cells maintained for 3-25 days *in vitro*. Cells were continuously perfused (1-2 mL/min) with a physiological saline containing (in mM) 140 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 10 glucose, 0.5 μM tetrodotoxin, pH 7.3. Ethanol was diluted in extracellular solution (final concentration 0.5-50 mM) and applied by bath perfusion. Recording pipettes (3-5 MΩ) contained (in mM) 140 KCl, 2 $MgCl_2$, 10 HEPES, 4 ATP, pH 7.3. GABA (10-50 μM) applied by brief pressure pulses directly to the soma elicited a current which reversed at 0 mV and was blocked by 50 μM bicuculline. In 60% of neurons examined (n > 100) ethanol caused a potentiation of the membrane current elicited by GABA. The threshold concentration of ethanol was 1 mM, and the effect of ethanol was maximal at 10 mM. In many cells, higher concentrations (40-50 mM) of ethanol inhibited GABA currents. These effects of ethanol were all reversible. Cells obtained from individual embryos had similar responses to ethanol, whereas cells obtained from different animals varied greatly in their sensitivity to ethanol. In cultures where ethanol enhanced GABA responses, excessive buffering of intracellular calcium (by including 5-10 mM BAPTA in the recording pipette) attenuated the effect of ethanol.

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636.12

THE EFFECTS OF ETHANOL ON CULTURED SEROTONERGIC NEURONS AND ASTROGLIA. D.K. Lokhorst and M.J. Druse Neuroscience Program, Loyola U. of Chicago, Stritch Schoolof Medicine, Maywood, IL 60153.

The effects of ethanol exposure on the development of serotonergic neurons were assessed by examining the influence of 50, 150 and 300 mg/dl of ethanol on cultured fetal rhombencephalic cells. Rhombencephalic cells were removed from rats on embryonic day 14, and cultured for 2 days in the absence of ethanol and an additional 4 days in the presence of ethanol. Cultured rhombencephalic neuronal and astroglial cells were assessed for serotonin (5-HT) uptake, 5-HT content, number of 5-HT immunoreactive cells, and DNA and protein content.

The results of these experiments demonstrate that exposure of cultured rhombencephalic neuronal cells to an ethanol concentration of 50 to 300 mg/dl does not affect 5-HT or DNA content, or the proportion of 5-HT immunoreactive cells. In addition, DNA content is unchanged in ethanol-exposed astroglial cells. However, 300 mg/dl of ethanol significantly reduces protein content and 5-HT uptake per well to approximately the same extent in both neuronal and astroglial cells. Thus, it appears that at the ethanol concentrations examined ethanol does not produce cell death. However, ethanol does inhibit protein synthesis in both neuronal and astroglial cells. This decreased protein synthesis is reflected in decreased 5-HT uptake by both cell types.

636.13

CALCIUM-DIACETYL HOMOTAURINE (Ca-AOTA) ALTERS ELECTROPHYSIOLOGICAL PROPERTIES OF HIPPOCAMPAL PYRAMIDAL NEURONS (HPNs) *IN VITRO*. Samuel G. Madamba, [1]Walter Zieglgänsberger,* [1]Marc L. Zeise* and George Robert Siggins. Dept. of Neuropharmacology and Alcohol Research Center, Research Institute of Scripps Clinic, La Jolla, CA 92037 and [1]Max-Planck-Institute for Psychiatry, 8000 München, F.R.G.

Ca-AOTA has shown promise in preventing relapse in abstinent alcoholics. However, its mechanism of action is unknown. As ethanol can alter synaptic transmission and several ionic conductances in hippocampal neurons, we examined the effects of superfusion of Ca-AOTA on rat CA1 HPNs in a slice preparation. We used standard intracellular recording or whole-cell "patch-slice" recording methods, in current- or voltage-clamp mode. Ca-AOTA had small concentration-dependent effects on membrane potential: 1-10 μM Ca-AOTA depolarized, hyperpolarized and had no effect in equal numbers of cells, whereas 100-500 μM Ca-AOTA slightly hyperpolarized most HPNs. Under voltage clamp (-36 to -47 mV holding potentials; 5-25 mV hyperpolarizing steps), Ca-AOTA (10-1000 μM) had little reproducible effect on M-current relaxations (which are reduced by ethanol), but caused an outward current and a large conductance increase. By contrast, Ca-AOTA elicited a net inward current (with conductance increase) in whole-cell recordings with TEA and Cs^+ in the internal and external medium. In conventional intracellular recordings with external TTX, 50-300 μM Ca-AOTA (like Cd^{++} 100 μM) blocked Ca^{++}-dependent action potentials and afterhyperpolarizations evoked by depolarizing current. In whole-cell voltage clamp with TEA and Cs^+, 50-100 μM Ca-AOTA reduced (by 5 to 57%; n= 6) the peak Cd^{++}-sensitive inward currents evoked by 20-65 mV depolarizing steps from holding potentials of -60 mV. These results suggest that Ca-AOTA, like ethanol, has multiple actions on HPNs, including activation of an uncharacterized K^+ conductance and inhibition of a Ca^{++} current. These mechanisms could account for the clinical efficacy of this agent. (Supported by USPHS (AA06420) and LIPHA, France.)

636.14

EFFECTS OF ETHANOL ON RAT HIPPOCAMPAL EPILEPTIFORM ACTIVITY. S.M. Cohen, D. Martin, R.A. Morrisett, W.A. Wilson, and H.S. Swartzwelder*. Departments of Pharmacology, Neurology and Psychology, Duke Univ. Med. Ctr. and V.A. Med. Ctr., Durham, NC 27705.

Activation of the NMDA receptor is a critical requirement in the induction of several forms of neuronal plasticity, including hippocampal electrographic seizures (EGSs). Electrophysiological and biochemical evidence strongly indicates that ethanol specifically blocks NMDA responses. Therefore, we examined the effects of ethanol on the expression and induction of electrographic seizures (EGSs) after kindling-like stimulation of the hippocampal slice.

It has been previously reported (Martin, et al. 1991 Alc. Clin. Exper. Res. in press), that ethanol has multiple effects on EGS expression. Ethanol 33-100 mM increased the threshold for seizure expression approximately 2-4 fold. The NMDA receptor antagonist, D-APV (50 μM) also increased seizure threshold by a similar amount. Lower concentrations of ethanol (10 mM) increased burst frequency and EGS duration. Increasing ethanol concentrations (33-60 mM) had little or no effect on the morphology of the EGSs. Very high concentrations (100-300 mM) suppressed the EGS in a dose-dependent manner.

In another set of experiments, ethanol had minimal effects on EGS induction produced by supra-maximal trains. Control slices were bathed in 75mM ethanol and conventional trains were given. Ethanol did not increase the number of trains required to elicit full EGSs.

These data suggest that phamacologic levels of ethanol have little or no effect on EGS induction or EGS morphology once epileptiform activity is established. However, ethanol clearly increases the threshold for expression of this seizure-like activity. Therefore, the effect of ethanol upon EGS threshold suggests antagonism of NMDA-mediated responses, since NMDA antagonists have a similar effect. The hippocampal EGS model therefore may provide a useful system to analyze the modulation of epileptiform activity by ethanol.

636.15

KCl-EVOKED GABA RELEASE UNCHANGED IN *IN VITRO* HIPPOCAMPUS AFTER CHRONIC FLURAZEPAM TREATMENT. E.I. Tietz and X-H. Xie. Department of Pharmacology, Medical College of Ohio, Toledo, Ohio 43699

Electrophysiological studies in CA1 region of *in vitro* hippocampal slices from benzodiazepine (BZ) tolerant rats showed significantly reduced paired-pulse inhibition 48 hr, but not 7 days after 1 week flurazepam (FZP) treatment. Together with the concomitant increase in the ½ decay time of the EPSP, these data suggested a decrease in hippocampal GABA-mediated inhibition in tolerant rats. Hitchcott et al., (1990) reported an increase in GABA release after chronic diazepam treatment. To determine whether altered GABA release could modify the electrophysiological responses measured in hippocampus, [^{3}H]GABA release was examined 48 hr after 1 week FZP treatment (100 mg/kg X 3 dy; 150 mg/kg X 4 dy). 200 μM hippocampal slices from treated (n=6) or control rats (n=6) were preincubated 10 min at 37°C in pregassed ACSF. Slices were incubated in 1.5 ml .23 μM [^{3}H]GABA (50 μM AOAA) for 30 min at 37°C under 95%O_2/5%CO_2. Slices were superfused (1 ml/min) with ACSF + 500 μM nipecotic acid. After 15 min, 20 mM KCl was injected into the chamber (1M, 20 μl/min) for 2 min. 30 μM EGTA blocked KCl-evoked release. [^{3}H]GABA release was calculated as a fraction of the amount remaining. There was no significant difference in the maximal (control:.015±.003;treated:.016±.004) or total fraction released between groups. GABA uptake into slices tended to be greater in control than treated slices but was not significantly different (679.6±87.6 vs. 542.7±69.7 pmoles/mg prot.). GABA uptake will be investigated further in hippocampal synaptosomes from chronic BZ treated rats. Supported by grants RO1-DA04075 and S07-RRO5700.

636.16

Ca^{2+} CURRENTS IN THE TERMINALS OF RAT NEUROHYPOPHYSIS ARE REDUCED BY DIFFERENT CHAIN LENGTH ALCOHOLS. X.Wang*, J.R.Lemos and S.N.Treistman. Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

Effects of ethanol and long-chain alcohols on voltage-activated calcium (Ca^{2+}) channels have been examined in acutely dissociated rat neurohypophysial terminals, which release vasopressin and oxytocin. Using "whole-cell" patch-clamp recordings, two types of high-threshold voltage-activated I_{Ca} (a fast-inactivating and a long-lasting current component) were present in the nerve terminals. We found that amplitudes of both current components were reduced in a dose-dependent manner (concentrations of 0.1-100 mM) after acute exposure to ethanol (EtOH), butanol (BuOH), hexanol (HxOH) and octanol (OcOH). The reduction did not result from a shift in their current-voltage or steady-state inactivation relationships. The order of effectiveness is OcOH > HxOH > BuOH > EtOH. These results, in contrast to those previously obtained with *Aplysia* neurons (Treistman & Wilson, PNAS, 84:9299, 1987), suggest the importance of EtOH's hydrophobicity in its blockade of Ca^{2+} channels in nerve terminals of the rat neurohypophysis. (Supported by PHS AA08003 and NSF 8919790).

636.17

EFFECTS OF ETHANOL AND CHLORDIAZEPOXIDE ON INHIBITORY PROCESSES IN THE FASCIA DENTATA AND HIPPOCAMPUS REGIO SUPERIOR Henriksen, S.J., Young, W.G. and Steffensen, S.C.*, Research Institute of Scripps Clinic, La Jolla, CA 92037

Acute intoxicating doses of ethanol producing blood alcohol levels of 120-200 mg% selectively increase paired-pulse (PP) inhibition in the dentate gyrus of anesthetized rats suggesting that ethanol facilitates recurrent inhibitory processes (Wiesner and Henriksen, *Neurosci. Letts.* 79:169, 1987). To further our understanding of the neuronal mechanisms underlying this phenomenon, we studied the effects of acute intoxicating levels of ethanol and the benzodiazepine (BZ), chlordiazepoxide, on extracellular field potentials and single-unit activity in the dentate gyrus and area CA1 of the hippocampus. In the dentate, ethanol: 1) had no effect on population excitatory postsynaptic potentials (pEPSPs), 2) decreased population spike (PS) amplitudes (28%), 3) increased paired-pulse (PP) inhibition, 4) decreased dentate granule cell (DGC) spontaneous activity (58%), 5) had no effect on putative interneuron spontaneous activity and 6) markedly increased post-field potential evoked interneuron discharges (IDs, 218%). In the dentate, chlordiazepoxide had no effect on pEPSPs or PS amplitudes, increased PP inhibition, decreased DGC (62%) and interneuron (72%) spontaneous activity and markedly decreased IDs (89%). In CA1, ethanol had no effect on pEPSP amplitudes, decreased PS amplitudes (29%), had no effect on PP responses, decreased pyramidal cell (PC) spontaneous activity (39%), had no effect on interneuron spontaneous activity and markedly increased IDs (97%). Chlordiazepoxide had no effect on pEPSPs, decreased PS amplitudes (26%), had no effect on PP responses, decreased PC spontaneous activity (41%) and had no effect on interneuron spontaneous activity or IDs. Our results suggest that the BZs decrease principal cell excitability by postsynaptic facilitation of inhibitory processes, whereas ethanol decreases principal cell excitability indirectly by increasing the excitability of inhibitory interneurons. This work was supported by NIAAA AA07365 and AA06420 to SJH.

636.18

***IN VIVO* ELECTROCHEMICAL STUDIES OF THE EFFECTS OF LOCALLY-APPLIED ETHANOL ON DOPAMINE NERVE TERMINALS IN RAT STRIATUM.** Y. Wang*, M.R. Palmer and G.A. Gerhardt. Depts. of Pharmacology and Psychiatry, Univ. of Colorado Health Sciences Center, Denver, CO 80262 and *Dept. of Pharmacology, National Defense Med. Ctr., Taipei, Taiwan, R.O.C.

Previous studies have shown that ethanol (EtOH) has effects on the dopaminergic system of the rat striatum, however, few studies have explored the direct effects of locally-applied EtOH on dopamine (DA) nerve terminal function. In the present study, we used high-speed (5Hz) chronoamperometric recording techniques using Nafion-coated carbon fiber electrodes coupled with pressure ejection of ETOH to investigate the effects of this drug on DA nerve terminals in the urethane-anesthetized rat. Local application of EtOH (100mM EtOH with 2.5 mM $CaCl_2$, 25-200nl) from a multibarrel micropipette did not produce a detectable change in the extracellular levels of DA in the anterior striatum. However, potassium (70mM)-evoked overflow of DA was seen to be reversibly inhibited (approx. 50%) by EtOH. In contrast, tyramine (2mM)-induced DA overflow was not altered by EtOH in the same brain region. In addition, the clearance/diffusion of locally-applied DA was reversibly enhanced by EtOH. This effect was partially antagonized by locally-applied nomifensine. Taken together, these data suggest that EtOH exerts its effects on DA-containing nerve endings in the rat striatum by causing an increase in DA uptake. (Supported by USPHS grants NS09199, AG06434, AA05915 and AA00102.)

636.19

ETHANOL INCREASES GABA MEDIATED CHLORIDE CURRENT IN CORTICAL NEURONS IN RAT BRAIN SLICES. W.R. Proctor and T.V. Dunwiddie, Veterans Admin. Medical Research Services and University of Colorado Health Sciences Center, Denver, CO.

Previous studies have shown very little effect of acute ethanol application on intracellular responses in rat **hippocampal** slices. In particular, ethanol did not enhance the $GABA_A$ receptor mediated chloride current recorded in CA1 pyramidal neurons; however, depolarizations elicited by local application of NMDA were significantly reduced by 80 mM ethanol superfusion. The present study was designed to examine the effects of ethanol on pyramidal neurons located in the *in vitro* **cerebral cortex**. Using conventional "sharp point" microelectrodes (60-80 MΩ), 80 mM ethanol application did not affect the membrane input impedance, but significant effects were observed on the resting membrane potential (RMP; 2-5 mV hyperpolarizations) and on EPSP responses (approx. 30% decrease in amplitude). Stimulus-evoked $GABA_A$-mediated IPSCs were studied with whole-cell recording from cortical neurons voltage-clamped at depolarizing potentials. Monophasic IPSCs recorded with this procedure were blocked by bicuculline, increased by pentobarbital, and enhanced by ethanol superfusion in a dose dependent manner over the range of 20 - 160 mM. In combination with the observation that ethanol modulates muscimol-induced chloride flux in cortical (but not hippocampal) microsacs, these results suggest that ethanol selectively interacts with the $GABA_A$ receptors expressed in cortex but not with those in the CA1 region of the hippocampus.

Supported by AA03527 and the V.A. Medical Research Services.

636.20

ETHANOL DOES NOT ALTER HYPERPOLARIZING RESPONSES TO BACLOFEN IN CA3 HIPPOCAMPAL NEURONS. G.D. Frye and A.S. Fincher*, Department of Medical Pharmacology, Texas A&M College of Medicine, College Station, Texas 77843-1114.

Previous behavioral studies suggest that $GABA_B$ receptors may be involved in the central actions of ethanol (Frye et al., J. Pharmacol. Exp. Ther. 237:478, 1986; Allan and Harris, Life Sci. 45:1771, 1989). To further test this hypothesis the acute and chronic effects of ethanol were examined on a cellular level model of $GABA_B$ receptor function. Intracellular recordings from CA3 neurons in hippocampal slices were used to measure baclofen-induced membrane hyperpolarization. Sequential superfusion of increasing concentrations of (+)baclofen (0.1-100μM), induced reversible, concentration-dependent hyperpolarization. In untreated cells, hyperpolarizing responses to near maximal concentrations of baclofen (30-100μM) were -23.3 ±3.4mV, while responses to baclofen (3μM; approx. ED_{50}) were -13.0±3.7mV. These responses were not changed by bath applied ethanol (30mM). Baclofen responses in cells from ethanol dependent rats or treatment matched controls also were not different from those in untreated cells. These results suggest that neither acute nor chronic ethanol treatment change $GABA_B$ receptor responses of CA3 pyramidal neurons to baclofen. Supported in part by PHS grants AA06322 and AA00101.

PSYCHOTROPHIC AGENTS: ANXIETY

637.1

ANXIOGENIC AND CARDIOVASCULAR EFFECTS OF CCK-4 IN MONKEYS ARE BLOCKED BY THE CCK-B ANTAGONIST LY262691. R. M. Palmour*, F. R. Ervin*, J. Bradwejn* and J. J. Howbert#. *Dept Psychiatry, McGill Univ., Montreal Que H3A 1A1 and #Lilly Research Labs, Eli Lilly & Co., Inidanapolis, IN 46285.

Cholecystokinin tetrapeptide (CCK-4), given i.v. to African green monkeys, has profound and dose-related effects on behaviors thought to reflect anxiety and panic. In awake male monkeys (~5 kg) with indwelling venous catheters, low doses of CCK-4 increase restless behaviors (scratch, fidget, pace), threat and alerting to external stimuli. These changes appear within 30 sec of administration, peak after 2 min and gradually decline through 20 min. Higher doses of CCK-4 engender frozen immobility during the initial 10 min and thereafter a gradual increase in restless behavior and arousal. Blood pressure and heart rate are increased at all doses during the first 5 min. The effect of LY262691 (LY), a non-peptide antagonist of brain type CCK (CCK-B) receptors, was tested on these behaviors. If administered s.c. 1 hr before CCK challenge, LY dose-dependently reduced ($p<.01$) threat, alerting to external stimulus and restless behaviors elicited by 5-10 μg CCK-4, with EC50's of 2.5-4 mg/kg, depending on the behavior. LY also reduced time immobile ($p<.01$) in monkeys given 20-30 μg CCK (EC50 6 mg/kg). LY completely blocked lip smacking, urination, drinking and penile erection typically observed in the first minute after CCK-4. At 12 mg/kg, LY alone reduced frequency of restless behaviors, but lower doses had no detectable effect on baseline behavior. In a separate experiment, 6 mg/kg LY blocked elevations in both systolic and diastolic blood pressure caused by 20 μg i.v. CCK-4. These findings suggest that the anxiogenic effects of CCK-4 are mediated through CCK-B receptors, and further support the idea that CCK-B antagonists may find a role in the clinical treatment of anxiety.

637.2

NEUROCHEMICAL AND PSYCHOPHARMACOLOGICAL PROFILE OF A NOVEL 5-HT1A RECEPTOR-SELECTIVE ANXIOLYTIC SUN 8399. I. Hirotsu, M. Harada*, K. Saito[1]*, M. Shibata[2]*, K. Nomura[2]*, T. Tatsuoka[2]*, T. Ohno* and T. Ishihara[1]*. Laboratories of Experimental and [1]Molecular Pharmacology, and [2]Medicinal Chemistry 1, Suntory Institute for Biomedical Research, 1-1-1, Wakayamadai, Shimamoto-cho, Mishima-gun, Osaka 618, Japan.

Anxiolytic actions of SUN 8399 (4-[4-[4-(2-pyrimidinyl) piperazin-1-yl] butyl]-2,3,4,5-tetrahydro-1,4-benzoxazepin-3,5-dione hydrochloride) was examined in various neurochemical and behavioral pharmacological tests.

In *in vitro* receptor binding assays, SUN 8399 displayed higher affinity for the 5-HT1A receptor site (IC_{50}=3.75 nM) than buspirone (IC_{50}=13.0 nM) and no significant affinity for benzodiazepine receptor (>10 μM). Moreover, SUN 8399 had much lower affinities for 5-HT2, 5-HT3, α1, α2 and D2 receptors (IC_{50}=9.3, 4.4, 0.85, 1.79 and 14.5 μM, respectively). Based on adenylate cyclase studies which measure 5-HT1A receptor-mediated effects in the guinea-pig hippocampus, the agonistic activity of SUN 8399 (10^{-5} M) was equivalent to that of 8-OH-DPAT, a selective 5-HT1A agonist, whereas the effect of buspirone was partial. SUN 8399 (3 mg/kg, i.p.) did not prevent 8-OH-DPAT (3 mg/kg, s.c.)-induced hypothermia in rats, but buspirone inhibited it. Anxiolytic activity of SUN 8399 (10-30 mg/kg, p.o.) was much more potent in the thirsty rat conflict test as compared with that of buspirone. In the social interaction test, SUN 8399 (10 mg/kg, p.o.) and buspirone possessed pro-social effects in rats which was not mimicked by diazepam. SUN 8399 (100 mg/kg, p.o.) lacked in mice and rats not only typical side effects such as sedation, hypnosis, muscle relaxation and ataxia of benzodiazepines but also antidopaminergic effects of buspirone.

Thus, it is concluded that SUN 8399 is a potent and 5-HT1A receptor-selective anxiolytic agent without undesirable effects of benzodiazepines and buspirone.

637.3

THE INTERACTION BETWEEN R(+)- AND S(-)-ZACOPRIDE. M.E.Kelly*, B.Costall*,D.M. Murphy*,R.J. Naylor*. (SPON: Brain Research Association) Studies in Pharmacology, University of Bradford, Bradford, BD7 1DP, UK

The R(+)-isomer of zacopride has been shown to be more potent than the S(-) to reduce aversive behaviour (Barnes et al., 1990). In addition S(-)zacopride can attenuate the actions of R(+) zacopride (R.J. Naylor, personal communication). This study investigates the interaction of R(+) and S(-)zacopride emphasising the duration of the pretreatment time.

Mice received S(-)zacopride (1μg/kg) 0,5,10,20,40 and 60 min before treatment with R(+) zacopride (1μg/kg), 40 min later the mice were tested in a light/dark box. Aversive responding was taken as the % time in the dark.

R(+)zacopride (1μg/kg) reduced aversive responding (time in the dark reduced 61% to 24%, P<0.01). S(-)zacopride (1μg/kg) given as a 0,5 or 10 min pretreatment failed to modify this response (time in dark 35%, 39% and 42% respectively). However, when S(-)zacopride was given as a 20,40 or 60 min pretreatment the reduction in aversive behaviour was abolished (time in dark 63%, 63% and 65% respectively).

This study confirms the ability of S(-)zacopride to antagonise the anxiolytic action of R(+)zacopride and emphasises the importance of selecting a suitable pretreatment time for interaction studies.

Barnes JM et al, Pharmac Biochem Behav (1990) 37,717-727

637.4

EFFECTS OF THE PUTATIVE ANXIOLYTIC AND ANTIDEPRESSANT COMPOUND WY-50,324 ON SEROTONERGIC BEHAVIORAL ACTIVITY FOLLOWING CHRONIC ADMINISTRATION. J. A. Moyer and R. F. Kucharik* Wyeth-Ayerst Research, CN 8000, Princeton, NJ 08543.

WY-50,324, a novel adamantyl piperazine derivative, has a high affinity for 5-HT1A and 5-HT2 receptors. *In vitro* and *in vivo* studies have demonstrated WY-50,324 to be a 5-HT1A partial agonist and a 5-HT2 antagonist. Behavioral characterization has indicated that WY-50,324 has an anxiolytic and antidepressant profile. The primary purpose of the current study was to assess 5-HT1A agonist activity of WY-50,324 during the course of repeated administration by assessing hypothermia and the serotonin syndrome. In addition, 5-HT1A and 5-HT2 antagonist activity after chronic (14 day) administration was determined by assessing the serotonin syndrome and head shake response, respectively. Finally, 5-HT1A and 5-HT2 *in vivo* sensitivity was ascertained after chronic (14 day) WY-50,324 treatment by administering selective agonists for these receptor subtypes and measuring behavioral responses associated with their activation. Standard 5-HT1A and 5-HT2 compounds were examined concurrently. In the rat, WY-50,324 (10.0 mg/kg i.p.) continued to produce a 5-HT1A partial agonist response in the serotonin syndrome as did buspirone (10.0 mg/kg i.p.). At the same doses, WY-50,324 and buspirone also produced a hypothermic response indicative of 5-HT1A agonism, but the magnitude of this response decreased over the course of repeated administration with both compounds. The 5-HT1A and 5-HT2 antagonist activity of WY-50,324 was still evident after chronic administration. As expected, the 5-HT2 antagonist, ritanserin, did not demonstrate 5-HT1A agonist or antagonist activity after repeated administration. Changes in receptor sensitivity, as measured by selective agonist challenges at 5-HT1A and 5-HT2 receptors, did not occur after chronic (14-15 day) treatment with WY-50,324 (10.0 mg/kg i.p.) or buspirone (10.0 mg/kg i.p.) in the rat. Chronic ritanserin (1.0 mg/kg i.p., 14-15 day), however, produced a supersensitive 5-HT1A hypothermic response. These results demonstrate that after repeated administration the *in vivo* pharmacological profile of WY-50,324 is unaltered i.e. 5-HT1A partial agonist and 5-HT2 antagonist activity are still evident.

637.5

EVALUATION OF THE EFFECTS OF PD 134308, A CCK-B ANTAGONIST, ON THE PUNISHED RESPONDING OF SQUIRREL MONKEYS. Powell, K.R.and Barrett, J.E., Dept. of Psychiatry, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Rd., Bethesda, MD 20889-4799. Lever pressing of squirrel monkeys was maintained by a fixed-interval 3-min schedule of food presentation during which every 30th response also produced a brief electric shock. Lever pressing was suppressed during this stimulus(conflict or punishment) compared to that occurring prior to the introduction of shock or, with some monkeys, during an alternate stimulus in which punishment did not occur. PD 134308, administered i.m. (0.03-3.0 mg/kg), increased punished responding but had no effect on non-punished responding. Peak increases of 150% of control occurred at 3.0 mg/kg. There was no indication of sedation at the highest dose of PD 134308 (10.0 mg/kg). By comparison, chlordiazepoxide (1.0-10.0 mg/kg) produced similar effects, except the magnitude of increases in punished responding reached approximately 200% of control performance levels and the higher doses reduced non-punished response rates. PD 134308 produces anxiolytic-like effects in this animal model of anxiety that would suggest potential clinical efficacy in humans of a novel class of compounds with actions at or modulated by cholecystokinin receptors.

637.6

EFFECTS OF BENZODIAZEPINE PARTIAL AGONISTS ON PUNISHED RESPONDING AND ON MIDAZOLAM DISCRIMINATION. S. Gleeson[1], J.M. Witkin[2], C.H. McDonald[1]*, and J.E. Barrett[1]. [1]Behavioral Pharmacology Lab., Uniformed Services University of the Health Sciences, Bethesda, MD 20814 and [2]Psychobiology Lab., NIDA Addiction Research Center, Baltimore, MD 21224.

Novel compounds acting as partial agonists at benzodiazepine (BZ) receptors show only some of the effects typical of full agonists, making them candidates for development as anxiolytics without accompanying sedation, muscle relaxation, or dependence liability. Several partial agonists were examined for anxiolytic activity and degree of similarity to the full agonist midazolam. The BZ derivatives Ro 16-6028 (bretazenil) and Ro 17-1812, and the β-carboline abecarnil produced large increases in punished responding of pigeons, similar to the effects of midazolam. Abecarnil and midazolam were effective across a similar dose range, while the effective dose range for the other drugs was broader. The similarity to midazolam was not apparent in the drug discrimination procedure, however. In pigeons trained to discriminate midazolam (1.0 mg/kg), Ro 16-6028, Ro 17-1812, and abecarnil only partially substituted (<80%) for midazolam, even at higher doses than required to produce peak anti-conflict effects. The results suggest that partial BZ agonists may exert anxiolytic effects across a broad range of doses that do not share subjective effects with full benzodiazepine agonists. Partially supported by DA 02873.

637.7

ANXIOLYTIC ACTIVITY OF FLESINOXAN IN ANIMAL MODELS. B. Olivier, A.M van der Poel*, J. Mos, J.A.M. van der Heyden and J. Schipper. Dept. Pharmacology, Duphar, P.O. Box 900 1380 DA Weesp, The Netherlands.

Flesinoxan selectively binds to the 5-HT_{1A} receptor (K_i= 1.7 nM). Flesinoxan has been studied in anxiolytic models in mice where exploration of a novel environment is suppressed by punishment. In the 4-plate test, where ambulatory activity is punished by a mild foot-shock, flesinoxan and other 5-HT_{1A} agonists show no anxiolytic effects in contrast to benzodiazepines. Similar results are obtained in the light-dark model, where exploration of mice is inhibited by bright illumination. In another anxiety model in mice, which is based on stress-induced hyperthermia, flesinoxan is active at doses of 1 mg/kg p.o. and higher. Also 8-OH-DPAT and buspirone are active in this model (0.3 mg/kg s.c and 10 mg/kg p.o.), whereas ipsapirone does not show an effect upto 20 mg/kg p.o.

Anxiolytic properties of drugs can also be detected by the so-called separation-induced ultrasonic vocalizations of infant rodents. Low doses of benzodiazepines reduce ultrasonic vocalizations in rat pups. 5-HT_{1A} agonists like buspirone and ipsapirone also reduce these separation induced anxiety calls. Flesinoxan is highly active (0.3-3 mg/kg i.p.) and far more potent than buspirone and ipsapirone. Ultrasonic vocalization emitted by adult rats in anticipation of electric footshocks are very effectively suppressed by flesinoxan (0.3 -1 mg/kg i.p.). Benzodiazepines are only marginally active in this test, except for alprazolam, which is fully effective at 3 mg/kg. In a conflict test in pigeons, it has been found that flesinoxan has anxiolytic activity at low doses (0.03-1) mg/kg (Barrett et al., J. Psychopharm. (1989) 3:64-69).

In conclusion, flesinoxan is a highly potent and selective 5-HT_{1A} agonist. Based on the animal pharmacology data there are strong indications that flesinoxan may have potential anxiolytic properties in man.

637.8

PHARMACOLOGICAL PROFILE OF THE 5HT$_{1A}$-ANXIOLYTIC EMD 56551 G.D. Bartoszyk, H. Böttcher*, H. Greiner*, J. Harting*, and C.A. Seyfried*. E. Merck, Pharm. Res., 6100 Darmstadt, FRG.

EMD 56551 (5-methoxy-3-[4-(4-(4-methoxyphenyl)-1-piperazinyl)butyl]indole) exhibited the following binding profile in vitro (receptor and IC_{50}, respectively): 5HT_{1A}: 4 nM; 5HT_2: 750 nM; D_2: 420 nM; affinity to 5HT_{1B}, 5HT_3, BZ, D_1, α_1 and α_2 was ≥ 1 μM. EMD 56551 inhibited forskolin-stimulated adenylate cyclase (IC_{50}: 2 nM), but antagonized the inhibitory effects of 1 μM 8-OH-DPAT in this model at 3 and 10 μM. 5HTP-accumulation in rat n.raphe was inhibited dose-dependently (threshold dose ≤ 0.1 mg/kg po). In the elevated plus maze and social interaction tests in rats, EMD 56551 was anxiolytic with bell-shaped dose-response curves (3-100 and 0.3-100 μg/kg sc, respectively). Foot-shock induced vocalization in rats was decreased with an ED_{95} of 100 μg/kg sc. In drug discrimination studies, EMD 56551 generalized to the 8-OH-DPAT cue with an ED_{50} of 0.6 mg/kg ip. Moreover, EMD 56551 inhibited two-way shuttle box avoidance and prevented haloperidol-induced catalepsy. The results characterize EMD 56551 as a selective partial 5HT_{1A}-agonist with a high potency and a broad dose range as regards anxiolytic activity.

637.9

IN VIVO VOLTAMMETRIC NEUROCHEMICAL PROFILE FOR THE TRIAZOLOBENZODIAZEPINE, ADINAZOLAM. F.T. Phelan and P.A. Broderick, Dept. Pharmacol., CUNY Medical School (Rm. J-910), Convent Ave. & W. 138th St., N.Y., N.Y. 10031, U.S.A.

Adinazolam is a benzodiazepine (BZD) agonist, an anxiolytic compound with demonstrated antidepressant activity (Amsterdam et al., *Psychopharmacol.* 88 (1986) 484). However, adinazolam does not affect either noradrenergic or serotonergic reuptake inhibition (Lahti et al., *Neuropharmacol.* 22 (1983) 1277). To elucidate adinazolam mechanistically, semiderivative voltammetry was used with stearate working electrodes to detect norepinephrine (NE) and serotonin (5-HT) in hippocampus (CA_1) of chloral hydrate anesthetized, male, virus free, Sprague Dawley rats. Specific details for the characterization of the NE and 5-HT electrochemical signals are published (Broderick, P.A., *Neurosci. Lett.* 95: (1988) 275). Additional studies showed that the α adrenoreceptor agonist, clonidine (30 mg/kg ip) significantly decreased the NE signal (36%, $p<0.05$), whereas the α adrenoreceptor antagonist, yohimbine (5 mg/kg ip) significantly increased the NE signal (24%, $p<0.05$). Moreover, the triazolobenzodiazepine, adinazolam (10mg/kg ip) significantly decreased the NE signal by 36.4% ($p<0.05$) and significantly decreased the 5-HT signal by 39.0% ($p<0.05$) at the end of one hour of study. The data are consistent with known mechanisms for the BZD's (Gallagher, D.W., *Eur. J. Pharmacol.* 49 (1978) 133; Sanghera, M.K. and German, D.W., *J. Neural Transm.* 57 (1983) 267). Importantly, the expected increased extracellular concentrations of NE and 5-HT, consistent with measurement of reuptake inhibitory mechanisms, do not occur. Thus, adinazolam regulates presynaptic NE and 5-HT release mechanisms. (Supported in part by an Upjohn grant).

637.10

SELECTIVE SEROTONIN RE-UPTAKE INHIBITORS DECREASE SCHEDULE-INDUCED POLYDIPSIA IN RATS. A. Woods*, C. Smith, M. Szewczak, M. Cornfeldt, P. Conway, and R. Corbett*. Department of Biological Research, Hoechst-Roussel Pharmaceuticals, Inc., Route 202-206, P.O. Box 2500, Somerville, NJ 08876.

Polydipsia was induced in food deprived rats by exposure to a fixed-interval feeding schedule (FI = 60 sec) for 150 min. per day. A polydipsic rat consumed a 3-4 times greater volume of water compared to a food deprived control rat. To determine if selective serotonin re-uptake inhibitors could decrease this schedule-induced polydipsia, animals were treated with either clomipramine (CMI), fluoxetine (FLU) or the noradrenergic re-uptake inhibitor, desipramine (DMI), as a negative control. Each of these agents were administered daily for at least 21 days at a dose of 5 mg/kg, ip, to polydipsic rats 60 min. prior to testing. Animals administered DMI showed no decrease in polydipsic behavior throughout the testing period. However, after chronic dosing, CMI and FLU significantly reduced polydipsia by approximately 50 percent. Since obsessive-compulsive disorder (OCD) and polydipsic behavior both involve an exaggeration of a normal behavior, the polydipsia model may be relevant for the prediction of compounds useful in the treatment of OCD. This idea is strengthened by the finding that CMI and FLU effectively reduce both polydipsic behavior in rats and clinical symptoms of OCD in humans, while DMI does not.

637.11

A COMPARISON OF BENZODIAZEPINE (BZ) RECEPTOR AGONISTS IN RAT BEHAVIORAL MODELS OF ANXIETY AND SEDATION. J.V.Cassella and M.D.Crago. Neurogen Corporation, Branford, CT 06405.

Drugs interacting with the BZ receptor reduce anxiety-related behaviors in animals and also produce a variety of side effects. Although some rat behavioral models have accurately predicted anxiolytic effects of these compounds, the measurement of anxiety-related behaviors can be confounded by concomitant sedation or motivational changes. This study compares the effects of the BZ receptor full agonists diazepam and alprazolam and partial agonists RO 16-6028 and CGS-9896 in three very different rat anxiety models (Elevated Plus Maze[EPM], Lick Suppression, and Fear Potentiated Startle) and an independent test of sedation (Locomotor activity). Diazepam (0.5-2.0mg/kg,IV) and alprazolam (0.06-0.5mg/kg,IV) dose-dependently reduced locomotor activity while RO 16-6028 and CGS-9896 depressed activity at some doses (0.125-1.0mg/kg,IV and 0.5-2.0mg/kg,IV, respectively). In similar dose ranges, all the compounds were efficacious in the EPM but sedative effects clearly interfered with the animal's performance in this task. All compounds increased the animal's willingness to take shocks in the lick task, with diazepam and RO 16-6028 clearly superior in efficacy. However, these two drugs also significantly increased drinking from a non-shocked spout. Performance in the Potentiated Startle paradigm also varied among the four test compounds. For the purposes of drug discovery a number of different behaviors, each possessing a minimal and varying sensitivity to different potential side effects should be employed. The overall profile of test results might best predict clinical efficacy of new test compounds.

637.12

BEHAVIORAL EFFECTS OF RO 15-4513 RELATED TO ITS INTRINSIC AGONIST ACTIVITY. A.H.Tang and S.R.Franklin. CNS Research, The Upjohn Company, Kalamazoo, MI 49001

Ro 15-4513 (RO) has pharmacological characteristics of a partial inverse agonist at the benzodiazepine (BZ) receptor. RO also reverses some, but not all, of the CNS effects of ethanol, BZ's, and barbiturates. It has a much higher affinity than other BZ's and β-carbolines for a recombinant $GABA_A$ receptor subtype identified in the cerebellum of rats (Luddens *et al.*, Nature 346:648, 1990). This study investigates the intrinsic behavioral effects of RO, and compares them to another BZ inverse agonist, FG 7142 (FG).

In rats trained to lever-press (VI-45 sec) for food reinforcement, both RO and FG suppressed respondings, with the former being 10X more potent. The effect of FG can be completely reversed by flumazenil, diazepam, or the BZ partial agonist, U-78875 (Tang *et al.*, Psychopharmacol. 101: Suppl. S56, 1990). The effect of RO on the food-reinforced behavior was incompletely antagonized by diazepam or flumazenil, although U-78875 was fully effective in reversing this effect of RO.

In rats trained in 2-way avoidance, RO dose-dependently impaired the avoidance response. FG produced only a minimum depressant effect. Again, flumazenil and diazepam partially reversed the avoidance depression by RO, but U-78875 produced a complete reversal. The results indicate that RO has intrinsic behavioral effects other than those of an inverse BZ agonist. U-78875 appears to be an antagonist for this intrinsic effect.

637.13

PERSISTENT MIDBRAIN HISTOLOGICAL ALTERATIONS IN ADULT MICE PRENATALLY TREATED WITH DIAZEPAM. A. Márquez-Orozco, M.C. Márquez-Orozco, M.A. Alcantara-Ortigoza* and A. Escobar*. Embryol. Dept. Sch. of Med., Inst. Invest. Biomed. UNAM, P.O.B. 70-553. México 04510 D.F. MEXICO.

Diazepam diffuses through the placental barrier of humans and mice, and accumulates in the mesencephalon, where it produces a delay in the neuroblastic differentiation of fetal mice, and an atypical chromatin distribution and less number of fibers in the neuroblastic nucleus. We investigated if these histological alterations of the fetal mesencephalon occur in adult mice. Single daily doses (2.7 mg/kg) of diazepam were s.c. administered to CD-1 strain female mice, between the 6th and 17th day of gestation. A control group received equivalent volumes of saline sol. The offsprings were wet-nursered by non-treated mice, weaned, and kept for 240 days. The motor activity and swimming patterns were periodically measured from the 6th day until the 8th month. Mice were deeply anesthetized, perfused with 10% formaline, and the brains were removed, fixed, and stained with current and modified fast-Golgy techniques. The sections were observed under a photonic microscope. The experimental animals showed atypical distribution of collicular and red nuclei neurones, less number of fibers, and accumulation of glial cells around vessels. These findings could explain the locomotor activity and swimming pattern alterations.

637.14

THE SPONTANEOUSLY HYPERTENSIVE RAT AS AN ANIMAL MODE OF ATTENTION-DEFICIT HYPERACTIVITY DISORDER. T. Sagvolden and B. Wultz. Dept. Neurophysiology, University of Oslo, N-0317 Oslo, Norway.
(SPON: European Brain and Behaviour Society).

About 3% of all children suffer from attention deficit hyperactivity disorder (ADHD). The primary purpose of the present research was to investigate the possibility of using the spontaneously hypertensive rat (SHR) as an animal model of ADHD.

Several experimental paradigms were used: open fields and various schedules of reinforcement.

The results showed that some behavioral changes in SHR are parallel to those of ADHD: SHR as well as ADHD children are hypoactive initially in a novel situation but develop a pronounced hyperactivity as time passes, there are some deficits in "attentive" behavior and both SHR and ADHD show a pronounced variability in their behavior.

SHR show reduced behavioral sensitivity to methylphenidate (Ritalin) compared to controls.

SHR is a promising animal model of ADHD also known as the "hyperkinetic syndrome".

LITERATURE: Sagvolden, T. & Archer, T. (Eds.) Attention Deficit Disorder: Clinical and Basic Research. Lawrence Erlbaum Associates, 1989.

637.15

COMPARATIVE STUDIES OF CALCIUM CHANNEL BLOCKERS EFFECTS ON CENTRAL FUNCTIONS AND THEIR PSYCHOLOGICAL DISORDERS. T.Shibuya, Y-Q.Zhang and Y.Watanabe. Dept. of Pharmacology, Tokyo Medical College, Shinjuku, Tokyo 160, Japan.

The modulation of Ca^{++} in the cerebellar granule cells of rats and the behavioral alterations resulting from treatment of Ca^{++}blockers were examined to evaluate the central effect of Ca^{++}blockers on the improvement of psychological dysfunctions. In hyperpotassium-induced Ca^{++} efflux, the fluorescence of Ca^{++} induced by Fura-2am in the granule cells was potentiated about 1.6 times higher than the steady state level. Fifty percent inhibition of this potentiation was seen in $5x10^{-7}$M of flunarizine (Flu), $2x10^{-6}$M of nicardipine (Nic), and concentrations higher than $5x10^{-5}$M of diltiazem, verapamil and nifedipine showed about 20% inhibition. In the behavioral studies, Flu and Nic demonstrated pharmacological properties similar to diazepam, but not chlorpromazine. Also both Flu and Nic treatment remarkably inhibited the hyperactivity of spontaneously hypertensive rats caused by mild audiogenic stress-induced fear, and also prolonged the duration of immobility in forced swimming of both male and female rats. These results suggest that the pharmacological properties of Ca^{++}blockers in the central nervous system are completely different from those in the peripheral nervous system, and some Ca^{++}blockers may be effective in the treatment of anxiety.

637.16

PHOSPHATIDYLINOSITOL HYDROLYSIS IN BRAIN MEMBRANES. X. Li, L. Song* and R. S. Jope. Dept. Psychiatry and Behavioral Neurobiology, Univ. of Alabama, Birmingham, AL 35294.

The hydrolysis of phosphatidylinositol (PI) was measured in brain membranes using the method of Wallace and Claro (J. Pharm. Exp. Therap. 255:1296, 1990) in which the hydrolysis of exogenous [^{3}H]PI is measured. [^{3}H]PI hydrolysis was stimulated by GTPγS concentration-dependently with a maximal stimulation of 2 to 3-fold obtained with 3 μM. NaF concentration-dependently stimulated [^{3}H]PI hydrolysis to a greater extent than did GTPγS, with a maximal effect of about 5-fold with 20 mM NaF.

Carbachol alone or with NaF did not stimulate [^{3}H]PI hydrolysis, but with GTPγS carbachol stimulated activity above that obtained with GTPγS alone. Pilocarpine, which is only slightly active in slices, stimulated [^{3}H]PI hydrolysis in the presence of GTPγS to a similar extent as did carbachol. Inclusion of excitatory amino acid agonists with GTPγS and carbachol did not inhibit [^{3}H]PI hydrolysis, in contrast to results in slices, but with GTPγS some agonists effectively stimulated [^{3}H]PI hydrolysis.

In membranes prepared from chronic lithium-treated rats (4 weeks) carbachol stimulated [^{3}H]PI hydrolysis to a similar extent as in controls but activation of G-proteins induced lower stimulation than in controls.

These results demonstrate that membrane preparations are useful to study the modulation of [^{3}H]PI hydrolysis.

637.17

PERTUSSIS TOXIN POTENTIATES SEIZURES INDUCED BY PILOCARPINE, KAINIC ACID AND N-METHYL-D-ASPARTATE. R. S. Jope and G. C. Ormandy. Dept. of Psychiatry and Behavioral Neurobiology, Univ. of Alabama, Birmingham, AL 35294.

Previous studies have shown that lithium potentiated the in vivo response to cholinomimetics in rats, resulting in seizures at otherwise nonconvulsant doses, but did not affect seizure activity induced by other chemical convulsants including kainic acid and NMDA (Ormandy et al., Exp. Neurol. 111:356, 1991). In vitro experiments have suggested that lithium interferes with receptor mediated second messenger production, possibly due to an action at G-proteins. The present study tested the hypothesis that selective inhibition of G-proteins by in vivo administration of pertussis toxin would induce effects similar to those of lithium.

Pertussis toxin (icv, 3 days) mimicked lithium in potentiating the convulsant response to pilocarpine in rats. The effect of pertussis toxin was dose-dependent and the extent of potentiation was over 13-fold, which was remarkably similar to lithium. The seizures were prevented by pretreatment with atropine, phenobarbital or diazepam. L-Phenylisopropyladenosine and MK-801 also demonstrated anticonvulsant activity, with MK-801 also protecting the rats against the rapid death associated with pertussis toxin/pilocarpine-induced seizures. Thus, seizures were cholinergically initiated and were controlled by the same drugs as were lithium/pilocarpine-induced seizures. The results illustrate that in several respects the response to cholinomimetics are modified in a similar manner by lithium and pertussis toxin. However, pertussis toxin lacks the specificity of lithium as it also potentiated the convulsant effects of kainic acid and NMDA.

637.18

SEIZURE-INDUCED PROTEIN TYROSINE PHOSPHORYLATION IN RAT BRAIN REGIONS. M. S. Baird, R. S. Jope and G. V. W. Johnson. Dept. of Psychiatry and Behavioral Neurobiology, University of Alabama, Birmingham, AL 35294.

Phosphorylation of protein tyrosines is an important modulatory process for cell signalling and other cellular functions. Rat brain regions were examined for altered protein phosphotyrosines, using Western blot analysis and microwave irradiation to limit postmortem alterations, after administration of two convulsants, lithium plus pilocarpine or kainic acid. Most phosphotyrosine proteins were unaltered by these treatments, but there was a large, specific increase in the tyrosine phosphorylation of a 40 kD protein. This elevation was evident in all three regions that were examined, the cerebral cortex, hippocampus and striatum. This increase occurred abruptly with the onset of generalized status epilepticus and remained elevated for at least 90 min. The majority of the tyrosine phosphorylated 40 kD protein was found in the cytosolic fraction. These results demonstrate a large, specific effect of chemically induced seizures on a single phosphotyrosine protein in rat brain.

EPILEPSY: BASIC MECHANISMS V

638.1

ANTICONVULSANT ACTION OF GABA BLOCKADE IN SUPERIOR COLLICULUS. A. Pazos, K. Japikse, K. Wetzler*, P. Pritchard*, R. Maggio* and K. Gale. Department of Pharmacology, Georgetown University Medical Center, Washington, D.C. 20007.

Blockade of GABA receptors, or stimulation of glutamate receptors, in superior colliculus (SC) has been shown to protect against maximal electroshock-induced tonic convulsions (Dean and Gale, Brain Res. 477:391-395) and pentylenetetrazol-induced spike-and-wave discharge (Redgrave et al., EJP 158:283-287). In the present study, we determined whether blockade of GABA receptors in SC could also protect against limbic motor seizures as characterized by facial and forelimb clonus with rearing.

Limbic motor seizures were induced by the unilateral focal application of bicuculline methiodide (BIC) (118 pmol in 120 nl) into area tempestas (AT), an epileptogenic site in the deep prepiriform cortex. Control rats (receiving bilateral infusions of saline into SC) all exhibited convulsive seizures following BIC in AT. Rats pretreated (5 min) with BIC (50pmol) bilaterally in the deep SC, were protected against the AT-evoked convulsive seizures. Unilateral application of BIC (50pmol) into the superficial SC, did not alter the convulsive response to BIC in AT.

These results are consistent with the observations made previously in other seizure models and suggest that a reduction of GABA transmission in the deep layers of the SC can decrease susceptibility to convulsive seizures evoked by activation of limbic circuits. As the deep SC is known to be a target of nigrotectal GABAergic projections, and, suppression of the activity of nigral neurons is anticonvulsant against a variety of seizures (including those evoked from AT), it is likely that the anticonvulsant action of BIC in SC is due to interference with the influence of the nigrotectal GABAergic projections.

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638.2

D1 Dopamine Receptor Agonists Induce Forelimb Clonus. D.R. Britton, K. Asin, L. Yahiro*, P. Curzon, L. Bednarz* J. Williams, Neuroscience Research, Abbott Laboratories, Abbott Park, IL 60064

The D1 dopamine receptor (D1) agonist, SKF38393, has been reported by several groups to potentiate pilocarpine seizures. We report that a variety of D1 agonists induce forelimb clonus in rats and mice independent of cholinergic agonist co-treatment. The compounds tested include isochromans (A68930, A68979, A70620) and the benzazepine, SKF82958. Each produced clonus with an ED_{50} value of from 3 to about 300-fold higher than its ED_{50} for inducing rotation in rats with unilateral 6-OHDA lesions. The response showed enantiomeric selectivity for the active enatiomer of A68930 and there was a significant correlation between potency in inducing clonus and IC_{50} values for stimulating adenylate cyclase or Ki values for binding to the D1 receptor *in vitro*. In male CD1 mice, A68930-induced clonus is sensitive to inhibition by a variety of anticonvulsants including diazepam, clonazepam, valproic acid and acetazolamide. It is also sensitive to D1-selective (SCH23390, A69024, SCH39166) but not D2-selective (haloperidol, YM09151-2) antagonists. Neither D2-selective (LY171555, bromocriptine) nor mixed D1/D2 (apomorphine) agonists produced clonus in the dose range tested. Furthermore, co-administration of LY171555 or bromocriptine provided apparent protection from clonus as evidenced by a rightward shift in the dose-response curve to A68930.

638.3

GAMMA-HYDROXYBUTYRIC ACID INDUCED SPIKE AND WAVE DISCHARGES IN RATS: ELECTROPHYSIOLOGICAL AND AUTORADIOGRAPHIC EVALUATION. P.K. Banerjee, O.C. Snead III and E. Hirsch. Div. of Neurology, Childrens Hospital of Los Angeles, Department of Neurology, Univ. of Southern California, Sch. of Med., Los Angeles, CA.

γ-hydroxybutyric acid (GHB), a naturally occuring compound, is synthesized from γ-aminobutyric acid (GABA) and induces bilaterally synchronous spike and wave discharges (SWD) in rats which simulate generalized absence seizures. The GHB model of absence seizure has been well characterized in our laboratory (Snead, Epilepsia 29:361, 1988). The purpose of the present study was to investigate the pattern of SWD generation in different layers of frontal cortex (FCtx) and thalamic nuclei. For this purpose, the animals were implanted with bipolar electrodes in two different layers of FCtx, dorsal hippocampus (CA1 field), ventrolateral (VL) and ventropostereolateral (VPL) nuclei of thalamus. The FCtx (layer one) bipolar recording was synchronous with the other cortical epidural (monopolar) electrode recordings while FCtx (layer two) did not seem to participate in the SWD. In the thalamus VPL synchronously fired with the cortical epidural recording during SWD induced by GHB but VL was found to be silent. Our biochemical findings using quantitative receptor autoradiography suggested that even though the highest density of GHB binding sites (labeled with ^{3}H-GHB) were present in the hippocampus (CA3>CA1>CA2), the bipolar recordings of hippocampus (CA1 field) were silent during GHB-induced SWD. Similarly, the bipolar recordings of VL were silent inspite of the fact that the GHB binding sites were more abundant in the VL than in the VPL, where SWD appeared synchronously with the cortical epidural recordings. Interestingly, the distribution pattern of ^{3}H-GHB in the brains from animals sacrificed dring GHB-induced SWD, showed a significant increase in ^{3}H-GHB binding in both the layers of FCtx (~120%). In the thalamus, the VPL binding rose to ~116% compared to GHB-naive VPL, whereas the increment in VL was approximately 80%. These findings suggest that GHB binding sites in thalamus and cortex are directly involved in the generation of SWD in the model of absence.

638.4

THE ELECTROPHYSIOLOGICAL BASIS OF EPILEPTIFORM MAGNETIC FIELDS IN NEOCORTEX: SPONTANEOUS ICTAL PHENOMENA SHI DI and DANIEL S. BARTH *Department of Psychology , University of Colorado, Boulder, CO 80309-0345 (U.S.A)*

In a previous report from our lab., electrical measurements of epileptiform cellular currents produced by physiologically evoked interictal penicillin spikes in rat somatosensory cortex were directly compared to the extracranial magnetic fields these currents generate. The present study uses the same methodology to extend these observations to spontaneous interictal and ictal phenomena in rat auditory cortex, and provides a more realistic empirical foundation for physical models with which to interpret noninvasive neuromagnetic recordings of human focal seizures. These data indicate that seizure foci under 1 x 1 cm^2 in cortical surface area are capable of producing magnetic fields that may be recorded at extracranial distances similar to those used in humans. Furthermore, physical models based on the dipole approximation appear to be appropriate for the interpretation of ictal magnetic field phenomena in neocortex.

638.5

IMPAIRMENT OF SPATIAL MEMORY AFTER SUSTAINED HIPPOCAMPAL STIMULATION: THE INFLUENCE OF CA1 CELL DAMAGE AND INTERICTAL SPIKING ACTIVITY. **A. Ylinen, J. Sirviö, T.F. Freund[1] and P. Riekkinen. Dept. of Neurol. and Pathol. Univ. of Kuopio, P.O.B. 1627, SF-70211 Kuopio, Finland and [1]Dept. of Funct. Neuroanatomy, Institute of Exp. Medicene. Hungarian Academy of Sciences, H-1450 Budapest, Hungary.**

Definits in learning/memory found in epileptic patients have been associated with morphological damage of the brain tissue and also with epileptiform electrical activity. Sustained stimulation of the perforant pathway (PP) input to the hippocampus has been shown to cause cell damage and also impairment in spatial learning/memory. In the present experiments we studied spatial learning/memory, hippocampal cell damage and appearance of interictal spikes after sustained stimulation (50 min) of PP in rats pretreated either with the GABAergic inhibition enhancing drug, vigabatrin (500 mg/kg) or with NMDA receptor antagonist, CGP 39551 (5 mg/kg). EEGs were recorded 1 week after the stimulation prior to water maze trials. Finally, the rats were perfused for histology. No defects of spatial learning/memory were found in vigabatrin pretreated rats (n=9). Also CGP 39551 treated rats (n=9) performed significantly better than placebo-treated (n=8) ($p < 0.001$), but slightly worse than nonstimulated controls (n=8). Vigabatrin rats showed neither cell damage in hippocampus nor interictal spiking in EEG, CGP 39551 rats showed cell damage but not spiking and placebo rats showed both. In conclusion, the degree of the impairment of learning/memory depends not only on the degree of cell damage but also on the amount of interictal epileptiform activity.

638.6

NONLINEAR DYNAMICS OF ICTAL EEG RECORDS. N.D. Schiff*, A. Canel*, D.R. Labar*, and J.D. Victor. Department of Neurology and Neuroscience, New York Hospital-Cornell Medical Center, New York, New York, U.S.A. 10021.

Mathematical models of the EEG posit nonlinear mechanisms at the level of cell populations. Ictal EEG records have qualitative features such as spike discharges which may reflect these underlying nonlinear dynamics. We sought to quantify nonlinear behavior in the ictal EEG by constructing nonlinear autoregressive models (NLAR). The nonlinear difference equation which represents the EEG in this model has a form which can be treated with methods of dynamical systems analysis.

The NLAR models consisted of linear autoregressive models augmented by a single quadratic or bilinear interaction term. By varying the lags associated with this term, we explored the pattern of nonlinear dynamics. NLAR analysis of ictal records from patients with petit mal seizures revealed a specific dynamical pattern. The pattern of interaction excluded simple cascades of linear and nonlinear systems as models, and indicated an interaction of signals separated by roughly 24 ms, at times from 40 ms to 60 ms in the past. Other patterns were seen in patients with other seizure types. In addition, we treated these models as dynamical systems and analyzed the stability of fixed points and two-cycles.

638.7

EFFECT OF YOHIMBINE AND CLONIDINE ON ELECTROSHOCK-INDUCED FOREBRAIN AND BRAINSTEM SEIZURES IN RATS. C. Wang, P. C. Iobe and R. A Browning, Southern Illinois Univ. Sch. of Med., Carbondale, IL 62901 and Univ. of Illinois Coll. of Med. Peoria, IL 61656

Recent studies have shown that electroshock-induced convulsions characterized by facial and forelimb (F&F) clonus represent a model of forebrain seizures, while tonic hindlimb extensor (HLE) convulsions represent a model of brainstem seizures (Browning and Nelson, Exp. Neurol. 93: 546, 1986). In an attempt to evaluate the role of noradrenergic receptors in the regulation of seizures, we have examined the effects of yohimbine, an alpha-2 antagonist and clonidine, an alpha-2 agonist on electroshock-induced F&F clonus (forebrain seizures) and tonic HLE (brainstem seizures) in male Sprague Dawley rats. F&F clonus was induced by a 24 mA, 0.2 sec current (the mean threshold) using a Whalquist stimulator and corneal electrodes, while tonic HLE was produced using the same instrument and a 150 mA, 0.2 sec current. Yohimbine (2.5 - 10 mg/kg, i. p.) produced a significant reduction in the incidence of HLE without affecting F&F clonus 30 min after drug administration. Clonidine (0.5 mg/kg, i. p.) had no effect on F&F clonus and produced a slight, albeit non-significant, inhibition of tonic HLE. The results support the hypothesis that noradrenergic receptors exert different effects in the regulation of forebrain and brainstem seizures. Whether the inhibitory effect of yohimbine on tonic HLE is mediated by an increase in norepinephrine release remains to be determined.

638.8

IP_3, CYCLIC AMP AND CYCLIC GMP IN RAT BRAIN REGIONS AFTER LITHIUM AND SEIZURES. A. G. Faulk, L. Song*, K. Kolasa and R. S. Jope. Department of Psychiatry and Behavioral Neurobiology, University of Alabama, Birmingham, AL 35294.

The concentrations of inositol 1,4,5 trisphosphate (IP_3), cyclic AMP, and cyclic GMP were measured in rat brain regions after acute or chronic lithium administration, as well as after the cholinergic agonist pilocarpine alone or in combination with lithium at a dose which induces seizures only in lithium pretreated rats.

Neither acute nor chronic lithium treatment altered the hippocampal or cortical concentrations of IP_3. Pilocarpine increased IP_3 after 20 min, and this response was enhanced in both acute and chronic lithium-treated rats. At 60 min after pilocarpine IP_3 was elevated further, and with pilocarpine plus lithium the IP_3 concentration remained elevated in the hippocampus but it decreased in the cortex.

Neither acute nor chronic lithium treatment altered the cyclic AMP. During seizures induced by pilocarpine cyclic AMP increased in the cortex with acute and chronic lithium and in the hippocampus with acute, but not chronic, lithium administration.

Acute and chronic lithium treatments both caused some reduction in cyclic GMP. Seizures induced by pilocarpine resulted in large accumulations of cyclic GMP with acute lithium treatment, but these increases were much less with chronic lithium administration.

In summary, chronic lithium treatment appeared only to reduce IP_3 and cyclic AMP concentrations after a long period of stimulation whereas both basal and stimulated cyclic GMP production were reduced by lithium administration.

638.9

NIGRAL GAMMA–VINYL GABA INFUSIONS AND FLUROTHYL-INDUCED SEIZURES: DEVELOPMENTAL EFFECTS IN RATS. S. G. Xu, E. F. Sperber, D. S. Garant, H. Y. Liu* and S. L. Moshé, Departments of Neurology, Neuroscience and Pediatrics, Albert Einstein College of Medicine, Bronx, NY 10461.

The GABAergic system within the the substantia nigra pars reticulata (SNR) is involved in the control of seizure as a function of age. In the present study, we investigated the effect of perinigral infusions of γ-vinyl GABA (GVG, an inhibitor of the GABA degradative enzyme, GABA transaminase) on clonic and tonic seizures induced by flurothyl in 16 day old rat pups and adult rats. Bilateral nigral infusions of GVG differentially suppressed seizures in the two age groups. In rat pups, nigrally administered GVG (5-20 μg/0.25 μl) increased the threshold for tonic seizures but not for clonic seizures in a dose-response manner. The optimal dose was between 5-10 μg because the dose of 20 μg produced marked sedation. In adult rats, only the dose of 20 μg elevated the thresholds for both clonic and tonic seizures. Lower doses (5-10 μg) did not protect adults against seizures, but there was a significant effect of GVG dose. In both age groups, the GVG effects were confined to the SNR. These results suggest that nigral infusions of GVG exert specific anticonvulsant effects as a function of age.

638.10

ANATOMICAL DIFFERENCES IN THE DEVELOPMENT OF GABA-B RECEPTORS AND RESPONSES. D.S. Garant, E.F. Sperber, P.K. Stanton, and S.L. Moshé, Departments of Neurology, Neuroscience, and Pediatrics, Albert Einstein College of Medicine, 1300 Morris Park Ave, Bronx, NY 10461 (USA).

In two-week-old rats the GABA-B agonist baclofen can attenuate seizures after either systemic administration or infusion into substantia nigra (SN), a structure implicated in seizure control. These treatments are ineffective against seizures in adult rats.

To determine whether receptor differences might explain this developmental disparity, baclofen-displaced ^{3}H-GABA binding to GABA-B sites was assayed in brain regions obtained from adult and 14- to 17-day-old rats. There were no significant differences in K_d between age groups. The SN of rat pups exhibited a 3- to 5-fold higher density (B_{max}) of GABA-B binding sites than the SN of adults. In contrast, the density of GABA-B sites was lower in hippocampal and cerebellar tissue from pups, both approximately two-thirds the density in corresponding adult structures. This anatomically selective increased density of GABA-B receptors in immature SN might underlie the greater anticonvulsant efficacy of baclofen observed in rat pups.

Whether this maturational difference in GABA-B receptors between SN and hippocampus results in a difference in responses to GABAergic stimulation, is being investigated by electrophysiologic studies.

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638.11

BRAINSTEM-EVOKED SYNCHRONOUS DISCHARGE IN THALAMIC NEURONS DURING SLEEP IN NORMAL AND PENICILLIN-TREATED CATS. M.N. Shouse, R. Szymusiak and D. McGinty. VAMC, Sepulveda, Dept. Anat. & Cell Biol., UCLA.

Some periods in the sleep-waking cycle are more seizure prone than others. One seizure prone period is the slow-wave sleep (SWS)-REM sleep transition. Discharge of pedunculopontine (PPT) and paramedian midbrain reticular formation (MRF) neurons accelerates during these transitions, while the thalamocortical EEG remains synchronized. Coincidence of moderate brainstem activation with thalamocortical EEG synchrony is hypothesized to contribute to enhanced seizure susceptibility during SWS-REM transitions.

In untreated cats, low intensity, single-pulse stimulation of the PPT or MRF delivered during waking or REM sleep typically evoked a single, short latency action potential in thalamic neurons. Stimulation during SWS commonly evoked a high frequency burst followed by a period of suppressed discharge and, in some cases, a second burst. High frequency bursts were more reliably elicited by PPT stimulation. Following systemic penicillin-G (100,000 IU/kg), brainstem stimulation during SWS reliably evoked high frequency bursts followed by 1-2 pause-burst cycles with a periodicity of 147 ± 12 ms (n=7). This was similar to the interburst interval of these same thalamic neurons during spontaneous EEG spike-wave discharges (156 ± 14 ms, n=7).

Enhanced PPT- or MRF-evoked synchronous thalamic discharge in SWS supports the hypothesis that brainstem activation during SWS-REM transitions contributes to enhanced seizure susceptibility.

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638.12

EFFECTS OF db-CAMP AND Na AND K CONCENTRATION IN MEDIUM ON THE Na,K-ATPase ACTIVITY AND INTRACELLULAR Na AND K CONTENTS OF CULTURED ASTROCYTES OF DBA and C57 MICE. D.M. Woodbury, S.Y. Chow, E.J.K. Noach and J. Li. Div. of Neuropharmacology and Epileptology, Univ. of Utah Sch. of Med. Salt Lake City, UT 84108.

The Na,K-ATPase activity in primary cultures of astrocytes derived from the cerebral cortex of audiogenic-seizure-susceptible mice (DBA) was higher than that of audiogenic-seizure-resistant mice (C57). Intracellular Na of DBA astrocytes was lower than that of C57 astrocytes whereas there was no difference in intracellular K. The Na,K-ATPase activity in astrocytes with db-CAMP pretreatment during the third week in culture was much lower than that without db-CAMP pretreatment in both DBA and C57 mice. Varying the concentration of K and Na in either culture medium or buffer solution altered the Na,K-ATPase activity and intracellular Na and K contents of cultured astrocytes. As K in medium increased from 1.2 to 10 mM, Na,K-ATPase activity gradually increased. It plateaued when K in medium was between 10 and 30 mM. The Na,K-ATPase activity declined as the K in medium exceeded 30 mM. These changes were accompanied by the expected changes in intracellular Na and K contents in both DBA and C57 mice. It appears that astrocytes from the hyperexcitable DBA mice are better able to transport Na than are the seizure-resistant C57 mice. Supported by NIH grants #NS21255 and #NS21834.

638.13

CHOLINERGIC MECHANISMS OF EPILEPTIC SEIZURE GENERATION IN THE RAT BRAIN. J.W.Cruickshank*, R.S.McLachlan, and S.M.Brudzynski. Departments of Physiol. and Clinical Neurol. Sci., Univ. of Western Ontario, London, Ontario, Canada N6A 5A5

Direct intracerebral application of cholinomimetic agents into the brain can cause motor seizures. The present study was designed to determine the types of acetylcholine receptors involved in cholinergic initiation of seizures from the basal forebrain and diencephalon in rats. Unilateral intracerebral microinjections of a mixed muscarinic and nicotinic agonist, carbachol (3μg), caused reproducible generalized seizures in 10 of 13 rats mostly from zona incerta. Local pretreatment with pirenzepine (7μg), an M1 receptor antagonist, abolished carbachol-induced seizures following 78% of injections compared to 14% following pretreatment with methoctramine (12μg), an M2 receptor antagonist. Pretreatment with the nicotinic blocker, mecamylamine (3μg), abolished seizures in 28%. Saline pretreatment prevented seizures in 23%. The results suggest that the activation of M1 muscarinic receptors is involved in the mediation of generalized seizures in the basal forebrain and diencephalic regions.

Supported by Ontario Mental Health Foundation.

638.14

CHOLINERGIC MODULATION OF SOMAN-INDUCED SEIZURES. J.H. McDonough and T.-M. Shih, Pharmacology Div., US Army Med. Res. Inst. Chem. Def., APG, MD 21010

Exposure to the nerve agent soman can produce prolonged recurring seizures and brain damage. Previous work has shown that the initiation and early phases of these seizures are mediated by cholinergic events. The purpose of this study was to determine how long seizure activity needed to progress before anticholinergic treatment became ineffective. Rats implanted with cortical and depth electrodes were pretreated with the oxime HI-6 (125 mg/kg, ip), to prolong survival, 30 min before soman (180 ug/kg, sc; an ED100 for eliciting seizures). EEG activity and behavior were monitored for the occurrence of seizure activity, operationally defined as the first continuous train of spike activity ≥ 10 sec. Scopolamine HBr (SCOP) was administered iv, over a range of doses, at selected times after seizure onset. At 2.5 and 5 min after seizure onset, low doses of SCOP (0.05-0.1 and 0.2 mg/kg, respectively) were capable of terminating the seizure; at 10 min after onset, only higher doses (≥ 0.8 mg/kg) of SCOP were completely effective. If the seizures progressed for 20 min, doses up to 3.2 mg/kg SCOP were incapable of stopping seizure activity. The results show that cholinergic control of soman-induced seizures is of a relatively brief, but discrete, duration, and that within this period of time there is a continual increase in the ED50 of SCOP.

638.15

LOSS OF GABA-MEDIATED INHIBITION IN LITHIUM-PILOCARPINE MODEL OF STATUS EPILEPTICUS. J.Kapur, E.W. Lothman[1] and R.J. DeLorenzo. Department of Neurology, Medical College of Virginia, Richmond, VA and [1]Department of Neurology, University of Virginia, Charlottesville, VA.

Convulsive status epilepticus (SE) is a neurological emergency. The pathophysiology of SE is poorly understood. This study uses a combination of electrophysiological and biochemical methods to compare the state of GABA-mediated inhibition in rats injected with lithium and pilocarpine to cause SE, with that in naive and lithium-treated controls. Paired pulse inhibition technique is used to evaluate GABA-mediated inhibition in the CA1 region of the hippocampus of urethane-anesthetized rats. There is a left shift of inhibition vs interpulse interval curve in test animals when compared with the curve from the two control groups. High affinity ^{3}H muscimol binding sites in rat forebrain synaptic plasma membrane (SPM) fraction are defined by generating a saturation curve. The SPM from control (naive or lithium-treated) groups showed a high affinity for ^{3}H muscimol and saturation of binding sites as reported in the literature. No high affinity binding site for ^{3}H muscimol or their saturation were detected in SPM derived from the test (SE) animals. These results demonstrate a loss of GABA-mediated inhibition in lithium-pilocarpine induced SE. Loss of GABA-mediated inhibition may result from post-translational modification of the GABA receptor and can play an important role in emergence of SE.

638.16

DIFFERENTIAL CHANGES IN EARLY AND LATE PHASE OF INHIBITION IN DENTATE GYRUS FOLLOWING SYSTEMIC ADMINISTRATION OF KAINIC ACID. Moshe Khurgel, E. Pagani* and N.W. Milgram. Life Sciences Division, Scarborough Campus, U. Toronto, Scarborough, Ontario, Canada, M1C 1A4.

Following activation of dentate gyrus granule cells by perforant path stimulation, both an early and a late phase of inhibition of the population spike measure can be demonstrated with the paired pulse technique. These two inhibitory phases are decreased by use and such changes in inhibition are thought to contribute to seizure development. We have monitored both phases during the development of status epilepticus following systemic administration of kainic acid. Subjects were freely moving rats which had been chronically implanted with perforant path stimulation electrodes and dentate gyrus recording electrodes. Consistent with previous reports, prior to the occurrence of the initial afterdischarge activity we found a progressive decrease in the early phase of inhibition measured at an interpulse interval (ipi) of 25 msec. In contrast, there was an unexpected increase in the late phase of inhibition which measured at a 300 msec ipi. We also observed a progressive broadening of the field potential, which was similar to a previously reported late NMDA component and can provide an explanation for the increase in the late phase of inhibition.

638.17

KYNURENATE AND ETHANOL WITHDRAWAL
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Ethanol withdrawal in dependent rats reduced the cortical content of kynurenic acid (KYNA) from 41.5±8 pmol/g tissue to 24.0±3 pmol/g tissue. KYNA is a tryptophan metabolite able to inhibit glutamate neurotransmission by interacting with glycine at the level of the NMDA receptor ion channel complex. In order to understand whether brain KYNA decrease was associated with the symptoms of ethanol withdrawal we administered nicotinylalanine (NAL) to dependent animals. We have previously shown that NAL administration (200 mg/kg i.p.) to rats significantly potentiates tryptophan (200 mg/kg i.p.) induced accumulation of KYNA in their brains. Furthermore NAL administration (100-400 mg/kg i.p.) to audiogenic susceptible DBA/2 mice prevented sound and kynurenine (300 µg/kg i.c.v) induced seizures. The administration of NAL (200-400 mg/kg i.p.) to ethanol dependent C57 black mice was able to prevent withdrawal seizures, tremors and changes in body temperatures, evaluated according to Ritzmann and Tabakoff, J. Exper. Ther., 199, 158-170, 1976.
It appears therefore that by correcting the withdrawal induced decrease of brain KYNA it is possible to prevent symptoms of ethanol withdrawal.

NEUROMUSCULAR DISEASES

639.1

FUNCTIONAL IMPROVEMENT DEMONSTRATED AFTER MYOBLAST TRANSFER THERAPY IN EXTENSOR DIGITORUM BREVIS MUSCLES OF BOYS WITH DUCHENNE MUSCULAR DYSTROPHY . J.A. Florendo, D.S. Kirby*, B.C. Schafer*, T.G. Goodwin*, and P.K. Law. Cell Therapy Research Foundation, Memphis, TN 38117.

Duchenne muscular dystrophy (DMD), an inherited, lethal disease of young males, has no known cure or treatment. Myoblast transfer therapy (MTT), intramuscular injections of normal myoblasts into diseased muscles, has been developed as a possible treatment to alleviate the symptoms of DMD. In this double-blind, first clinical trial of MTT, eleven male volunteers with DMD, aged 5 to 11, received normal myoblast injections in randomly selected extensor digitorum brevis (EDB) muscles. Sham-injected EDBs served as control muscles. All the subjects received Cyclosporine (CsA) for the duration of the trial, and no adverse reactions occurred. This study aimed to demonstrate functional improvement in DMD muscles after MTT.

To evaluate muscle function of EDB muscles, an apparatus was designed and a procedure was developed whereby isometric twitch and maximal voluntary contraction (MVC) tensions could be measured. Twitch tensions were evoked by supramaximal, indirect electrical stimulation. A study of eleven normal adult volunteers was conducted to demonstrate the reproducibility of the technique before testing the DMD volunteers. Six measurements of twitch and MVC amplitudes were obtained before and at 3 and 6 months after MTT on test and control DMD muscles. After MTT the test muscles demonstrated functional improvement, whereas the control muscles showed a loss in strength. (Supported by MDA, Sandoz, and Walgreens.)

639.2

THE EFFECT OF AMITRIPTYLINE ON MUSCLE WEAKNESS AND MYOTONIA IN MYOTONIC DYSTROPHY. S. Milner-Brown, Department of Kinesiology. University of Colorado, Bouler, CO 80309.

The objective of this study was to quantify the degree of muscle weakness and myotonia in 12 patients with myotonic dystrophy (MD), and to quantitatively determine the effects of a 6-month therapeutic trial of the antidepressent drug amitriptyline. Muscle weakness was quantified by comparing the 5-sec maximum voluntary contraction (MVC) in newtons (N)/bodymass (Kg) of 12 patients and 20 healthy subjects; knee extensor, elbow flexor, and first dorsal interosseous (FDI) muscles were compared. Myotonia was quantified by measuring relaxation times (RT) at the end of the 5-sec MVC produced by FDI, as the time taken for the MVC to decrease to 50% and 75% (referred to as 1/2 and 3/4 RT). The results were as follows: (1) the mean muscle strength of each of the 3 muscles of the patients was significantly ($p < 0.001$) reduced compared with healthy subjects; knee extensors 3.3 ± 1.9 N/Kg vs 7.8 ± 1.4 N/Kg, Elbow flexors 1.25 ± 0.6 vs 3.3 ± 0.9 N/Kg, FDI 0.27 ± 0.05 vs 0.46 ± 0.08 N/Kg; and (2) 1/2 and 3/4 RT means of the MD patients (vs healthy subjects) were significantly ($p < 0.01$) prolonged; 328 ± 87 vs 135 ± 28 msec and 44.5 ± 116 vs 185 ± 51 msec.

After the 6-month therapeutic trial of amitriptyline (50 mg/day), the mean muscle strength of FDI improved from 0.27 to 0.33 N/Kg ($p < 0.05$); both the mean 1/2 RT and 3/4 RT were reduced from 328 to 142 msec and from 445 to 187 msec, respectively ($p < 0.01$). It is concluded that amitriptyline may provide therapeutic benefit to patients with myotonic dystrophy.

639.3

AMINOGUANIDINE ON NERVE BLOOD FLOW, VASCULAR PERMEABILITY, ELECTROPHYSIOLOGY, AND OXYGEN FREE RADICALS. J. D. Schmelzer*, M. Kihara*, J. F. Poduslo, G. L. Curran*, K. K. Nickander*, and P. A. Low. Dept. Neurol., Mayo Clinic, Rochester, MN 55905

Since advanced glycosylation endproducts have been suggested to mediate hyperglycemia-induced microvascular atherogenesis, and because aminoguanidine (AG) prevents their generation, we examined whether AG could prevent or ameliorate the physiologic and biochemical indices of streptozotocin (STZ) induced experimental diabetic neuropathy. Four groups of adult Sprague-Dawley rats were studied: Group I, STZ + 25 mg/kg/d AG; Group II, STZ + 50 mg/kg/d AG; Group III, STZ alone; Group IV, Controls. We monitored conduction serially in sciatic-tibial and caudal nerves, nerve blood flow, oxygen free radical activity (conjugated dienes and hydroperoxides), and the permeability coefficient-surface area product to ^{125}I-albumin. STZ diabetes (Group III) caused a 57% reduction in nerve blood flow, abnormal nerve conduction and amplitudes, and a 60% increase in conjugated dienes. Nerve blood flow was normalized by 8 weeks with AG (Groups I, II) and conduction was significantly improved, in a dose-dependent manner, by 16 and 24 weeks in sciatic-tibial and caudal nerves, respectively. The permeability coefficient was not impaired, suggesting a normal blood nerve barrier function for albumin, and oxygen free radical indices were not ameliorated by AG. We suggest that AG reverses nerve ischemia and more gradually improves their electrophysiology by an action on nerve microvessels. AG may have potential in the treatment of diabetic neuropathy.

639.4

HIND LIMB PARALYSIS INDUCED BY INTRASPINAL CORD INJECTION OF COLCHICINE: IMPROVED RECOVERY BY CALCIUM CHANNEL BLOCKERS. **M.Baraldi, P.Zanoli*, P.Guidetti*, C.Truzzi*. Chair of Pharmacology and Pharmacognosy, Modena University, 41100 Modena, Italy.**

The intraspinal cord injection of small doses (5-10 ug/rat) of colchicine (C) at the lumbar level induces urine retention and hind limb paralysis in a dose related fashion. The onset of paresis occurs 1 day after the injection of 5 ug of C. By 2-3 days the hind limbs are completely flaccid. Thereafter the condition begin to improve behing almost normal by 21-24 days. Hence this model was used as a tool to study the neurochemical mechanisms of the paralysis due to spinal cord injury and to test new pharmacological treatments. The motor and sensory deficits in C treated rats were evaluated 1,3,6,8,10,13 and 15 days after C injection by actimetric system and neurological scores. By day 3 after the C injection, when the paralysis reaches its maximum score, we found by RIA a decrease of Sub P in the spinal cord (0.51 vs 7.82 pmol/mg prot. of controls) without changes of Dynorphin. This finding seems to be in line with the reduction of Sub P described in spinal cord injury and with the notion that Sub P antagonists may produce paralysis after their intrathecal administration. By radioreceptor binding assay we found also a reduction of the NMDA-related receptors labeled by ^{3}H-TCP and of sigma receptors labeled by ^{3}H-SKF 10047 associated with an enhancement of calcium binding sites labeled by ^{3}H-PN 200-110 in the spinal cord of C treated rats (35.3 vs 16.1 fmol/mg prot. in controls). Since both Sub P and glutamatergic system are involved in calcium homeostasis, we performed pharmacological challenges by chronically injecting the NMDA noncompetitive antagonist MK 801 or calcium channel antagonists (PY 108-068 and PN 200-110). While MK 801 worsened the spontaneous recovery, the calcium channel blockers strongly facilitated the motor and the neuronal recovery of the C- induced paralysis.

639.5

ELEVATED MECHANOSENSITIVE CHANNEL ACTIVITY IN INTACT SKELETAL MUSCLE FROM *mdx* MOUSE. A. Franco Jr., and J.B. Lansman. Department of Pharmacology, UCSF, San Francisco, CA. 94143.

Cell-attached recordings were made from the surface of *mdx* myotubes and acutely dissociated intact fibers. Mechanosensitive channels in skeletal normal muscle are closed at rest and open when the membrane is stretched. However, a subset of the mechanosensitive channels observed in *mdx* myotubes are highly active at rest. The channels which show the highest resting activity in *mdx* myotubes are open at rest but close, rather than open, with stretch.

As an extension of our earlier results we also examined the activity of mechanosensitive channels in fibers acutely dissociated from the flexor digitorum brevis (FDB) muscle. In contrast to the two forms of mechanosensitive channel activity seen in myotubes, a single class of mechanosensitive channels are observed in FDB fibers. However, surveying the surface of FDB fibers for channel activity, similarly shows that mechanosensitive channel activity is higher in *mdx* FDB fibers when compared with normal fibers.

A process was observed in ~15% of the patches containing channels in *mdx* myotubes and FDB fibers, in which stretch first activated channels which then subsequently became inhibited with stretch. This may be interpreted as a switch in mechanosensitivity from being activated to inactivated with stretch. Thus, although FDB fibers do not show stretch-inactivated channel from the onset of recording, as is the case for myotubes, they do, however, show channels that are inactivated with stretch after a process of conversion. There is, therefore, a correlation between the percentage of channels which are inhibited with stretch and the elevation in resting activity in *mdx* muscle.

639.6

DECREASED CELLULAR ENERGY CHARGE AND INTRACELLULAR ENZYMES IN THE CARDIAC AND SKELETAL MUSCLES OF CHF-146 DYSTROPHIC HAMSTERS (DH) WITH CARDIOMYOPATHY. R.K. Handa, S.K. Bhattacharya, P.L. Johnson, M.P. Gupta, and T.A. Adamec*. Surgical Research Lab, Univ. of Tenn., Memphis, TN 38163.

Membrane-mediated excessive intracellular calcium accumulation (EICA) plays a fundamental pathogenetic role in hereditary muscular dystrophy in animals, as well as in Duchenne muscular dystrophy (DMD). Since efflux of intracellular enzymes (ALD, CPK and LDH) with high plasma activities, decreased adenine nucleotides (ADN) with reduced cellular energy charge (CEC) are the inevitable biochemical fate of the dystrophic tissue with EICA, these variants were measured in the ventricular myocardium (HT) and rectus femoris muscle (RF) of 1 and 12-month-old male DH, and CHF-148 normal hamsters (NH). Glucose-6-phosphate (G6P) level was also monitored to assess the efficacy of glycolytic pathways in DH. Plasma ALD, CPK and LDH were increased in both young and old DH, however the plasma activity fell significantly with age. Correspondingly, these intracelluar energy producing enzymes were significantly diminished both in HT and RF of older DH, but not in younger animals. G6P was reduced progressively in DH ($p<0.05$). In respose to accelerated Ca^{2+}-pumping in dystrophic tissue, ADN and CEC were significantly lower in the HT and RF of older DH, but not in younger hamsters showing considerably less EICA. Thus, ADN and CEC inversely correlated with EICA in DH. **We conclude that** membrane-mediated efflux of intracellular enzymes and depletion of cellular ADN and CEC occur in DH. The glycolytic pathway in DH appears to be suppressed significantly. (Supported by NIH AR-38540)

639.7

INCREASED LIPID PEROXIDATION AND ANTIOXIDANT ENZYME ACTIVITIES IN THE CHF-146 DYSTROPHIC HAMSTERS (DH). M.P. Gupta, S.K. Bhattacharya, P.L. Johnson, T.A. Adamec*, and R.K. Handa. Surgical Research Lab, Univ. of Tenn., Memphis, TN 38163.

Membrane-mediated chronic cellular degeneration plays a fundamental pathogenetic role in hereditary muscular dystrophy. We have shown EICA, efflux of intracellular enzymes and aberrant sarcolemmal dystrophin distribution in DH. We studied whether this genetic membrane insufficiency is associated with enhanced lipid peroxidation and altered antioxidant enzyme activities in muscle of 1 and 12-month-old male DH and CHF-148 strain age and sex matched normal hamsters (NH). Lipid peroxidation (LP), glutathione (GSH), glutathione peroxidase (GSH-Px) and glutathione reductase (GR) were measured in post 10,000 X g supernatant of rectus femoris muscle. Compared to NH, LP increased by 38% ($p<0.01$) and 43% ($p<0.02$), concomitant to decreased GR activity of 43% ($p<0.003$) and 34% ($p<0.001$) in young and old DH, respectively. However, GR activity was higher in older DH ($p<0.01$), compared to younger. GSH level increased by 38% in older DH ($p<0.01$), perhaps in response to cellular adaptive mechanism and regeneration, but not in younger DH. GSH-Px activity was comparable in younger hamsters, but a 21% drop was noted in older DH ($p<0.02$). **We conclude that** chronic pathobiology in DH is inherently linked to cellular antioxidant enzyme-mediated lipid peroxidation of membranes. Increased LP in dystrophic muscle is accompanied by decreased GR and GSH-Px activities. Elevated GSH content in older DH appears to provide insufficient protection against inevitable peroxidative damage and progressive cellular degeneration. (Supported by NIH Grant #AR-38540)

639.8

SKELETAL MUSCLE LACTATE DEHYDROGENASE IS DECREASED IN MURINE MOTOR NEURON DISEASE. R. N. Mandler and J. M. Baca*. Dept. Neurology, The University of New Mexico School of Medicine, Albuquerque, NM 87131.

Lactate dehydrogenase (LDH) is the glycolytic enzyme that converts pyruvate into lactate and regenerates NAD^+ in active skeletal muscle. Skeletal muscle LDH activity decreases after surgical denervation. Based on this fact we explored whether LDH might be decreased in affected skeletal muscles of the Wobbler mouse, a natural murine model of denervation due to degeneration of spinal cord motoneurons. LDH activity was determined by an enzymatic spectrophotometric assay performed on homogenized biceps muscle samples from matched pairs of Wobbler and normal littermates at 3, 7, and 15 weeks after birth. In each age group, LDH activity was lower in the Wobbler. The lowest activity occurred in the oldest age group. Differences were statistically significant by paired t-test in the 7-week and 15-week age groups ($p< 0.05$), with no difference in the 3-week age group. These results demonstrate a progressive alteration of muscle energy metabolism in a natural model of motor neuron disease.

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639.9

Differential staining of rat pudendal motoneurons with an antibody specific to somatic motoneurons. David G. Wells[1], Arlene Y. Chiu[2] and Cynthia J. Forehand[1]. [1]Dept. of Anat. and Neurobiol., Univ. of Vermont, Burlington, VT 05405 and [2]Div. of Neurosci., Beckman Research Inst. of the City of Hope, Duarte, CA 91010.

In amyotrophic lateral sclerosis (ALS), somatic motoneurons, but not preganglionic neurons, undergo degeneration. In humans with ALS, the nucleus in the sacral cord that innervates the external sphincters of the anus and urethra (Onuf's nucleus), is spared (Mannen et al., J. Neurol. Neurosurg. Psychiatry 40:464, '77). This observation has led to the suggestion that these neurons are similar in some way to preganglionic neurons.

Recently, an antibody (MO-1) has been developed that specifically recognizes cholinergic somatic motoneurons in the rat brain stem and spinal cord. This antibody does not stain preganglionic neurons (Urakami & Chiu, J. Neurosci. 10:620, '90). We have applied this antibody to the rat lumbosacral spinal cord to investigate the antigenicity of pudendal motoneurons that correspond to Onuf's nucleus. In the rat, Onuf's nucleus is segregated into two distinct motoneuron nuclei: the dorsomedial (DM), which innervates the external anal sphincter and m. bulbocavernosus, and the dorsolateral (DL), which innervates the external urethral sphincter and the m. ischiocavernosus (Schrøder, J. Comp. Neurol. 192:567, '80).

All motoneurons that contribute to the sciatic nerve abundantly express this antigen; this is not the case in either DM or DL. Two populations of cells were observed in both nuclei. One group was similar to preganglionic neurons in that they expressed the antigen minimally, if at all. The second group of cells, interspersed with the first group, clearly expressed the antigen, but to a lesser extent than motoneurons that project via the sciatic nerve. Whether these two groups reflect differential staining between sphincter and non-sphincter motoneurons is currently being investigated by retrograde labeling in conjunction with MO-1 immunohistochemistry.

639.10

SPECIFICITY OF THE LAMBERT-EATON MYASTHENIC SYNDROME (LEMS) AUTOANTIBODY FOR NERVE TERMINAL Ca CHANNELS. S.J. Hewett and W.D. Atchison, Dept. of Pharmacol./Toxicol., Mich. State Univ., E. Lansing, MI 48824

LEMS is a presynaptic, neuromuscular disorder characterized by impaired nerve-evoked release of acetylcholine. An autoantibody to prejunctional Ca^{2+} channels has been suggested to mediate the pathogenesis of this disease. We demonstrated previously that acute exposure of rat forebrain synaptosomes to plasma from a patient with LEMS reduced depolarization-dependent uptake of $^{45}Ca^{2+}$ (Soc. Neurosci. Abst. 15: 1035, 1989). The objective of this study was to assess the specificity of the autoantibody by comparing the ability of acute application of IgG isolated from the plasma of a patient with LEMS to reduce depolarization-dependent uptake of Ca^{2+} and Na^+ into synaptosomes. Prior to the initiation of uptake, synaptosomes were incubated under pure O_2 for 90 min with varying concentrations of control or LEMS IgG. Synaptosomes were then incubated for 1 sec with resting (5 mM KCl) or depolarizing (41.25 mM KCl) solution to determine depolarization-dependent uptake of $^{45}Ca^{2+}$. Alternatively, following a 10 min incubation with veratridine (150 μM), 5 sec influx of $^{22}Na^+$ was determined in the absence and presence of tetrodotoxin. Net K^+-stimulated $^{45}Ca^{2+}$ uptake was inhibited 36.5±14.5% and 44.5±9.8% by 2 and 4 mg/ml LEMS IgG, respectively. The same concentrations of LEMS IgG did not affect voltage-dependent uptake of $^{22}Na^+$ into veratridine-depolarized synaptosomes. It is possible that normal activation of Ca channels was prevented by IgG-induced alterations in synaptosomal membrane potentials (E_m). Accordingly, we measured E_m using the fluorescent, potentiometric dye (DiS-C2(5)) following acute application of 2 mg/ml LEMS or control IgG. No alterations in dye fluorescence were evident. These results suggest that IgG from patients with LEMS bind selectively to Ca^{2+} channels of presynaptic nerve terminals. (Supported by the Muscular Dystrophy Association.)

639.11

FUNCTIONAL ASPECTS OF PREGANGLIONIC SYMPATHECTOMY BY CHOLINESTERASE ANTIBODIES. V. Moser[1], S. Padilla*[2], P. Hammond*[3] and S. Brimijoin[3]. [1]ManTech Environ. Tech. and [2]US Environ. Prot. Ag., Res Tri. Pk. NC 27709 and [3]Mayo Clinic, Rochester MN 55905.

Systemically administered antibodies to acetylcholinesterase (AChE) cause a unique complement-mediated destruction of preganglionic sympathetic nerve terminals (Brimijoin and Lennon, PNAS 87: 9630, 1990). To further assess neurological integrity in this disorder, 19 rats given murine monoclonal AChE-antibodies or normal mouse IgG (1.5 mg, i.v.) were repeatedly examined over 4 mo with a neurobehavioral test battery (FOB and motor activity). Antibody-treated rats developed permanent eyelid-drooping (ptosis) within 4 hr, reflecting loss of preganglionic sympathetic terminals. Persistent pupillary constriction (miosis) was noted, in accord with previous evidence of preserved parasympathetic function. Weight gain was also depressed. Essentially normal functions included neuromuscular performance (e.g., grip strength, landing foot-splay, gait), excitability (e.g., arousal, reactivity), locomotor activity, and sensorimotor responsiveness to specific stimuli. These results confirm the autonomic focus of immunologic damage. Enzyme assays at 4 mo showed reduced AChE activity in sympathetic ganglia (35%) and adrenal glands (30-40%), while choline acetyltransferase activity, a marker of cholinergic cytoplasm, was largely absent. The enzymatic data were nearly identical with those from an earlier study, one week after antibody injection. Parallel failure of functional and biochemical recovery demonstrates the permanent nature of the neural lesions induced by AChE antibodies. (NS18170).

639.12

GLOBAL BLOCKADE OF CATECHOLAMINE-RELEASE AFTER PREGANGLIONIC SYMPATHECTOMY BY ACETYLCHOLINESTERASE ANTIBODIES. S. Brimijoin[1] and G. M. Tyce[2], Depts. of [1]Pharmacology and [2]Physiology, Mayo Clinic, Rochester MN 55905.

Previous results in rats treated with antibodies to acetylcholinesterase (AChE) demonstrated complement-mediated destruction of preganglionic terminals in sympathetic ganglia with survival of postganglionic neurons. To assess the resulting deficit of sympathoadrenal function, experiments were designed to measure stress-induced release of catecholamines. Rats (6 per group) received injections of murine monoclonal antibody to AChE, or normal mouse IgG (1.5 mg). Four days later a cannula was placed in the femoral artery. On day 5 a 1.5 ml basal blood sample was taken from the awake, unrestrained animals. Later that day the rats were subjected to 10 min of mild stress by forced immobilization in padded restrainers, a second blood sample was drawn, and the rats were killed by pentobarbital overdose. Catecholamine determinations by HPLC in all basal samples showed very low levels of norepinephrine and epinephrine (< 0.2 ng/ml) and moderate levels of the deaminated product, 3,4-dihydroxyphenylethylene glycol (DOPEG, 0.7-1.1 ng/ml). In controls, immobilization-stress increased plasma norepinephrine and epinephrine up to 25-fold (net increase 2.0 & 4.6 ng/ml respectively). The norepinephrine-response in antibody-treated rats was much smaller (15% of control) and the epinephrine-response was nearly abolished (5% of control). Antibody-treatment also markedly reduced the stress-induced rise in DOPEG, although it did not lower basal levels. We conclude that preganglionic immunosympathectomy leads to sustained blockade of neurotransmission throughout the sympathetic nervous system. (Supported by NS18170).

639.13

MECHANISM OF SELECTIVE PREGANGLIONIC SYMPATHECTOMY BY ACETYLCHOLINESTERASE ANTIBODIES. P. Hammond*[1], V.A. Lennon[2], and S. Brimijoin[1]. Depts. of [1]Pharmacology, [2]Neurology, and [2]Immunology, Mayo Clinic, Rochester MN 55905.

Preganglionic sympathetic nerve terminals are selectively destroyed in rats treated with monoclonal antibodies to acetylcholinesterase (AChE). Previous work demonstrated rapid loss of AChE and ultrastructurally defined synapses from ganglionic neuropil, but morphological and biochemical abnormalities at motor endplates were minor. The present experiments were undertaken to clarify the basis for selective immunological attack on ganglionic terminals. Immunofluorescence cytochemistry of superior cervical ganglia, 12 hr after iv injection of AChE antibodies, showed abundant punctate deposits of murine IgG presumably bound to AChE-rich synapses. Unexpectedly dense IgG deposits were also found at motor endplates, demonstrating antibody-access to these structures as well. Monoclonal antibody to component C3 revealed intense complement-fixation in perikaryal ganglionic neuropil. This evidence for complement-mediated lysis of ganglionic terminals is consistent with our earlier finding that ganglionic damage is prevented by depletion of complement with cobra venom factor. However, specific C3-immunofluorescence was also found at the neuromuscular junction, where it colocalized with rhodamine-labeled α-bungarotoxin. The basis for a selective cytodestructive lesion of sympathetic terminals remains uncertain. Topologic features of the neuronal target enzyme may render the preganglionic plasma membrane particularly vulnerable to complement-mediated lesions. (Supported by Grants NS18170 and NS23537).

639.14

EFFECTS OF ACETYLCHOLINESTERASE ANTIBODIES ON CHOLINERGIC SYSTEMS IN NEWBORN RAT BRAIN. Z. Rakonczay* and S. Brimijoin, Dept of Pharmacology, Mayo Clinic, Rochester, MN 55905.

Acetylcholinesterase (AChE), an extracellular, membrane-associated enzyme, is a possible target of autoimmunity. When injected systemically in adult rats, antibodies to AChE irreversibly damage some peripheral cholinergic structures (preganglionic sympathetic neurons), but brain and spinal cord are protected by the blood-brain barrier. Because barriers to IgG may be more permeable in immature mammals, we undertook to determine whether AChE-antibodies would enter brain and disrupt central cholinergic systems in newborn rats. Antibodies (ZR 2,3,4,5,6) were given *ip* (equal mixture, 50 µg IgG each), 1 day postnatally (d1). Control animals received saline solution or normal mouse IgG. Body and brain weights and cholinergic markers (AChE, choline acetyltransferase, ChAT) were measured at days 3, 7, 12, and 21 in serum, brain and other tissues. The outward effect of antibody was an almost complete cessation of growth. Antibody-treatment also caused an immediate 90% decrease of serum AChE which was sustained through d21. Brain AChE decreased by 30% at d3, reflecting virtual disappearance of the tetrameric G4 form. At d7, this form began to recover but was still only half as abundant as in controls. Body and brain weights also remained subnormal. At d12, the pattern of AChE molecular forms in whole brain extracts was nearly the same as in controls, but total AChE and ChAT activity were still reduced by 50%. Persistent deficits of G4 AChE were noted in cerebral cortex at d12 and d21. This finding may be evidence for delayed maturation of AChE-expressing cortical neurons. (Supported by NS18170).

639.15

AN ELECTRON MICROSCOPIC STUDY OF NEUROMUSCULAR ALTERATIONS IN PARANEOPLASTIC PHENOMENON. H.J. Finol, I. Rodríguez*, A. Márquez*, I. Montes de Oca* and B. Muller*. Sciences, Medicine and Dentistry Faculties, Universidad Central de Venezuela, Apartado 47114, Caracas 1041 A, Venezuela.

Several clinic and pathologic alterations have been described in skeletal muscle in patients suffering from cancer with no muscle metastases. Some of these changes have been considered of neural origin. In support of this point of view, we describe in this work the nerve and muscle ultrastructural pathology in a case of paraneoplastic phenomenon associated with a carcinoma of cervix. A needle biopsy was taken from quadriceps femoris muscle in a 65 year-old female who presented proximal muscular weakness and wasting, associated with a carcinoma of cervix. The patient was treated only with radiotherapy. The changes observed were: some muscle fibers showed different degrees of atrophy and others looked normal. Atrophied fibers presented in motor end-plates destruction of axon terminal with preservation of subsynaptic regions. Neural damage was also observed in myelinated fibers outside the end-plate. Vascular alterations and mononuclear cell infiltration formed by lymphocytes, macrophages and mast cells were also seen. Our findings suggest that muscular damage in this case probably has a dual etiopathogenic mechanism, vascular and neural. Supported by CDCH of UCV, Fundación Polar and The British Council Venezuela.

639.16

ABNORMAL ISOMETRIC MUSCLE FORCE CONTROL IN REFLEX SYMPATHETIC DYSTROPHY. C.J. Hunker* and M. Backonja. Dept. of Neurology, U. of Wisconsin, Madison, WI 53792.

The etiology and specific pathophysiology of movement disorders associated with reflex sympathetic dystrophy (RSD) are unknown; central nervous system mechanisms and psychogenic origins have been proposed. Freund and colleagues (1978, 1984) have shown that submaximal isometric contractions provide a means of relating muscle force control to involuntary motoneuronal activity. Hunker et al. (1991), have demonstrated that submaximal isometric contractions can distinguish pathological from somatoform tremors. Maximal and controlled voluntary contractions were examined in 10 RSD patients via isometric wrist and thumb/index finger tasks along with accelerometer recordings from affected and unaffected limbs. RSD patients presented muscle weakness and motor abnormalities of dyskinesia, dystonia, synkinesia and 6.0 Hz tremor localized to the affected limb. Since movement disorders of central origin result from abnormal motor unit activity, involuntary muscle force aberrations are salient during submaximal isometric contractions; in contrast, psychogenic movement disorders, which are not due to involuntary motor unit activity, are absent under isometric conditions. These data support putative CNS pathophysiological substrates for the movement disorders associated with RSD.